DIFFERENCES IN 5-HT6 C267T GENE RECEPTOR POLYMORPHISM BETWEEN PEOPLE WITH SCHIZOPHRENIA AND HEALTH CONTROLS FROM THE BATAK TRIBE

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Abstract: Schizophrenia is a chronic mental disorder that affects human psychosocial functioning. Approximately 24 million people worldwide are estimated to suffer from this disorder, with the onset typically occurring in late adolescence or early twenties. People with Schizophrenia (PWS) have a higher risk of premature death, in which environmental and psychological factors influence the development. Furthermore, the serotonin system plays a vital role in the pathogenesis of schizophrenia, and the serotonin 5-HT6 receptor gene is associated with its development. The "serotonin-dopamine" hypothesis states that an increase in dopaminergic and serotonergic neurotransmission in subcortical areas and a decrease in activity in the prefrontal cortex cause positive and negative symptoms.

Method: This study used an analytical comparative method with an observational analysis design. The samples were selected using consecutive non-probability sampling, with 145 PWS patients and 145 healthy controls from the Batak ethnic group. The analytical tests used were Chi-Square and logistic regression.

Results: The PWS group was dominated by male subjects, with 93 people (64.1%), while in the healthy control group, there were 126 males (86.9%). The median age was 35 years and 24 years in both groups respectively. The median onset of disease in the PWS group was 25 years, and the median length was eight years. The T allele was the most frequently found in the PWS group, with 218 occurrences (75.2%), while in control, it was found 216 times (74.5%) with a p-value of 0.84. In the logistic regression analysis, the p-values for genotypes CC vs TT and CT vs. TT were 0.673 and 0.963, respectively.

Conclusion: This study did not find a relationship between the 5-HT6 C267T gene with schizophrenia in the Batak ethnic group and their control group.

Keywords: Batak Ethnic Group, Serotonin, 5-HT6 Gene, Polymorphism

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Introduction

Schizophrenia is a severe psychiatric disorder marked by persistent symptoms and profound challenges in psychosocial functioning [1]. According to the World Health Organization (WHO), in 2022, approximately 24 million people worldwide, or 1 in every 300 people, are affected by this disorder. This equates to 1 in 222 adults (0.45%), and the onset mainly occurs during late adolescence and early twenties. Furthermore, the onset tends to occur earlier in males than females, and PWS are 2 to 3 times more likely to die prematurely than the general population. Other aspects correlated with the development of schizophrenia include social factors such as urbanization and migration. The prevalence in the US population ranges between 0.6% and 1.9%. Males tend to experience the first onset in their early 20s, while females usually experience it in their late 20s or early 30s [2]. Schizophrenia is also a severe mental disorder associated with developmental brain impairment caused by genetic or environmental factors. Cytokines play a crucial role as primary regulators of immune and inflammatory responses, impacting the functioning of dopaminergic, noradrenergic, and serotonergic neurotransmission systems [3]. The development of this disorder is influenced by a combination of genetic and non-genetic factors, including environmental and psychological influences, which can lead to alterations in the chemical composition and structure of the brain. Several theories have been proposed as risk factors for schizophrenia, ranging from neurodevelopmental, neurodegenerative, and neurotransmitter disorders [4,5].

The serotonergic system plays a crucial role in the pathogenesis of schizophrenia, as evidenced by the effects of Lysergic Acid Diethylamide (LSD) on enhancing serotonin effects, which produce visual hallucinations, a rare condition in schizophrenia. The discovery of numerous subtypes of 5-HT receptors and their broad impact on various neurotransmitters and behaviours revived interest in the role of 5-HT in schizophrenia. A noteworthy discovery is that lysergic acid diethylamide (LSD) and certain antipsychotic medications, like chlorpromazine, clozapine, and olanzapine, exhibit binding solid affinity to this receptor. These findings indicate that the gene associated with this receptor may be regarded as a potential candidate gene for susceptibility to schizophrenia [6-8]. Furthermore, serotonin (5-hydroxytryptamine, 5-HT) is one of the first neurotransmitters involved in schizophrenia, even before dopamine (DA). It is implicated in the core etiological aspects, including delusions and hallucinations (positive symptoms) and negative symptoms such as withdrawal, anergia, flat affect, avolition, anhedonia, and decreased cognitive function [9].

The human 5-HT6 receptor gene (HTR6) has been mapped to chromosome 1p35±p36.13 and encodes for a receptor protein positively linked to adenylate cyclase. Genomic clone comparisons for the human 5-HT6 receptor revealed a silent RsaI polymorphism (C267T) in the coding region. A study in Taiwan found a significant association between the genotype of this gene and schizophrenia, but they did not find any association with aggressive behaviour [8]. Therefore, this study aims to investigate the differences in the serotonin receptor 5-HT6 gene C267T variant between the PWS and control groups of the Batak Tribe in Indonesia.

Study analysis and methods

Study Analysis

This study used a comparative analytical method with an observational analysis design. The non-probability consecutive sampling was used, while the total sample size was 145 PWS and 145 control individuals of the Batak Tribe. The analytical tests utilized were Chi-Square and logistic regression.

