DIFFERENCES IN INTERLEUKIN-4-590 C/T VARIANTS BETWEEN BATAK PEOPLE WITH SCHIZOPHRENIA AND HEALTHY CONTROL

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Background: Schizophrenia is a severe mental disorder characterized by disturbances in thinking, perception, emotion, and behaviour. Although the exact causes are unknown, research suggests that genetic and environmental factors contribute to its development. Besides, the interleukin-4 (IL-4) 590 C/T polymorphism comprises a variation in the DNA sequence at position 590 of the IL-4 gene. The IL-4 gene encodes interleukin-4, a cytokine involved in immune response and inflammation. This genetic polymorphism has been associated with various medical conditions, including neurological and psychiatric disorders. The relationship between IL-4 590 C/T polymorphism and schizophrenia has been previously examined.

Method: This comparative analytic research used a cross-sectional design to compare the Batak people with Schizophrenia (ODS) group and healthy control. DNA isolation and examination of Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) were performed.

Results: The Chi-Square test with Yates' correction showed no significant differences in the frequency of allele occurrence between ODS and control groups (p=0.1). Additionally, logistic regression analysis indicated a p-value of 0.415 for genotype (CC vs CT), with an odds ratio (OR) of 0.743 and a 95% confidence interval (CI) ranging from 0.364-1.517. The p-value for genotype CC vs. TT was 0.002, with an OR=0.433 and a 95% CI of 0.257-0.730.

Conclusion: This research identified a significant relationship, specifically in the TT genotype, when compared to CC

Keywords: Schizophrenia, Interleukin-4, Polymorphism, Alleles, Genotype

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1. Introduction

Schizophrenia is a complex disorder characterized by positive, negative, and cognitive symptoms. The positive symptoms manifest as psychotic symptoms such as visual or auditory hallucinations, paranoia, delusions, confusion, and thought disturbances, which are absent in healthy individuals. The negative symptoms are present in healthy individuals but decreased or absent in schizophrenic patients, including deficits in social interaction, apathy, lack of motivation and excitement, poor speech, and persistent behaviour. Meanwhile, the cognitive symptoms encompass impaired executive function and memory, as well as inattention or attention deficit [1]. The prevalence of schizophrenia varies significantly across countries and regions. Furthermore, it is estimated at 0.7%, 0.31% -0.59%, 0.18%, and 0.14% in the United States, Australia, Indonesia, and North Sumatra, respectively [2.3].

A systematic review found that schizophrenia accounts for 1.7% of disabled individuals annually worldwide. Individuals with schizophrenia experience a range of challenges, including poor recovery, reduced life expectancy, and increased mortality rates across all age groups. Moreover, they have higher rates of comorbid physical illnesses, such as coronary heart disease, stroke, diabetes mellitus, respiratory problems, and certain types of cancer being the leading causes of death, with suicide accounting for 15% of all cases. Understanding the epidemiology of schizophrenia and its implications is crucial for reducing the economic burden and improving the prognosis of patients [3,4].

Recent hypotheses suggest that neuro-inflammatory and immune factors are associated with schizophrenia. Increased concentrations of pro-inflammatory cytokines have been observed in the serum of people with Schizophrenia (ODS) in response to inflammation or stress [5]. Interleukin-4 (IL-4), an important anti-inflammatory cytokine, regulates the Th2-type immune response. Additionally, it stimulates T-helper cell differentiation into Th2 cells, inhibits Th1 cell development, and influences antibody production and immunoglobulin class switching [6,7]. The human interleukin-4 gene, located on chromosome 5q31-5q33, has been identified as a candidate gene for schizophrenia [6].

Takabayashi et al. (2000) reported IL-4 gene polymorphisms at the -33 bp (base pair) position from the first ATG codon and 33 bp from the transcription initiation site. Cytokine abnormalities, including IFN-, IL-2, IL-4, IL1RA, TNF-α, and IL-4, were shown in schizophrenic patients, suggesting an inflammatory syndrome in schizophrenia [7-9]. However, contradictory results have been previously stated. For instance, Watanabe et al. (2008) found no significant differences between ODS and healthy control concerning the IL-2 -330 T/G genotype relationship and IL-4 -590 C/T promoter variants [10]. Therefore, this research aims to investigate the potential differences in IL-2 -330 T/G variants and their relationship with schizophrenia.

2. Statistical analysis and methods

2.1. Statistical analysis

This comparative analytic research employed a cross-sectional design to compare the Batak ODS group with healthy control group, involving DNA isolation and examination of PCR-RFLP.

