The Comparison of *Catechol-o-methyltransferase* VAL158MET on Schizophrenic Bataknese Patients

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Abstract

Background: Schizophrenia is a mental disorder that is common throughout the world. Schizophrenia is estimated to occur in 24 million people or one in 3000 people (0.32%). In Indonesia, it is estimated that around 6.9% of family members have schizophrenia. Genetic and epidemiological studies have linked environmental factors to genetic factors, including place of birth, infections, complications during pregnancy and delivery, effects of medications, and stress. From a genetics perspective, one hypothesis is through catechol-O-methyltransferase (COMT) polymorphism, where there are two forms, namely the soluble form (S form) and the membrane-bound form (M form) located on chromosome 22q11. The COMT gene polymorphism is a single nucleotide polymorphism (SNP) rs4680, which can change Val to Met at codon 158 (Val158Met), which occurs from the M form of the gene. This gene polymorphism is widely considered to have a strong relationship with schizophrenia.

Methods: This is an analytical comparative study with a cross-sectional design, in which 400 samples were recruited into case groups of Bataknese people with schizophrenia who have been hospitalized in the installation unit in North Sumatera Mental Hospital and a control group of blood-donors members at North Sumatera Red Cross. The study was carried out from October 2022 to March 2023. Blood sample collection was carried out via *consecutive nonprobability sampling type*, while *Polymerase Chain Reaction – Restriction Fragment Length Polymorphism* (PCR-RFLP) on the examination of COMT polymorphisms was performed.

Results: The appearance of Val alleles (N=351 times) was found more in control groups than in the cases group, while the Met alleles appeared 89 times in the PWS groups. The genotype variations of ValVal were found to be the highest variant in Batak PWS groups for 123 (61.5%), and interestingly, there were similar variants of ValMet in both groups, accounting for 34.0%. In comparison, the genotype of ValVal and MetMet had a p-value of 0.013, with OR=0.232, CI 95% between 0.085-0.633. Meanwhile, the comparison of ValMet and MetMet showed a p=0.808, OR=0.271, with CI between 0.097-0.760.

Conclusion: Individuals with Val alleles display possible mechanisms to develop schizophrenia compared to those with Met alleles. As far as the genotype of Val158Met is concerned towards the risk factors of developing schizophrenia, no significant difference was found between the Batak PWS groups and the control group in terms of genotype appearance.

Keyword: Catechol O-Methyltransferase, Indonesia, Polymorphism, Single Nucleotide

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Introduction

Schizophrenia can be found in 24 million of the population with a ratio of 1:3000 individuals (0.32%) globally; similarly, in Indonesia, in every a million house members, 6.9% of relatives have been confirmed to suffer schizophrenia or psychosis mental disorders [1]. *Genome-wide association studies* (GWAS) compared the genotype and allele frequencies from thousands to millions of *single nucleotide polymorphism* (SNP) by recruiting subject groups that have been diagnosed with schizophrenia (PWS) and control group, and their studies have identified genes that are relevant among those groups [2]. Another study has suggested that the polygenic interaction in complex molecular states had a relation to the pathogenesis of schizophrenia, which was dopamine dysregulation [3].

Catechol-O-methyltransferase (COMT) is a crucial enzyme due to its contribution to dopamine catabolism and its possible complex mechanism in causing schizophrenia. The COMT enzymes contribute to establishing and balancing the dopamine levels in the brain, especially in the *prefrontal cortex* (PFC) [4]. Genetic variation of COMT could be a schizophrenic pathogenesis as the COMT is a catabolic enzyme, contributing to regulating the central neurotransmission of dopamine; in terms of pathogenesis, the enzymes appear in two forms, i.e., *soluble form* (S-form) and *a membrane-bound form* (M-form), primarily expressed in brain [5]. The COMT is located in the 22q11 chromosome, which may cause complex syndrome if there are any disruptions, which may develop into schizophrenia and any psychosis states. The polymorphism forms of the COMT gene may appear by which is in *single nucleotide polymorphism* (SNP) rs4680. This could alter the Val codes into Met (Val158Met) from the M-form form that has been extensively studied to have a relation to schizophrenia [6].

