






# Candidate IL-2 -330 T/G Polymorphism in Javanese with Schizophrenia

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**Abstract. Background:** Schizophrenia is a complex disorder involving multiple genes with mild to moderate effects and non-genetic risk factors such as environmental and psychological influences. A significant reduction in interleukin-2 -330 T/G production by peripheral lymphocytes is an immunological finding replicated in various countries. A study of interleukin-2 -330 T/G polymorphism in schizophrenic patients is still very limited and conducted in a few countries with different results. A study comparing interleukin-2 -330 T/G polymorphisms in Javanese with schizophrenia and healthy controls has also not been completed in Indonesia, particularly in North Sumatra Province.

**Method:** Sampling was performed using the non-probability purposive method. A total of 120 Javanese cases with schizophrenia and 120 healthy control at Prof. RSJ. M. Ildrem Medan, Indonesia, were recruited. The interleukin-2 -330 T/G polymorphism was examined by the PCR method.

**Result:** The genotype frequency of Javanese cases was 23.3% for GG, 44.2% for TG, and 32.5% for TT. The healthy control group's GG, TG, and TT genotypes were 35%, 27.5% and 37.5%, respectively. The OR comparison to the GG and TG genotypes was 0.611 (95% CI 0.276–1.352,  $p = 0.224$ ) and 0.892 (95% CI 0.505–1.574,  $p = 0.693$ ). Furthermore, T allele was higher than G allele and healthy control, respectively ( $p = 0.229$ , OR = 0.813 (95% CI of 0.581- 1.218)).

**Conclusion:** There was no significant difference between the frequency of allele occurrence in Javanese cases with schizophrenia and healthy control. In addition, there was no significant relationship between the GG and TG genotypes of the interleukin-2 - 330T/G polymorphism and healthy control.

**Keywords:** schizophrenia · polymorphism · interleukin-2 · Javanese · healthy control

## 1 Introduction

Schizophrenia affects 20 million individuals globally, this state is characterized by distorted thinking, perception, emotion, language, low self-esteem, and behavior. Disorders experienced by schizophrenic people include hallucinations (hearing voices or seeing things that are not present) and delusions (fixed, false beliefs). Many studies have been performed to determine the causes of schizophrenia, which have been suspected to range

from developmental, neurodegenerative processes, and neurotransmitter disorders to infectious or autoimmune processes [1, 2].

Cytokines have been shown to interact with neurons affecting nerve transmission. In binding to specific receptors on the surface of neurons, it can modulate the secretory activity of these cells concerning catecholamines or neuropeptides. Therefore, concentrations of various cytokines can be increased or decreased in people with schizophrenia. Interleukin-2 (IL-2), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- $\alpha$ ) mediate immune and inflammatory responses. It activates cytokines that play a key role in the central nervous system and are actively transported into the central nervous system but also released from activated glial cells. Furthermore, interleukin-2 is a growth factor for T-, natural killer (NK)-, and B cells. In people with schizophrenia, elevated levels of the soluble interleukin-2 receptor (sIL-2R) have been observed, and interleukin-2 and interferon-gamma (IF- $\gamma$ ) production has been reported to be significantly higher in schizophrenic people [3, 4].

Samojedny et al., 2013 in Poland found statistically significant differences in genotype and allele frequency distribution for the interleukin-2 polymorphism. The presence of the TT genotype and T allele correlated with an increased risk of paranoid schizophrenia. The functional significance of the selected interleukin-2 gene polymorphism was predetermined. Meanwhile, a genetic interleukin-2 T (Thiamin) turning to G (Guanine) polymorphism was encountered at sequence 330 bp, in the region containing the transcription factor NFAT binding site. This study also reported that the GG genotype was associated with high levels of interleukin-2, while the TT and GT genotypes were associated with decreased cytokine production [4, 5].

Pro-inflammatory studies related to ethnicity have also been conducted in Indonesia, specifically in North Sumatra, such as Amin et al. in 2019, with the title Tumor Necrosis Factor- $\alpha$  levels and their relationship with cognitive function in schizophrenia with Malayan-Mongoloid tribes. Studies on gene polymorphisms in cytokines of schizophrenic people are still limited. Based on a literature search, no studies in Indonesia, particularly North Sumatra, examined the comparison between interleukin-2 -330 T/G polymorphisms in Javanese with schizophrenia and healthy control [6].

