Biomacromol. J., Vol.12, No. 1, 16-22, January 2026.

Biomacromolecular Journal

www.bmmj.org

Association between single nucleotide polymorphisms in PTEN and susceptibility to schizophrenia in an Iranian population

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(Received 5 February 2023, Accepted 14 May 2023, Published 30 January 2026)

ABSTRACT

Schizophrenia is a mental disorder that affects the world's population, and genetic and environmental factors play a role in its development. Polymorphism research can serve as a marker for disease in identifying individuals at higher risk of developing the disease. This study was conducted with the aim of investigating PTEN gene polymorphisms (rs12569998, rs2299939) and the risk of schizophrenia (SCZ). DNA was isolated from 108 schizophrenic blood samples and 108 control group samples, and then the Tetra ARMS-PCR technique was used to investigate rs12569998 and rs2299939 gene polymorphisms. The frequency of genotypes, alleles, and the Hardy-Weinberg equilibrium were evaluated in this case-control study. AC, CC, and AC+CC (OR = 0.84, P = 0.85; OR = 0.8, P = 0.88; OR = 0.83, P = 0.86) and GT, TT, GT+TT (OR = 0.52, P = 0.45; OR = 0.33, P = 0.23; OR = 0.5, P = 0.4) were the results of the PTEN gene's SNP analysis in rs2299939 A/C and rs12569998 T/G. There was no significant difference between SCZ and the control group ($p>0.05$). According to this study, these two SNPs are not risk factors for SCZ, and the frequency of specific genotypes and alleles among other genes should be examined to diagnose SCZ. Further research with larger sample sizes in different genetic populations is recommended to validate the current data.

Keywords: PTEN gene, polymorphism, schizophrenia

INTRODUCTION

Psychiatric pathology such as schizophrenia (SCZ) in late adolescence or early adulthood, characterized by highly heterogeneous symptoms for example hallucinations, delusions, speech or behavior disorder, and impaired cognitive ability, requires accurate and correct identification, which is time-consuming, expensive and complicated [1]. Despite extensive research on schizophrenia, the cause is unknown. However, genetic factors are a significant factor in schizophrenia [2], and 80% of the phenotypic variation in incidence (1% of the global population) of schizophrenia is attributed to genetic factors [3]. In addition to hereditary factors, inflammation, and immunity, unusual brain structures, disorders during pregnancy, and environmental factors are highly effective in the pathogenesis and etiology of this disease [4, 5].

It is now believed that environmental variables, gene polymorphism, and copy number variation interact intricately to impact the developing

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central nervous system and synaptic circuits, ultimately resulting in a variety of disorders [6]. At 108 gene loci, 128 SNPs have been found by genome-wide association studies (GWAS) [4, 7].

According to a 2022 study, the polymorphism may act as a genetic risk factor for schizophrenia at both behavioral and neurological levels. [8]. On chromosome 10q23.14, the tumor suppressor gene phosphatase and tensin homolog (PTEN) encodes a 403 amino acid dual-specificity lipid and protein phosphatase and controls the conserved PI (3)K-AKT-mTOR pathway. It performs specific roles in the growth and upkeep of the nervous system, including apoptosis, controlling cell migration, survival, proliferation, metabolism, and growth [9]. Under the impact of astrocytic processes, PTEN expression is markedly elevated following spinal cord damage. An imbalance in the post-injury synaptic plasticity pathways is reflected in the alterations seen following injury. As a result, methods of altering PTEN gene expression are promising [10]. According to Takeuchi et al.'s research, PTEN affects neurogenesis and synaptic functioning in different ways. For instance, PTEN loss in post-mitotic neurons causes cellular hypertrophy, whereas PTEN deficiency in neural stem/progenitor cells (NSPC) promotes proliferation [11]. PTEN loss causes neuronal hypertrophy, which includes many aberrant axons, lack of appropriate axonal projections, and enlargement of soma, dendrites, and axons (12). Numerous SNPs make up the highly polymorphic PTEN gene, and studies have demonstrated that PTEN can produce novel isoforms [12]. Wang and colleagues' work on the PTEN gene (rs786204926) showed that the polymorphism is an important factor in the chemosensitivity of breast cancer. [13]. Liu et al. (2016) shown that the PTEN gene's rs701848, rs2735343, and rs112025902 polymorphisms may be linked to depression and its symptoms [14]. Due to its high incidence and substantial socioeconomic burden, schizophrenia must be treated [15]. There are few effective treatments for schizophrenia, and further research into the underlying pathology is required to advance this field and properly manage its symptoms. This theory that the pattern evolution of nerves is a key factor in the genesis of schizophrenia is supported by clinical research, evidence of brain pathology, genetics, and nerve imaging studies [16].