According to a study in Korea in 2005 that investigated the relationship between Val58Met gene polymorphism of COMT and brain morphological disorders in chronic schizophrenia, there was a relationship between Val alleles and schizophrenia, in which a significant reduction of volume at the anterior cingulate cortex (ACC), left amygdala-uncus, right middle temporal gyrus (MTG), and left thalamus was observed. Compared to the Met alleles, the Val displayed more reduction; therefore, the Val158Met polymorphism from COMT genes contributes significantly to the morphological disorders in the brain of schizophrenic patients [7]. Han and his co-workers in 2006 reported that the Met alleles from COMT genes allowed an increase in tonic activities of dopamine cognitive stability, leading to inflexibility of cognition among schizophrenic patients [8].

As an ethnicity, the Bataknese is the most populous ethnicity in North Sumatra. This ethnicity is a long descendant of Proto-Malay based on the Asian Demography, authorized by Ananta Aris in 2015. This means that the ethnicity remains practising similar language and traditions that have lasted for thousands of years with authentic practices [9].

Studies investigating the relationship between genes and schizophrenic factors remain limited, especially the relation of COMT genes in Indonesian samples, as this gene is an important factor in releasing dopamine in the prefrontal system [10]. An individual with Valin (Val) allele homozygote has higher activity of COMT with a lower concentration of dopamine in extracellular levels. Meanwhile, the individual with Metionin (Met) tends to have a lower activity of COMT, in which the Met158Met enzymes have a lower degradation rate, around a third compared to Val158Met enzymes. Thus, the high bioavailability of dopamine is incredibly common within the synaptic path [11].

Subject and Method

This comparative analytic study compares the PWS cases of Bataknese patient groups and healthy Bataknese as the control group. The schizophrenia Bataknese patients were recruited at the Inpatient Installation Unit of the Regional Mental Hospital of North

Sumatera Prof. Dr. M. Ildrem, whereas the healthy Batak control samples were recruited from the Indonesia Red Cross chapter Medan, and both of the sample recruitments were carried out during the period from October 2022 to March 2023. The inclusion criteria for the schizophrenia group were those who (1) have been diagnosed based on Indonesian Guidance for Diagnosis Mental Disorders Vol. III (2) were 18-55 years old, (3) have at least two generations of immediate family members belonging to the Batak ethnic group, (4) were cooperative, and (5) were willingly interviewed (via PANSS assessment). The inclusion criteria for the control group were (1) 18-55 years old, no history of psychiatric disorders according to MINI ICD-10, and having at least two generations of immediate family members belonging to Bataknese. After obtaining ethical consent from the board and written consent from the research participants, demographic data were collected, and blood samples were taken. The blood samples were collected, and DNA isolation and analysis were performed using Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) technique with NlaIII restriction enzyme. The frequency of polymorphism results would then be statistically analyzed. The difference between the Val158Met variant alleles among the Batak ethnic schizophrenia group and the healthy Batak control group was statistically tested using chi-square with Yates correction, obtaining p-value and odds ratio (OR) through chi-square test to estimate the risk. As this study consisted of two phenotypes, Val (G)/ Met (A) and three genotype variables (not a 2x2 table), which consist of Val/Val, Val/Met, and Met/Met, the chi-square test condition could not be applied, therefore; the relationship between genotype and the occurrence of schizophrenia would be analyzed using logistic regression, with the MetMet genotype was selected as the reference.

Results

Demographic data revealed that most subjects in both the Batak ethnic group with PWS (person with schizophrenia) and the healthy Batak control group were males. Among the Batak PWS group, there were 145 males (72.5%), while in the healthy Batak control group, there were 163 males (81.5%). The median age was 35 in the Batak PWS group and 24.5 years old in the healthy Batak control group (p=0.001). The median onset of illness in the Batak PWS group was 25 years. The mean duration of illness in the Batak PWS group was 8.91 years with a standard deviation of ±5.16 years. The mean total PANSS (Positive and Negative Syndrome Scale) score in the Batak PWS group was 96.42, with a standard deviation of ±7.85.

Table 1. Demographical Characteristics

	Groups		
Variable	ıup 00)	ıtrol ıup 200)	*P value*
Sex			
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 Illness[a]	8.91±5.16	-	
PANSS total score[a]	96.42±7.85	-	

In the Val158Met variant, the distribution of alleles varied between the PWS group and the Batak control group. The frequency of the Val allele appeared to be higher in the control group, occurring 351 times (50.3%), while the Met allele occurred 89 times (51.1%) in the Batak PWS group. The obtained p-value was 0.73, indicating no significant difference. The odds ratio (OR) was 0.94, with a confidence interval (CI) ranging from 0.67 to 1.31.