## 2 Patients and Methods

### 2.1 Patients and Study Design

This one-time unpaired categorical comparative analytical study compared groups of Javanese with schizophrenia and healthy control using a cross-sectional method. This study was conducted at the Inpatient Installation of the North Sumatra Mental Hospital (R. S. J. Provsu) Prof. M. Ildrem for 4 months in 2021. Patients included fulfilled the inclusion and exclusion criteria. Inclusion criteria included Javanese with schizophrenia diagnosed based on the PPDGJ-III criteria, having a PANSS score of 80-120, aged 18-45 years, two generations of first-degree relatives and being Javanese, cooperative, and willing to be interviewed. Meanwhile, the exclusion criteria were having a history of other psychiatric disorders, neurological diseases, endocrine disorders, autoimmune diseases, and a history of using alcohol and other addictive substances. The samples

used were 120 Javanese with schizophrenia (82 male and 38 female) and 120 healthy control (92 male and 28 female).

## 2.2 Methods

In PCR and allele-specific PCR examination, primers were obtained and blasted to confirm the primer and enzyme sequences, as well as reverse primers that could identify the site. The examination was conducted at the Technical Implementation Unit of the integrated laboratory, Faculty of Medicine, University of North Sumatra. Blood sampling was carried out by withdrawing 5 ml from the anterior cubital vein. Subsequently, the blood was put into a vacutainer containing ethylenediamine tetraacetic acid (EDTA) and stored at 4 to -80 °C until DNA isolation was carried out. The method used for DNA isolation was salting out, and standard methods extracted genomic DNA from the samples subjected to freezing. The single base polymorphism at position -330 in the interleukin-2 promoter region was read by PCR sequence-specific primers (PCR-SSP), namely oligonucleotide primers (forward): 5'CTGACATGTAAGAAGCAATC-TAT3' and (reverse): 5'CTCAGAAAATTTCTTTGTCC3' for the G allele examination. The T allele examination was read with PCR sequence-specific primers (PCR-SSP), namely oligonucleotide primers (forward): 5'TTCACATGTTTCAGTGTAGTTTTAT3' and (reverse): 5'TGTTACATTAGCCCACACTTA3'.

## Statistical Analysis

The variables analyzed were interleukin-2 -330 T/G polymorphism, and the next analysis was a comparative test to look for differences in the allele distribution in Javanese with schizophrenia and healthy control. The results showed that the two variables were normally distributed, and the conditions for  $\chi^2$  were fulfilled. Hence, a Chi-Square test was performed. The Kolmogorov-Smirnov test was used for a sample size of more than 50 for each group to test normality, and the Odds Ratio (OR) was calculated [7, 8].

## 2.3 Results

Among 120 Javanese with schizophrenia and 120 healthy controls, the number of male was 82 (68.3%) and 92 (76.7%), with the mean age of 34 and 31 years, respectively, indicating they dominated more than females. The median disease onset was 25 years, and the median length of illness was 9 years in Javanese with schizophrenia. The PANSS score on Javanese with schizophrenia was 101 in Table 1.

The frequency of G and T allele occurrence in Javanese with schizophrenia was 39.2% and 60.8%, while in healthy control was 45% and 55%, respectively. Chi-square analysis showed a p-value = 0.229, meaning there was no significant difference between the frequency of allele occurrence, and the OR obtained was 0.813 with a CI of 0.581-1.218 (Table 2).

According to the Hardy-Weinberg formula, if the frequencies of the alleles A and a (from the biallelic locus) are p and q, then  $(p+q) = 1$ . This means  $(p+q)^2 = 1$  too, it is true that  $(p+q)^2 = p^2 + 2pq + q^2 = 1$ . This  $p^2$  formula corresponds to the genotype frequencies of AA homozygous,  $q^2$  to aa, and  $2pq$  to Aa. 'AA, Aa, aa' are the three possible genotypes for locus biallelic like most SNPs, the number of frequencies should

**Table 1.** Demographic characteristics

	Javanese	Control	P-value
	(n = 120)	(n = 120)	
Gender			
Male	82 (68.3)	92 (76.7)	
Female	38 (31.7)	28 (23.3)	<0.001*
Age (years)	34 (26–50)	31 (20–43)	<0.001**
Onset	25 (17–35)		
Length of illness	9 (4–18)		
PANSS	101 (81–110)		

\* *Chi-square with continuity correction.* \*\* *Mann-Whitney U*

**Table 2.** Comparison between the allele of the interleukin.2 –330 T/G gene and schizophrenia in Javanese with schizophrenia and healthy control obtained by the chi-Square procedure

Allele	Javanese with schizophrenia (n = 120)	Control (n = 120)	P-value	OR (95% CI)
G	94 (39.2)	108 (45)		0.813
T	146 (60.8)	132 (55)	0.229	(0.581–1.218)

**Table 3.** HWE value in Javanese ODS and healthy control interleukin-2 polymorphism –330 T/G

	ODS Javanese	p value
		Healthy Control
<i>Chi square</i>	0,16	0,231

*If  $P < 0.05$  - not consistent with HWE. Not accurate if  $< 5$  individuals in any genotype group.*

be 1. In this study, a test was carried out according to the steps above, using the help of an online software with the following results

The frequency of GG, TG, and TT genotypes in Javanese with schizophrenia was 23.3%, 44.2%, and 32.5%, while in healthy control was 35%, 27.5%, and 37.5%, respectively. Based on the logistic regression analysis, the chi-square value for the genotype (GGvsTT) was  $p = 0.224$  with  $OR = 0.611$  and  $CI\ 0.276\text{--}1.352$ , while for (TTvsGG) was  $p = 0.693$  with  $OR = 0.892$  and  $CI\ 0.505\text{--}1.574$  (Table 3) (Table 4).