SNP is often used as a biomarker and can play a role in the diagnosis of schizophrenia [17]. PTEN plays a role in the development and maintenance of the nervous system, but a direct investigation of the PTEN gene and SCZ has not yet been performed. The aim of this study was to investigate molecular analysis to determine the association between the risk of schizophrenia in the Iranian population and PTEN gene polymorphisms (rs12569998, rs 2299939).

MATERIAL & METHODS

Subject Population

In this case-control study, 68 men and 40 women were identified as having schizophrenia (SCZ), while 93 men and 15 women who had no history of neurological disorders and no schizophrenia diagnosis were gathered as controls. The sample size was calculated based on the Cochran formula. In this calculation, 100 patient samples and 100 control group samples were estimated with an error level of 5%. Every subject was solely of Iranian descent. Following diagnosis, participants were chosen from the Farabi, Modares, and Isfahan Psychiatric Hospitals as well as the Behravan Welfare Center in Yasuj. Three psychiatrists evaluated each patient's mental health in accordance with the DSM-V criteria for schizophrenia and psychotic symptoms [18]), and the files of patients with schizophrenia were reviewed against screening standards. All participants completed an informed consent form at the time

of enrollment, and the Declaration of Helsinki governing human subjects applied to all operations.

Inclusion Criteria

The control group had no personal or family history of neuropsychiatric disorders or any abuse of addictive drugs. The diagnosis was based on the guidelines for schizophrenia and psychotic disorders.

Exclusion criteria

Patients who, in addition to schizophrenia, had a chronic physical illness or other mental disorders, had psychosis due to medication use, and files that had incomplete information and were not completed after contacting the patients' families due to lack of response were excluded from the study. Demographic characteristics were gathered using a structured questionnaire for two groups: patients and healthy individuals. This included details such as age, contact information, education level, medical history, family history, marital status, weight (measured using a clinical scale), height (in centimeters), and waist circumference (measured with a tape just above the superior iliac crest while the subject was standing). A volume of 3 ml of venous blood was collected using EDTA-coated tubes and stored at -80°C until further analysis.

Genotyping of PTEN Polymorphisms rs12569998 and rs2299939 to control the quality of the extracted DNA, DNA was examined using a Nano Drop optical absorption device (samples with an OD between 1.8 and 2 were confirmed) (USA Perkin Elmer xenon flash Tube Model 550-s). Electrophoresis technique was also used to control the accuracy of DNA extraction (DNA samples were run on an electrophoresis gel and after staining with ethidium bromide and photographing with a gel dock, the accuracy of the band formed on the gel was confirmed by comparison with a molecular marker). The primers were designed by OLIGO primer analysis software Version 7 (http://www.ncbi.nlm.nih.gov). To examine the PTEN polymorphisms rs12569998 and rs2299939, the tetra primer-ARMS-PCR technique was used. The sequences of outer and inner primer pairs are shown in Table 1.

