ASSOCIATION OF THE MCP-1 -2518 A/G POLYMORPHISM
WITH SCHIZOPHRENIA RISK: INSIGHTS FROM A BATAK ETHNIC GROUP STUDY

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Abstract

Background: A genetic variation called the monocyte chemoattractant protein-1 (MCP-1) -2518 A/G polymorphism can affect a person's inflammatory response and MCP-1 gene expression. Several studies suggested this polymorphism to be potentially linked to the risk of developing schizophrenia, a complex and multifactorial condition involving the interaction between genetic and environmental factors. Recently, individuals carrying the G allele of the MCP-1 -2518 A/G have been implicated with increased susceptibility to schizophrenia compared to those homozygous for the A allele. However, other factors contributing to the development need to be considered. Further studies are required to understand better the relationship between the MCP-1 -2518 A/G and schizophrenia risk.

Method: 400 individuals, including 200 schizophrenia patients and 200 healthy Batak controls, were sampled using a non-probability purposive sampling method. The RFLP and PCR techniques were used to study the MCP-1 -2518 A/G polymorphism.

Result: With a p-value of 0.2, an odds ratio (OR) of 0.82, and a 95% confidence interval (CI) spanning from 0.62 to 1.09, the frequency of the A and G alleles did not differ substantially between the schizophrenia and control groups. However, the genotypes (AG vs GG) showed a significant correlation with an OR of 3.167, a 95% CI (confidence interval) of 1.889 to 5.311, and p 0.01. With p= 0.157, an OR of 1.557, and a 95% confidence interval (CI) spanning from 0.085 to 0.633, there was no statistically significant connection between AA and GG.

Conclusion: The MCP-1 -2518 A/G polymorphism may be linked to a risk of schizophrenia, according to this study. To validate these findings and clarify the underlying mechanisms connecting MCP-1 and schizophrenia, additional research was needed.

Keywords: schizophrenia, polymorphism, MCP-1, Batak tribe, control group

Introduction

Schizophrenia is one of the most complex brain disorders, with severe and persistent psychotic manifestations, cognitive dysfunction and psychosocial impairments. The symptoms are usually unnoticed in early life but worsen during childhood and show some degree of improvement with age. People with schizophrenia (PWS) generally face lifelong challenges due to the ongoing need for medication and the increasing burden [1]. Furthermore, it is among the top 10 mental diseases globally, with a global incidence of about 20 million people. It often starts between 15 and 30, peaking in onset between 15 and 25 for males and 25 to 35 for females. Overall, females with schizophrenia have a better prognosis than males [2,3]. In Indonesia, schizophrenia ranks third among the top 10 mental disorders, affecting 6.9% of a thousand households with a family member experiencing schizophrenia or psychosis, and is more commonly found in rural than urban areas. Among the 90% of patients receiving treatment for schizophrenia, the age range spans from 15-55 years [2,4].

Numerous studies have revealed biological anomalies such as genetic, perinatal, neuroanatomical, neurochemical, and others that increase the risk of schizophrenia, which is impacted by biopsychosocial variables. Individuals who migrate from one country to another or are members of ethnic minority populations in urban areas may be more at risk due to psychological and socioenvironmental factors. [5]. Chronic inflammatory processes can occur in the periphery of the body in PWS, and cognitive abnormalities are connected to immune system changes related to inflammation in the brain. Recent meta-analyses have revealed elevated levels of chemokines and monocyte chemoattractant protein and general inflammation markers (C-reactive protein [CRP] and leukocyte count), cytokines (interleukin [IL]-6, -8, -10, interferon-gamma [IFN-gamma], and tumour necrosis factor-alpha and chemokines and monocyte chemoattractant protein [MCP] 1/CCL2) in PWS compared to controls [6].

