





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

Fitri Ana Ima S<sup>1</sup>, Mustafa M. Amin<sup>1\*</sup>, Elmeida Effendy<sup>1</sup>

<sup>1</sup>Department of Psychiatry, Faculty of Medicine, Universitas Sumatera Utara

\*Corresponding author: [mustafa.mahmud@usu.ac.id](mailto:mustafa.mahmud@usu.ac.id)

**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

## 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-HT<sub>6</sub> receptor gene (*HTR6*) has been mapped to chromosome 1p35±p361p36.13 and encodes for a receptor protein positively linked to adenylate cyclase. Genomic clone comparisons for the human 5-HT<sub>6</sub> 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-HT<sub>6</sub> 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-HT<sub>6</sub> 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 MgCl<sub>2</sub>, 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'-AACTTCTTCTGGTGTCGCTCTTC-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'-AACTTCTTCTGGTGTGCTCTTC-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 1. Demographic characteristics

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

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

Variable of MCP-PWS	Control (n=200)	P-value	OR (95% CI)
1-2518 A/G(n=200) Allele			
C	72 (48%)	0.84	0.962(0.663-1.403)
T	218 (75.2%)		

Table 3. Comparison between the genotypes of the 5-HT6 variant (C267T) in the PWS and

Genotype	control group of the Batak Tribe			OR (95% CI)
	PWS	Control	P-value	
	(n=145)	(n=145)		
CC	9(6.2%)	11(7.6%)	0.673	1.222 (0.481-3.106)
CT	54(37.2%)	52(35.8%)	0.880	0.963 (0.591-1.570)
TT	82(56.6%)	82(56.6%)	Comparison	

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

	P-value	
	PWS	Control
<i>Chi square</i>	0,97	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

1. Na KS, Jung HY, Kim YK. The role of pro-inflammatory cytokines in the neuroinflammation and neurogenesis of schizophrenia. Vol. 48, Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2014. p. 277–86.
2. Patel KR, Cherian J, Gohil K, Atkinson D. Schizophrenia: Overview and treatment options. P.T. 2014;39(9):638–45.
3. Srinivas L, Vellichirammal NN, Alex AM, Nair C, Nair I V., Banerjee M. Pro- inflammatory cytokines and their epistatic interactions in genetic susceptibility to schizophrenia. J Neuroinflammation [Internet]. 2016;13(1):1–12. Available from: <http://dx.doi.org/10.1186/s12974-016-0569-8>
4. Aricioglu F, Ozkartal CS, Unal G, Dursun S, Cetin M, Müller N. Neuroinflammation in schizophrenia: A critical review and the future. Klin Psikofarmakol Bul. 2016;26(4):429–37.
5. Pandey GN, Ren X, Rizavi HS, Zhang H. Proinflammatory cytokines and their membrane-bound receptors are altered in the lymphocytes of schizophrenia patients. Schizophr Res [Internet]. 2015;164(1–3):193–8. Available from: <http://dx.doi.org/10.1016/j.schres.2015.02.004>
6. Chiu HJ, Wang YC, Liou JH, Chao CH, Lee H, Tsai KY, et al. Serotonin 6 receptor polymorphism in schizophrenia: Frequency, age at onset and cognitive function. Neuropsychobiology. 2001;43(3):113–6.
7. Meltzer HY, Massey BW. The role of serotonin receptors in the action of atypical antipsychotic drugs. Curr Opin Pharmacol [Internet]. 2011;11(1):59–67. Available from: <http://dx.doi.org/10.1016/j.coph.2011.02.007>.
8. Tsai SJ, Chiu HJ, Wang YC, Hong CJ. Association study of serotonin-6 receptor variant (C267T) with schizophrenia and aggressive behavior. Neurosci Lett. 1999;271(2):135–7.
9. Karki R, Pandya D, Elston R.C, Ferlini C. Defining “mutation” and “polymorphism” in the era of personal genomics. 2015. <https://doi.org/10.1186/s12920-015-0115-z>.
10. Sanchez AO, Lacort ML, Quiles CM, Gimeno GS, Domingo JD. Epidemiology of schizophrenia and its management over 8-years period using real world data in Spain. BMC Psychiatry. 2020; 20:149 Karki R, Pandya D, Elston RC, Ferlini C. Defining ‘mutation’ and ‘polymorphism’ in the era of personal genomics. BMC Med Genomics [Internet]. 2015;8(1):1–7. Available from: <http://dx.doi.org/10.1186/s12920-015-0115-z>
11. Kronfol Z, Remick DG. Cytokines and the brain: Implications for clinical psychiatry. Am J Psychiatry. 2000;157(5):683–94.

12. Na KS, Jung HY, Kim YK. The role of pro-inflammatory cytokines in the neuroinflammation and neurogenesis of schizophrenia. *Prog Neuro-Psychopharmacology Biol Psychiatry*. 2014;48:277–86.
13. Luo Y, Pang L, Zhao Y, Guo C, Zhang L, Zheng X. Gender difference in the association between education and schizophrenia in Chinese adults. *BMC Psychiatry*. 2020; 20:296.
14. Dubertret C, Hanoun N, Adès J, Hamon M, Gorwood P. Family-Based Association Study of the Serotonin-6 Receptor Gene (C267T Polymorphism) in Schizophrenia. *Am J Med Genet-Neuropsychiatry Genet*. 2004;126 B(1):10–5
15. Vogt RI, Abarbanell SD, Neidt H. Investigation of the human serotonin 6 (5- HT6) Receptor Gene in Bipolar Affective Disorder and Schizophrenia. *Am J Med Genet-Neuropsychiatry Genet*. 96:217–221 (2000)

**Open Access** This chapter is licensed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits any noncommercial use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made.

The images or other third party material in this chapter are included in the chapter's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the chapter's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.