PTEN polymorphisms was analyzed using tetra primer-ARMS-PCR method, including 10 µl PCR premix (Cina Colon – Iran) according to the manufacturer's recommended protocol, 1.5 µl template DNA and 1 µl of each primer; also, 3.5 µl DNase-free water was added to the Pre-Mix into a 0.2 ml microtube. PCR reaction was performed in a thermocycler (Thermal Cycler, Thermo Fisher Scientific Invitrogen-US).According to the protocol, the initial denaturation was carried out at 95 C° for 5 min and 40 cycles at the set temperatures and times for the denaturation at 95oc f for 30 s, extension at 95oc for 30 s, annealing 56 C° for rs2299939, 54 C° for rs12569998 for 30 s and 72 C° for 30 s (35 cycles) and finally at 72 C° for 10 minutes. Amplified T-ARMS-PCR product was used for genotypes identification by electrophoresis. After being loaded on 4% agarose gel and stained with 0.5 µg/ml, ethidium bromide was observed under UV light.

Statistical Analysis

Demographic characteristics were means ± SD (standard deviation). Statistical analysis was done via using the student's t-test and chi-square and Fisher's exact test. The Kolmogorov-Smirnov single-sample test was used to determine whether the data had a normal distribution. We estimated odds ratios (OR) and 95% confidence intervals (95% CI) to investigate the association between polymorphisms in rs12569998 (T/G) and rs2299939 (A/C) at the PTEN gene with the risk of SCZ. Data were evaluated with SPSS (Version 25.0). $p < 0.05$ was considered to be the significance level for all statistical analyses.

RESULTS

Characteristics of Samples

According to the study, 93 males (86%) and 15 females (14%) were designated as the control group, while the SCZ group comprised 68 individuals (63%) and 40 individuals (37%), respectively. The average age of the control group was 37.52±10.776 years, whereas the patients had a mean age of 43.71±11.035 years (p=0.489). Table 2 presents a comparison of the demographic characteristics between the two groups. Genotype profiles were observed with T-ARMS-PCR method inpatient and control groups in different patterns in the form of bandson gel electrophoresis including two bands with 167 bp and 413 bp lengths for (TT) genotype, three bands with167, 300 and 413 bp for (GT) genotype and two bands in 300 bp and 413 bp regions for (GG) genotype (Figure 1).

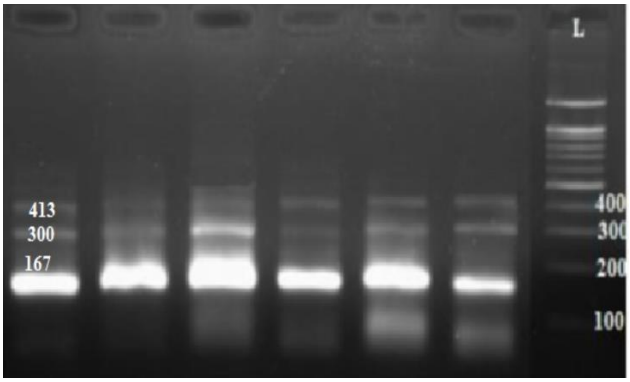

Figure 1. The 2% agarose gel image of some samples to check the polymorphism re12569998 (T/G) PTEN gene. L: Marker, all samples are heterozygote (GT).

Two bands with a length of 120 bp and 276 bp for genotype (AA), three bands with 120 bp, 216 bp and 276 bp for genotype (AC) and two bands in the regions of 216 bp and 276 bp for (CC) genotyping in rs39229 appeared (Figure 2).