**Table 4.** Comparison of genotypes of interleukin-2 -330 T/G polymorphism in Javanese with schizophrenia and healthy control obtained by the logistic regression procedure

Genotype	Javanese with schizophrenia (n = 120)	Control (n = 120)	P-value	OR (95% CI)
GG	28 (23.3)	42 (35)	0.224	0.611 (0.276–1.352)
TT	39 (32.5)	45 (37.5)	0.693	0.892 (0.505–1.574)
TG	53 (44.2)	33 (27.5)	Comparison	

### 3 Discussion

Most of the samples were male, both in Javanese with schizophrenia and healthy control. There was no significant difference in gender between the two groups, but there was a significant age difference. The median age from Javanese with schizophrenia is 34 years, with a minimum of 26 years and a maximum of 50 years. The research by Charlson et al. in 2018 [9] with the title “Global Epidemiology and Burden of Schizophrenia” reported global data on the condition’s prevalence. The study stated that no gender difference was observed globally. Approximately 70.8% of the cases were found in the 25–54 year age group, with the highest prevalence in the 40s and decreasing in the older age group. The median onset in Javanese with schizophrenia was 25 years, with a minimum of 17 years and a maximum of 35 years. Moreover, the median length of illness is 9 years, with a minimum and maximum of 4 years and 18 years [9].

The median total Positive And Negative Syndrome Scale (PANSS) score in Javanese with schizophrenia is 101, with a minimum and maximum of 81 and 110. Kozma et al. [10] conducted a study on 1028 schizophrenia patients in 2010 in the United States. It examined the factors leading to hospitalization of a person with schizophrenia, where the patient was followed for 52 weeks. The total PANSS score and the total Personal and Social Performance Scale (PSPs) were the rating scales analyzed. The PANSS score was divided into 3 parts such as low (<75), medium (75–94), and high (>95). The hazard ratio for hospitalized patients with a high total PANSS score was 5.45 ( $p < 0.001$  95% CI 2.59–11.46), and 2.31 ( $p = 0.01$  95% CI 1.11–3.20) in patients with a medium total PANSS score compared with a low score [9, 10].

This study stated that the frequency of G and T allele occurrence in Javanese with schizophrenia was 39.2% and 60.8%, respectively, while in healthy control was 45% and 55%. Chi-square analysis showed a  $p$ -value = 0.229, meaning that the allele had a non-significant relationship with schizophrenia. The OR obtained was 0.813 with a 95% CI of 0.581–1.218. Therefore, schizophrenic Javanese with G allele had 0.813 times the odds of experiencing the condition compared to individuals with T allele. The OR was below 1, hence, G allele was a protective factor for the occurrence of schizophrenia, if at all. These results are related to the distribution of allele frequencies but not in line with Samojedny et al., 2013 [4]. The study involved 115 people with

schizophrenia and 135 healthy control with a mean age of  $43.3 \pm 12.6$  and  $41.3 \pm 9.0$  years, respectively. The proportions of G and T alleles were 44.3% and 55.7%, while in healthy control were 55.6% and 44.4%, respectively. The p-value was 0.05, meaning there was a significant difference between the frequency of allele occurrence and the incidence of schizophrenia. However, these results are consistent with Watanabe et al. (2008) in Japan, which involved 536 patients with schizophrenia and 510 healthy control with a mean age of  $40.1 \pm 14.2$  and  $37.4 \pm 10.2$  years, respectively. Furthermore,  $p > 0.05$  was found, meaning there was no significant difference between the frequency of allele and schizophrenia occurrences [4, 11].

