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Original Article

Polymorphism of *TPH2* Gene rs120074175 Is Not Associated with Risk Factors of Schizophrenia

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ABSTRACT

Context: Polymorphism on tryptophan hydroxylase 2 (*TPH2*) gene rs120074175 can cause the synthesis of neurotransmitter serotonin in the brain to reduce up to 80%. Reduced serotonin in the brain can cause dopamine release to occur continuously. Excess dopamine in the brain may cause positive symptom of schizophrenia. **Aim:** The aim of this study was to investigate the genotype distribution of *TPH2* rs120074175 gene on patients with schizophrenia at Prof. Dr. Soerojo Magelang Psychiatric Hospital, Indonesia, and the relationship between the genetic polymorphism of the *TPH2* rs120074175 gene against risk factors of schizophrenia. **Settings and Design:** This was a cross-sectional study. **Materials and Methods:** The method used was amplification refractory mutation system–polymerase chain reaction (ARMS-PCR). Whole blood from healthy subjects and patients with schizophrenia, Wizard genomic deoxyribonucleic acid (DNA) purification kit (Promega, Fitchburg, Wisconsin), PCR master mix (Promega), ARMS-PCR primers, ddH_2O, agarose (Thermo Scientific, Seoul, South Korea), Tris, Acetic Acid, EDTA (TAE) 1X, ethidium bromide, loading dye 6×, and DNA ladder (Thermo Scientific) were the materials used. **Statistical Analysis:** Hardy–Weinberg equilibrium and chi-square (χ^2) tests were used. **Results:** The results showed that both groups (healthy subjects and patients with schizophrenia) at the Prof. Dr. Soerojo Magelang Psychiatric Hospital have a wild-type GG genotype (100%) without anyone having a mutant A allele. **Conclusion:** *TPH2* rs120074175 gene polymorphism was not associated with risk factors for schizophrenia.

KEYWORDS: *Genetic polymorphism, schizophrenia, tryptophan hydroxylase 2 gene*

INTRODUCTION

Schizophrenia is a mental illness that is usually characterized by hallucinations, delusions, loss of motivation, and withdrawal from the social environment.[1] It has an onset ranging from 18–30 years for men and 20–30 years for women, where men have a greater risk for developing schizophrenia compared to women. Although schizophrenia has a prevalence of only 1% in the entire population of the world,[2] this disease is included among the 10 diseases that cause disability.[3] People who are affected by schizophrenia tend to have a shorter age compared to the general population, which is due to the high possibility to develop other diseases, such as liver failure or diabetes, and suicidal acts.[4]

The onset of schizophrenia is very closely related to the balance of dopamine in the body. Dopamine is a neurotransmitter that affects human behavior, memory, and cognition.[5] The dopamine pathway in the brain can be divided into four pathways, namely the mesolimbic, mesocortical, nigrostriatal, and tuberoinfundibular

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pathways. The presence of dopamine imbalance in all four pathways can be one of the possible causes of schizophrenia. Excessive dopamine activity in the mesolimbic pathway can cause positive symptoms of schizophrenia, and a lack of dopamine in the mesocortical pathway can cause negative symptoms of schizophrenia.[6-9] The imbalance of dopamine in the brain can be caused by several factors, one of which is the result of gene polymorphism.[10]

The polymorphism that occurs in the tryptophan hydroxylase-2 (*TPH2*) gene can be one of the causes of dopamine imbalance in the brain. The *TPH2* is a gene that encodes the tryptophan hydroxylase enzyme in the brain stem, where this gene is located on chromosome 12q21.1.[11,12] This enzyme plays a role in regulating the synthesis of neurotransmitters serotonin (5-hydroxytryptamine) in the brain.[13-15] Serotonin has a function in regulating the dopamine release in the brain. Polymorphisms that occur in *TPH2* gene can decrease the function of genes and reduce serotonin synthesis in the brain. These gene polymorphisms can occur at various points, rs4570625, rs7305115, rs4290270,[12] C1473G, and rs120074175.[11] TPH2 gene polymorphism at rs120074175 occurs at the 1463th base sequence, which changes base of guanine (G) to adenine (A) and reduces gene function, such that serotonin synthesis in the brain stem will decrease up to 80%. Decreased function of these genes will make patients have more anxious attitudes and more tendencies to commit suicide.[11] In addition, reduced serotonin levels in the brain can cause dopamine release to occur continuously, so that dopamine overexpression due to *TPH2* gene polymorphism can be one of the possible causes of schizophrenia, especially in positive symptoms of schizophrenia owing to dopamine overexpression on the mesolimbic pathway. Therefore, we investigated the role of *TPH2* rs120074175 gene polymorphism as a risk factor of schizophrenia.