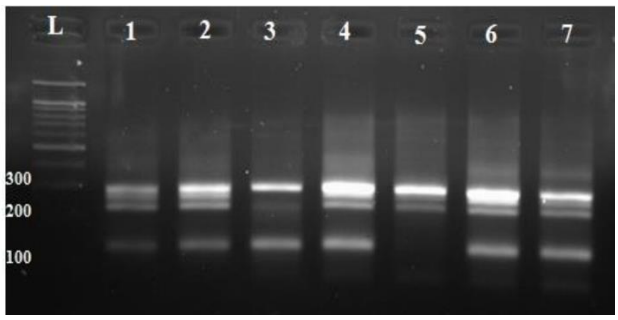

Figure 2. The 2% agarose gel image of some samples to check the polymorphism re2299939 (A/C) PTEN gene. L: Marker- 1,2,4,6,7 heterozygote (AC), 3 dominant homozygous (AA), 5 recessive homozygous (CC).

Genotypic Frequency

The chi-square test was used to determine the frequencies of the AA, AG, and GG genotypes for the rs12569998 (T/G) and rs2299939 (A/C) polymorphism locus of PTEN. SCZ and the control group did not differ significantly (p>0.05) (Table 3). In the present study, rs12569998 TT (13.2%) (11.1%), GT (56.2%) (85.2%), and GG (30.6%) (3.7%) genotypes were appeared in control and patient samples, respectively. The frequency of homozygous and heterozygous genotypes in patients and control groups was not significantly different (p=0.45). However, the frequency of the GT genotype in the patient group was higher than the control group. (OR=0.52, 95%CI=0.046-3.76) (p=0.45). (OR = 0.33, P = 0.23(TT); OR = 0.5, P = 0.4 (GT+TT)). The rs2299939 genotypes AA (11.1%), AC (73.1%), and CC (11.1%) were observed in control samples, while in SCZ they were present at a rate of 13, 72.2%, and 14.8%. The frequencies of homozygous and heterozygous genotypes in both the patient and control groups did not show a significant difference according to the $\chi 2$ test (p = 0.85). This relationship was further validated by Fisher's exact test. Nonetheless, the sick group had a greater frequency of the CC genotype than the control group (OR=0.80, 95%CI=0.25-2.54) (p=0.88). OR = 0.83, P = 0.86 (AC+CC); OR = 0.84, P = 0.85 (AC).

DISCUSSION

Millions of individuals worldwide are impacted by neurological illnesses, which are significant contributors to mortality and disability. Examining the relationship between polymorphisms and SCZ risk is crucial since it sheds light on the mechanisms and offers a prognostic index. The genotype and allelic frequency of rs12569998 T/G and rs2299939 A/C in the PTEN gene did not significantly differ between the SCZ and healthy groups in the current investigation. A significant genetic influence in schizophrenia susceptibility is played by polymorphisms found in a number of risk loci [5]. The homozygous CC mutant in SCZ exhibited a 1.33-fold increase compared to the control group, as indicated by our findings in rs2299939 A/C. Data collected from 108 patients and 108 controls revealed that the combined (AC+CC) genotypes were not associated with an elevated risk of SCZ (p=0.86). At rs12569998T/G, the frequency of homozygous GT did not show a significant difference between the control and SCZ groups, but was about 1.04 times higher in the SCZ group (p=0.45). No significant relationship between polymorphisms and the risk of SCZ was observed, and it indicates that this polymorphism is not related to SCZ susceptibility in the Iranian population. Our study showed that in rs12569998T/G and rs2299939 A/C of PTEN gene, the frequency of some genotypes in the SCZ group was higher than the control group.

Similar to our research, in the study by Sargazi et al., the cereal-like transcription factors (GRHL), rs2486668C/G was not associated with SCZ susceptibility, while rs545809A/T was associated ($P < 0.05$). They demonstrated that the rs545809A/T allele can influence the risk of SCZ and raises the risk of SCZ by 2.33 times in the Iranian population [19].