The genotype of the interleukin-2 -330 T/G polymorphism in this study was a combination of the G and T alleles, consisting of GG, TG, and TT. The frequency in Javanese with schizophrenia was 28 (23.3%), 53 (44.2%), and 39 (32.5%), respectively, while in healthy control was 42 (35%), 33 (27.5%), and 45 (37.5%). Based on the logistic regression analysis results, the p-value for the GG genotype was 0.224 with OR = 0.611 and 95% CI of 0.276-1.352, meaning that there were differences in the GG genotype in the incidence. The p-value for the TG genotype was 0.693 with OR=0.892 and 95% CI of 0.505-1.574, meaning that there was no difference in the TG genotype. These results are inconsistent with the Polish population reported by Frydecka et al. 2013 [5]. The study involved 151 and 279 of schizophrenic patients and healthy control with a mean age of  $38.0 \pm 11.9$  and  $38.7 \pm 8.8$  years, respectively. The GG, TG, and TT genotypes in people with schizophrenia were 10.7%, 46.3%, and 43%, respectively, while in control were 9.9%, 43.1%, and 47%. A p-value = 0.72 was found, meaning there was a significant relationship between polymorphisms in the Polish population. However, these results are inconsistent with Watanabe et al. 2008 in Japan, which conducted a study of the same gene in a Japanese population. It involved 536 and 510 schizophrenic patients ( $40.1 \pm 14.2$  years) and healthy control ( $37.4 \pm 10.2$  years) with a p-value of 0.701. Since the p-value  $> 0.05$ , there was no significant difference between the genotype polymorphism and the incidence in the Japanese population [3, 11].

## 4 Conclusions

Most subjects were male, and there was no significant difference in gender between the two groups with  $p=0.193$ . In schizophrenic Javanese, the median age was 34, with a minimum and maximum of 26 and 50 years. In the healthy control, the median age was 31 years, with a minimum and maximum of 20 years and 43 years. Therefore, there was a significant difference in the age of the two groups with  $p = 0.044$ . Onset ( $p=0.188$ ), length of illness ( $p = 0.574$ ), and PANSS score ( $p = 0.16$ ). There was no significant difference between the frequency of allele occurrence with a p-value = 0.229. Individuals with T allele had the same odds of having schizophrenia as those with G allele. The allele of IL-2-330T/G polymorphism was not a risk factor (OR = 0.813) for schizophrenia.

The frequency of GG, TG, and TT genotypes in the Javanese with schizophrenia was 28, 53, and 39, while the frequency was 42, 33, and 45, in healthy control. The p-value for the GG genotype was 0.224 with OR = 0.611 (95% CI 0.276- 1.352), while the p-value for the TG genotype was 0.693 with OR=0.892 (95% CI 0.505-1.574). Therefore, there

was no significant relationship between the GG and TG genotypes of the interleukin-2 -330T/G polymorphism.

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**Transparency Declarations.** Competing interests: None to declare.

## References

1. World Health Organization. Schizophrenia. <http://www.who.int>. 2019.
2. Srinivas L. et al. Pro-inflammatory cytokines and their epistatic interactions in genetic susceptibility to schizophrenia the role of inflammation. *Journal of Neuroinflammation*. 2016. doi <https://doi.org/10.1186/s12974-016-0569-8>
3. Muller N, Weidinger E, Leitner B, Schwarz M.J. The role of inflammation in schizophrenia. 2015. doi: <https://doi.org/10.3389/fnins.2015.00372>
4. Paul-Samojedny M, Owczarek A, Kowalczyk M, Suchanek R, Palacz, Kucia K, et al. Association of Interleukin 2 (IL-2), Interleukin 6 (IL-6), dan TNF-Alpha (TNF- $\alpha$ ) Gene Polymorphisms With Paranoid Schizophrenia In A Polish Population. *The Journal of Neuropsychiatry and Clinical Neurosciences*. 2013; 25: p. 72-82.
5. Frydecka D et al. Genetic variants in transforming growth factor-b gene (TGFB1) affect susceptibility to schizophrenia. 2013. <https://doi.org/10.1007/s11033-013-2662-8>.
6. Amin M.M, Rasyid A, Effendy E, Amir N, Suryandari D.A. The level of tumour necrosis factor-alpha and its relationship to the cognitive function of Malayan- Mongoloid patients with schizophrenia. 2020. <https://doi.org/10.17392/1108-20>.
7. Dahlan M. Besar Sampel Dan Cara Pengambilan Sampel. edisi ke-3. Jakarta: Salemba Medika; 2010.
8. Madiyono B, Moeslichan S, Sastroasmoro S, Budiman I, Purwanto SH. Perkiraan Besar Sampel. In: *Dasar-Dasar Metodologi Penelitian Klinis*. Jakarta: Sagung Seto; 2011.
9. Charlson FJ, et al. Global Epidemiology and Burden of Schizophrenia: Findings From the Global Burden of Disease Study 2016. 2018. <https://academic.oup.com/>. doi: <https://doi.org/10.1093/schbul/sby058>.

10. Kozma CM, Dirani RG, Canuso CM, Mao L. Predicting hospital admission and discharge with symptom or function scores in patients with schizophrenia: pooled analysis of a clinical trial extension. 2010. doi: <https://doi.org/10.1186/1744-859X-9-24>.
11. Watanabe Y, et al. Association study of interleukin 2 (IL2) and IL4 with schizophrenia in a Japanese population. 2008. <https://doi.org/10.1007/s00406-008-0813-z>.

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