Studies on chemokines in schizophrenia are still limited, with most focusing on chemokine levels in sick males. Small proteins called chemokines are produced in tissues in response to damage or infection. Among them, MCP-1 has been extensively investigated in schizophrenia [7,8]. MCP-1 acts as a mediator in attracting monocytes and macrophages to inflammatory sites. Patients with a variety of neuroinflammatory conditions, such as multiple sclerosis, ischemic stroke, and human immunodeficiency virus (HIV)-1 encephalitis, have been found to have elevated levels of MCP-1 in their blood. [9]. The -2518 A/G polymorphism affects monocyte MCP-1 production and transcriptional activity in the distal regulatory region. In South Korea, Pae et al. (2004) reported significant differences in the alleles and genotypes of the MCP-1 -2518 A/G between PWS and healthy controls. Similarly, The monocyte MCP-1 synthesis and distal regulatory region transcriptional activity are impacted by the -2518 A/G polymorphism. [9,10]. The MCP-1 gene has three exons and two introns and is found on chromosome 17q11.2-q21.1. The precursor molecule of MCP-1 consists of a hydrophobic N-terminal signal peptide of 23 amino acids. Upon signal peptide cleavage, the protein forms a monomeric polypeptide containing 76 amino acids. In the Tunisian population, the MCP-1 -2581G/-362G haplotype combination confers protection against schizophrenia, with a more pronounced protective effect associated with the -2518G allele and the -362G variant [11]. Therefore, this study aimed to compare the MCP-1 -2518 A/G polymorphism between PWS and the Batak population group.

Patients and Method
Patients and Study Design
This comparative analytical study employed a case-control design. The total sample consisted of 400 individuals, including 200 (145 males and 55 females) PWS and 200 (163 males and 37 females) controls, selected using non-probability purposive sampling.
Method
The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was used to identify the genotypes of MCP-1 at the -2518 A/G promoter site. The 25μl PCR reaction mixture contained 100ng of DNA, 2.5 mmol/L of magnesium chloride, 200 nmol/L of dNTPs, 12.5ng of primers, and 0.5 Taq DNA

polymerase enzyme units. The promoter polymorphism at the -2518 position was determined at 139 base pairs (bp) using the forward primer: 5' GCT CCG GGC CCA GTA TCT 3' and the reverse primer: 5' ACA GGG AAG GTG AAG GGT ATGA 3'. A 94°C initial denaturation step was followed by 30 cycles of 94°C, 55°C, and 72°C for 30 seconds, 30 seconds, and 7 minutes, respectively, during the PCR cycle conditions. The PCR products were then subjected to overnight incubation at 37°C with the addition of the PvuII enzyme for MCP-1 restriction. The restriction fragment was 182.54 bp (allele A), and the uncut 236 bp fragment (allele G). The genotypes were ascertained by examining the PCR results on a 3% agarose gel stained with ethidium bromide.

Result

    The two groups had no gender-related statistically significant variations in the demographic variables (p=0.495). The PWS group's median age was 35 years, while the control group's was 24.5 years; this age difference was statistically significant (p=0.001). The median age of disease onset in the PWS group was 25, with a minimum value of 17 years and a maximum of 34 years. With a standard deviation of 5.16 years, the sickness lasted an average of 8.91 years. According to Table 1, the mean total PANSS score was 96.42, with a standard deviation of 7.85. Between the PWS group and the control group, there were no appreciable changes in the frequency of allele occurrence (p=0.2). The odds ratio (OR) was 0.82, with a confidence interval (CI) ranging from 0.62 to 1.09, as presented in Table 2. The most common genotype in the PWS and healthy groups was AG, with a sample size of 89 (44.5%) and 134 (67.0%) subjects, respectively. Logistic regression analysis showed a p-value of 0.157 for the genotype (AA vs. GG). The OR was 1.557, with a 95% CI ranging from 0.085 to 0.633. The p-value for the genotype (AG vs. GG) was <0.01. The OR was 3.167, with a CI ranging from 1.889 to 5.311, as presented in Table 3.