Eight SNPs for the Alzheimer's PTEN gene were examined, but no indication of a connection to the illness was discovered. The findings demonstrated that the risk of Alzheimer's disease cannot be influenced by genetic variation in the PTEN gene [20]. Phillips et al.'s research revealed no link between colon cancer and the PTEN gene. There was no statistically significant association between four SNPs (rs2299939, rs12357281, rs2248293, and rs926091) in two groups [21]. None of the 26 SNPs, including rs2299939 and rs2248293, were shown to be substantially associated with testicular germ cell tumor (TGCT) in the Andreassen et al. research. The only PTEN gene that was substantially linked to TGCT was (rs11202586) (OR % 1.16, 95% CI % 1.06–1.28, P=0.040) [22]. In addition, rs1625579 in MiR137 has no connection in schizophrenia, according to Sun et al., while rs107822 in MiR219-1 is significantly associated ($p<0.05$), and the source C allele, TC, and CC genotypes are risk factors for schizophrenia [23]. There are certain biological pathways linked to schizophrenia. Phosphatase and tensin homolog regulation affects activation of the PI3K/AKT/mTOR pathway. PTEN stability and phosphatase biological activity are controlled by this process [24]. Some human problems associated with PTEN mutations have been identified, including neurological diseases and glioblastoma, which affect the PI3K/AKT pathway [25]. Overactivation of These alterations could be due to PTEN over activating the AKT signaling pathway. The lipid metabolic route in schizophrenia may have PIK4CA as a novel therapeutic target [26]. Examination of the rs2299939 and 12569998 polymorphisms and SCZ risk using various genetic models revealed no correlation between SNPs and Iranian population susceptibility to SCZ. The patient group's increased prevalence of specific genotypes and alleles, however, might be regarded as a contributing cause to SCZ.

Two PTEN polymorphisms were identified in our study, as indicated in Table 3. These

polymorphisms demonstrated that rs12569998 with GT and CC genotype at rs2299939 could not be a risk for schizophrenia because these relationships were not significant: rs2299939: with OR=0.8 95% CI=0.25-2.54; P=0.88, rs12569998: with OR=0.8 95% CI=0.25-2.54; P=0.45.

In the PTEN gene, the C allele of rs701848, the C allele of rs2735343 and the T allele of rs112025902 were associated with an increased risk of depression OR = 3.814, 95% CI: 3.093–4.703, P = 0.001; OR = 2.642, 95% CI: 2.152–3.242, P = 0.001; OR= 2.882, 95% CI: 2.347–3.539, P = 0.001; respectively. This polymorphism was related with the risk of depression and depressive [14].

However, in mental disorders, the molecular processes underlying cellular dysfunction, survival function, or neuronal death are not well understood. Cell proliferation, migration, death, as well as the pathophysiology and architecture of an intracellular network essential for neuronal synaptic function, are influenced by the PI3K, AKT, and GSK3 pathways.[27]. The relationship between schizophrenia and PTEN is not fully understood. Further functional studies will provide new evidence for the role of the rs12569998 T/G and rs2299939 A/C SNPs of PTEN in the pathogenesis of SCZ and help to elucidate the disease mechanisms. It is hoped that the results of this study will provide a basis for further research. The present study did not show significant difference between PTEN polymorphism (rs12569998 and rs2299939) and SCZ. In addition, it is suggested that other PTEN polymorphisms be investigated in comprehensive studies in a larger Iranian population or other populations, and more accurate results can be obtained by using diverse ethnic groups in future research.

ACKNOWLEDGMENTS

The authors wish to thank the University of Sistan and Baluchestan, and the patients and healthy subjects who willingly participated in this study.

FUNDING

We have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DECLARATION OF INTEREST STATEMENT

The authors declare no relevant financial interests. This research was registered with the number 11507 in the Research Vice-Chancellor of the University of Sistan and Baluchestan.

AUTHOR CONTRIBUTION

Dor Mohammad Kordi Tamandani, Research conception, Protocol/project development, Review, Project administration, analysis and interpretation.

Nasim Naeimi, Manuscript editing, Research conception, design, Methodology, Validation, Investigation, Performing the experiments, Data acquisition, Statistical analysis, Writing Data Software, analysis and interpretation.

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