2.2. Method

The interleukin-4 genotype at the promoter position -590C/T was identified using the PCR-RFLP method. A 2 mL blood sample was incubated through PCR for 10 minutes before adding Taq polymerase (MBI). The PCR reaction mixture consisted of 1 mmol/L MgCl2, 0.2 mol/L dNTPs, one µM primer, and two enzyme units. The forward (5' ACT AGG CCT CAC CTG ATA CG 3') and reverse (5' GTT GTA ATG CAG TCC TCC TG 3 ') primers were used to amplify the promoter variants at position -590C/T, yielding fragments of 150 bp, 82 bp, and 118 bp. The PCR cycle conditions included denaturation at 94°C for 5 minutes, followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds. Genotypes were determined using a 3% agarose gel stained with ethidium bromide. The interleukin-4-590C/T PCR product (253 bp) was then digested with FaqI (BsmFI) (Fermentation) at 37°C for 3 hours and separated on the stained 3% agarose gel. The

expected fragment sizes were 253 bp for the -590T allele and 209 bp and 44 bp for the -590C allele, respectively.[6]

2.3. Result

Most ODS and healthy control subjects from the Batak ethnic group were males. In ODS group, there were 105 males (74.4%), while healthy control consisted of 102 males (72.3%). The median age in the ODS group was 34 years, ranging from 19-51, while it was 25.9 years in the control group, ranging from 20-40. The median onset of illness was nine years, ranging from 1 to 23, as presented in Table 1. The T allele was the most frequently observed in ODS (163 times, 57.8%) and control groups (174 times, 64.8%). The Chi-Square test with Yates' correction showed no significant differences in the frequency of allele occurrence between both groups (p=0.1), as indicated in Table 2. The most common genotype in the ODS group was CT (n = 79 times, 56.1%), while in healthy control of the Batak ethnic group, it was TT (n= 65 times, 46.2%). The logistic regression analysis indicated a p-value of 0.415 for genotype CC vs. CT, with an OR=0.743 and a 95% CI of 0.364-1.517. The p-value for CC vs. TT was 0.002, with an OR=0.433 and a 95% CI of 0.257-0.730, as presented in Table 3.

Table 4.1 Demographic characteristics of the Batak ODS and healthy control

	Batak tribe ODS (N=141)	Control (N=141)	p-value
Gender			
Male	105 (74.4%)	102 (72.3%)	
Female	36 (25.6%)	39 (27.7%)	0.686*
Age (Years)	34 (19-51)	25.9 (20-40)	<0.001**
Onset	24.7 (17-34)		
Long Sick	9 (1-23)		

Table 4.2 Distribution differences between IL-4-590 C/T alleles

Alleles	ODS	Control	p-value	OR (IK 95%)
C	119 (42.2%)	99 (35.2%)	0.1	1.35 (0.96–1.89)
T	163 (57.8%)	183 (64.8%)		

Table 4.3. Differences in the variants of IL-4-590 C/T in the Batak ODS and healthy control

Genotype	ODS	Control	p-value	OR (IK 95%)
CC	20 (14.2%)	23 (16.3%)		
CT	79 (56.1%)	53 (37.5%)	0.415	0.743 (0.364-1.517)
TT	42 (29.7%)	65 (46.2%)	0.002	0.433(0.257-0.730)

3. Discussion

In general, the results showed no statistically significant differences in the occurrence of C and T allele variations (p=0.1), with an OR of 1.35, indicating no significant disparity in allele distribution between ODS and control groups. These aligned with the research conducted by Schwarz et al., which reported no relationship between allele

frequencies in the two groups. Similarly, Danilow et al. found no relationship between alleles and genotype in 182 schizophrenic patients and 215 healthy individuals in Poland [6].

This research indicated a higher prevalence of the CT genotype in ODS group compared to TT, which was more common among healthy control. Individuals with the TT genotype had a 0.43-fold higher risk of developing schizophrenia. These results were consistent with the significant association that Schwarz reported between IL-4-590 CC and schizophrenia in Germany. The research by Danilow et al. and genetic association analyses conducted in the Polish population did not detect a significant association between IL-4-590C/T polymorphism and schizophrenia. The discrepancies in the stated results might be due to variations in populations and sample sizes investigated. Additionally, it is important to consider ethnic similarity in genetic association research to avoid confusion caused by differences in population structures [6,11].

4. Conclusion

In conclusion, a significant relationship was discovered, specifically in the TT genotype when compared to CC, but no significant differences were found in the occurrence of alleles. This contributes to the growing body of knowledge on the IL-4 -590C/T polymorphism and its potential association with schizophrenia. Therefore, further research incorporating larger sample sizes and diverse populations is recommended to elucidate the genetic basis of this complex disorder more comprehensively.

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