Method

DNA examination was conducted using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method to identify the 5-HT6 genotype at the C267T promoter position. About 1 mL of blood was boiled using the PCR technique for 10 minutes before adding Taq polymerase (MBI). The PCR mixture reaction consisted of 1 mmol/L MgCl2, 0.2 mol/L dNTPs, 1 µM primer, and 2 units of the enzyme. The length of the amplified PCR of the promoter polymorphism at the C267T position using forward primer: 5'-AACCTCTTCTCTGCTGCTCTTC-3' and
reverse primer: 5'-ATGAGCAGGTAGCGGTCCAGGC-3' was at 200 bp, and after treatment with the restriction enzyme RsaI, the following fragments were identified: 82 bp and 118 bp using forward primer: 5'-AACTTCTTCTTGGTGTGCTCTTTC-3' and reverse primer: 5'-ATGAGCAGGTAGCGGTCCAGGC-3'. The PCR cycle conditions were denaturation at 95°C for 1 minute, followed by 62°C for 1 minute, and 72°C for 1 minute, in 35 cycles. The genotype was determined using a 2% agarose gel stained with ethidium bromide.

**Result**

Most of the PWS subjects were males, with 93 individuals (64.1%), while in the healthy control group, there were 126 (86.9%). The median age was 35 in the PWS group and 24 in the control group. The median onset of disease in the PWS group was 25 years, and the median duration was eight years, as shown in Table 1.

The most frequently found allele in the PWS group was T (n=218; 75.2%), while in the control group, it occurred 216 times (74.5%), with a p-value of 0.84 (Table 2). Logistic regression analysis revealed a p-value of 0.673 for the CC vs. TT genotypes and 0.963 for CT vs. TT, as described in Table 3.

<table>
<thead>
<tr>
<th>Group</th>
<th>Variable</th>
<th>PWS of Batak Tribe (n=145)</th>
<th>Control (n=145)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>93 (64.1%)</td>
<td>126 (86.9%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>52 (35.9%)</td>
<td>19 (13.1%)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>35 (19-51)</td>
<td>24 (20-40)</td>
<td></td>
</tr>
<tr>
<td>Onset</td>
<td>25 (17-34)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Length of Disease</td>
<td>8 (1-25)</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1. Demographic characteristics**

<table>
<thead>
<tr>
<th>Variable of MCP-PWS 1-2518</th>
<th>Control (n=200)</th>
<th>P-value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/G (n=200)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>72 (48%)</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>218 (75.2%)</td>
<td>216 (74.5%)</td>
<td>0.84</td>
</tr>
</tbody>
</table>

**Table 2. Comparison between alleles of the 5-HT6 variant (C267T) in the PWS and control groups of the Batak Tribe**
Table 3. Comparison between the genotypes of the 5-HT6 variant (C267T) in the PWS and control group of the Batak Tribe

<table>
<thead>
<tr>
<th>Genotype</th>
<th>PWS (n=145)</th>
<th>Control (n=145)</th>
<th>P-value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>9 (6.2%)</td>
<td>11 (7.6%)</td>
<td>0.673</td>
<td>1.222 (0.481-3.106)</td>
</tr>
<tr>
<td>CT</td>
<td>54 (37.2%)</td>
<td>52 (35.8%)</td>
<td>0.880</td>
<td>0.963 (0.591-1.570)</td>
</tr>
<tr>
<td>TT</td>
<td>82 (56.6%)</td>
<td>82 (56.6%)</td>
<td>Comparison</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Result of HWE of the 5-HT6 variant (C267T) in the PWS and control group of the Batak Tribe

| P-value |
|---------|---------|---------|
| PWS     | 0.97    |         |
| Control | 0.49    |         |

If P < 0.05 - not consistent with HWE.

Not accurate if <5 individuals in any genotype group

Discussion

Based on the results, most subjects were male, which is consistent with a cohort study by Sanchez et al. in Spain that found the prevalence of schizophrenia in males to be almost twice as high as in females [10]. These findings differ from other studies that reported no difference in prevalence between genders, although the incidence of schizophrenia was higher in males. Access to mental health services and differences in help-seeking behaviours between males and females can also affect the perceived prevalence of schizophrenia [11,12]. A study in China showed that the prevalence of schizophrenia was higher in females due to lower mental health services and more significant stigma against women in the country [13].

This study found no difference in the 5-HT6 gene allele (C267T) between the PWS and control groups. This finding is consistent with studies conducted in Germany and France, which found a relationship between alleles and schizophrenia [14,15]. In contrast, a study conducted in Taiwan linked the 5-HT6 gene (C267T) to schizophrenia and aggressive behaviour. They found a relationship between alleles and schizophrenia, but no relationship was observed between the 5-HT6 gene (C267T) and aggressive behaviour [7]. These inconsistent study results indicate the involvement of ethnic differences in polymorphism. Environmental factors and lifestyle may also play a significant role in the development of schizophrenia, leading to variations in results between different studies. Therefore, a more extensive and precise study is needed to evaluate different factors and potential interactions in the development of schizophrenia.

The study found no significant difference between genotypes and schizophrenia, consistent with previous studies [6,8,14]. Genetic variation in the 5HT6
gene only contributes limitedly to the development of schizophrenia. However, failing to find a significant relationship may reflect false negative findings due to the small sample size and low statistical power. The C267T variant is also a silent mutation, which does not cause a change in the amino acid sequence. Therefore, additional gene variants not detected by C267T polymorphism analysis may affect the development of schizophrenia. The effect of the 5HT6 genotype on schizophrenia may not be the same in different schizophrenia groups [6].

**Conclusion**

This study found no relationship in the 5-HT6 C267T gene between PWS and healthy controls in the Batak tribe. This might be due to sample size, environment, and culture differences.

**References**