Table 1. Demographic characteristics

Variable	PWS (n=200)	Control (n=200)	P-value
Gender			
Male	145 (72.5%)	163 (81.5%)	0.495*
Female	55 (27.5%)	37 (18.5%)	
Age	35.00 (19.00-51.00)	24.50 (20.00-40.00)	0.001**
Onset[a]	25.00 (17.00-34.00)	-	
Duration of Disease	8.91±5.16	-	
Total PANSS score[a]	96.42±7.85	-	

Chi-square with continuity correction

Table 2. Differences between MCP-1 Gene Polymorphism Allele -2518 A/G in PWS and Healthy Controls from the Batak Tribe

Variable Allele MCP-1 - 2518 A/G	Group		P-value	OR (95% CI)
	PWS (n=200)	Control (n=200)		
A	189 (47.3%)	2O8 (52%)	0.2	
G	211 (52.8%)	192 (48%)		0.82 (0.62-1.09)

Table 3. Differences between MCP-1 -2518 A/G Gene Polymorphism Genotypes in PWS and Healthy Controls from the Batak Tribe

Variable Genotype MCP-1 -2518 A/G	Group		P-value	OR (95% CI)
	PWS (n=200)	Control (n=200)		
AA	50 (25%)	37 (18.5%)	0.157	1.557 (0.843-2.874)
AG	89 (44.5%)	134 (67%)	< 0.01	3.167 (1.889-5.311)
GG	61 (30.5%)	29 (14.5%)		

Discussion

The sample used was predominantly composed of males, and there were observed differences in age between the two subject groups. The results were consistent with previous studies that evaluated the trends in the incidence and disability-adjusted life years (DALYs) of schizophrenia globally. Differences in gender, including greater schizophrenia levels, were found in males compared to females in 2017. The number of cases in males reached approximately 6.51 million, while in females, it was around 6.14 million cases. This was supported by similar observations in a cohort study involving over three million subjects aged 15-64 in Spain by Sanchez et al. The prevalence discovered in males reached 64.3%, almost twice as high as 35.7% in females, and was also higher in all age groups examined [13].

The age analysis results were consistent with most studies indicating that the onset of schizophrenia most commonly occurs in the age range of 21-25 years, with a delay of 3-5 years in females compared to males. In the 40-50 age group, even though less common, the onset of schizophrenia is more frequent in females (66%-87%) [14,15].

No association was found between alleles in both the PWS and control groups. This was in line with a previous study involving 123 inpatient PWS and 114 healthy control subjects, which also reported no significant differences in allele distribution between the ODS and control groups [13]. Similarly, other studies in Korea stated no association between alleles and genotypes in both groups. These hypothesized that the distribution of genotypes and alleles may demonstrate appreciable changes in the contribution of MCP-1 -2518 promoter polymorphism to the clinical heterogeneity of schizophrenia when people with positive and negative symptoms of schizophrenia are taken into account. [16].

The current results contrasted with a case-control study conducted in Tunisia, which indicated significant differences between PWS and Control groups for the allele, with the G allele being more frequently found in the control group than in PWS [10]. The observations suggested that different inclusion criteria could influence the results obtained because the selected populations had different characteristics. Environmental factors and lifestyle could play a significant role in the development of schizophrenia, leading to variations in results among different studies. Therefore, more extensive and meticulous studies are needed to evaluate the different factors and their potential interactions.

This study observed an association between genotypes, with the heterozygous genotype showing significant differences compared to GG. This was consistent with a previous study that discovered a significant association between MCP-1 -2518A/G genotype in the PWS and control groups. Furthermore, carriers of MCP-1 -2518G heterozygous and homozygous genotypes were detected to have a lower risk of developing schizophrenia than non-carriers of the variant [17]. Another study that supported these findings identified a correlation between people who had positive and negative symptoms but not schizophrenia. At least in the Korean population, it was reported that the MCP-1 -2518 promoter polymorphism may be connected with the severity of schizophrenia symptoms but not its development. [16].

Conclusion

In conclusion, no significant association was found between the MCP-1 -2518 A/G polymorphism and schizophrenia in the studied population. However, there was an association between the genotypes, specifically the heterozygous, suggesting a potential role in the clinical heterogeneity of schizophrenia. Further study is needed with larger sample sizes and diverse populations to understand better the genetic factors influencing schizophrenia development.

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