Table 2. Allele Variants Val158Met Distribution between Two Groups

	Groups			
Variable Alel Val158Met	PWS Group (n=200)	Control Group (n=200)	P value	OR (95% IK)
Val	311 (49.7%)	31 5 (50.3%)		
Met	89 (51.1%)	85 (48.9%)	0.73	0.94 (0.67-1.31)

The genotype variations between the PWS group and the Batak control group display similarly. The most common genotype variation in both groups was ValVal, with a frequency of 123 (61.5%). The ValMet variant also showed similarity between the two groups, accounted for 34.0% in both. Logistic regression analysis resulted in a p-value of 0.013 for the genotype (ValVal vs. MetMet), indicating a significant difference. The odds ratio (OR) is 0.232, with a 95% confidence interval (CI) ranging from 0.085 to 0.633. The p-value for the genotype (ValMet vs. MetMet) is 0.808, indicating no significant difference. The odds ratio (OR) is 0.271, with a CI ranging from 0.097 to 0.760.

Table 3. Genotype Differences of Val158Met between PWS and Control Group

Variable Genotype Val158Met	Group PWS Group (n=200)	Control Group (n=200)	P value	OR (95% IK)
ValVal	123 (61.5%)	123 (61.5%)	0.013	0,232 (0.085-0.633)
ValMet	68 (34.0%)	69 (34.5%)	0.808	0.271 (0.097-0.760)
MetMet	9 (4.5%)	8 (4.0%)	Comparison	

Table 4. Hardy-Weinberg value between Val158Met PWS and Control Group

	Nilai p	
	ODS	Kontrol
Chi square	0,059	0,043

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

Discussion

This study found significant differences between the Batak PWS group and the healthy Batak control group. Meta-analysis studies by Singh et al. are consistent with the findings of this study, showing a predominance of males among individuals with schizophrenia [12]. Another supporting study by Al-Asmary et al. in Saudi Arabia found a higher prevalence of schizophrenia in males (123 individuals) compared to females (49 individuals). The male-to-female ratio in this study was 1:2.5, and schizophrenia in females seems to be often associated with an older age of onset [13]. The median duration of illness in the PWS group was 8.9 years. Although schizophrenia can occur in all age groups, the theoretical onset of schizophrenia is most commonly observed between the ages of 15-25 for males and 15-30 for females; some literature also categorizes early onset schizophrenia as occurring between the ages of 13-17 or late onset between the ages of 40-55 [14]. The onset of schizophrenia can also be

associated with advanced parental age, particularly paternal age, due to a decline in reproductive cell quality caused by ageing [15].

This study examined the frequency of the Val and Met alleles in the Batak PWS group and the Batak healthy control group. The chi-square analysis yielded a p-value of 0.79, indicating a significant difference in allele frequencies between the two groups. An odds ratio (OR) of 0.94 suggests that individuals with the Val allele have the same likelihood of developing schizophrenia as individuals with the Met allele, indicating that the distribution of the two alleles is similar when the odds ratio is close to or equal to 1. In this study, an OR of 0.94 (close to 1) suggests no association between the Val158Met allele variant and the risk of schizophrenia. This finding is consistent with a study by Ohmori et al. in Japan in 1998, which found no significant difference in the occurrence of the Val and Met alleles between the PWS and control groups [7].

The logistic regression analysis yielded a chi-square value of 0.013 for the genotype (ValVal vs. MetMet) with an OR of 0.232. The chi-square value for the genotype (ValMet vs. MetMet) was 0.808 with an OR of 0.271. These results indicate no association between the genotypes (ValVal vs. MetMet) and (ValMet vs. MetMet) and the risk of schizophrenia. These findings are consistent with a study conducted by Han et al. in 2005, which found no significant difference in the frequency of Val and Met genotypes between patients and the control group (p=0.76) [8].

Conclusion

Individuals with the Val allele have an equal likelihood (odds) of experiencing schizophrenia compared to individuals with the Met allele. The Val158Met allele variant does not indicate any association with the risk factor for schizophrenia. There was no significant difference in the frequency of genotype occurrence between the Batak PWS group and the Batak healthy control group. These findings suggested no association between the genotypes (ValVal vs. MetMet) and (ValMet vs. MetMet) with the risk of schizophrenia. The occurring polymorphism may continue to be inherited, resulting in different frequencies of polymorphism in each ethnic group.

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