Subjects and Methods

Subjects

In this case-control study, a total of 46 patients with unrelated schizophrenia and 51 control subjects were recruited. All patients were enrolled from Prof. Dr. Soerojo Magelang Psychiatric Hospital, Indonesia with following two criteria: (1) age around 18–65 years and (2) a newly diagnosed patients with schizophrenia and/or those under therapy for less than 1 year. Patients who abused alcohol/drugs and/or have brain disease, whose medical records cannot be traced, who have other psychiatric disease, and who are not willing to approve informed consent were excluded from this study.

Healthy control subjects self-reported that they were free from physical diseases as well as from individual and family history of mental illness. All participant information was anonymized during the analyses, and all patients signed informed consent to participate. The study protocol and process were assessed and approved by the ethics committee at Prof. Dr. Soerojo Psychiatric Hospital (KEH/001/1/2019).

DNA extraction and genotyping

Whole blood samples were obtained and genomic DNA was isolated using Wizard Genomic DNA Purification Kit (Promega, Fitchburg, Wisconsin). Genotyping of *TPH2* rs120074175 gene was carried out using amplification refractory mutation system–polymerase chain reaction (ARMS-PCR) with four primers and PCR Master Mix (Promega). Forward positive control primer (F) was -5'-GTG GTA TAT TTT GCA GCA CGC CCT T-3'-, reverse positive control primer (R) was -5'-ATT GAC TGA ACT GCT GCT GCT AAG CCC C-3'-, G allele-specific primer (P_G) was -5'-GCA GGG ACT TTG CAA AGT CAA TTA CTC G-3'-, and A allele-specific primer (P_A) was -5'-TAG GGA TTG AAG TAT ACT GAG AAG GAA T-3'-.

Statistical analysis

The allele frequencies of the obtained data were analyzed statistically by Hardy–Weinberg (df = 1). The chi-square (χ^2) statistic test was used to analyze the correlation of genetic polymorphism with the number of patients with schizophrenia and healthy subjects.

Results

According to ARMS–PCR results, all subjects from this study either control subjects or patients with schizophrenia had wild-type GG genotype (100%) without anyone having a mutant A allele [Table 1, Figure 1].

Discussion

There have been many relationship studies of *TPH2* gene polymorphism against schizophrenic disorders, at single-nucleotide polymorphism (SNP) rs4570625, rs7305115, rs4290270,[12] and rs7963803,[16] but no studies that directly relate SNP rs120074175 against schizophrenic disorders are available similar to this study. In an *in vitro* experiment, SNP rs120074175 showed that the presence of polymorphisms at this SNP can cause a decreased serotonin synthesis (up to 80%) in the brain. Reduced serotonin levels in the brain

can cause the dopamine release to be out of control. This excess of dopamine neurotransmitters in the brain is very closely related to positive symptoms of schizophrenic disorders.[17] This result was in agreement with a similar study by Zhang *et al*.[11] In this study, patients who had mutant A alleles tend to be more depressed, and had tried to commit suicide at least once. Therefore, the presence of a polymorphism at rs120074175 is expected to be one of the possible causes of positive symptoms in schizophrenia.

In this study, it has been stated that all patients with schizophrenia did not have mutant A alleles. The rarity of polymorphism that occurs at rs120074175 was also in agreement with other studies against other psychiatric diseases [Table 2]. Such as study by delorme where age, sex, and race had been adjusted between the control group and patient group,[20] was also still unable to find any polymorphism at rs120074175. So it can be concluded that the polymorphism that occurs at SNP rs120074175 is a rare polymorphism.[20]

Our study failed to confirm the result from previous study,[11] that might due to different of patient race and number of samples used. Majority patients from previous study was Caucasian and this study was Deutero Malay, then this study only used 46 samples patient compared to previous study which used 87 samples patient. The results of this study cannot be continued into the calculation of statistical analysis because no mutant allele is present in patients. Therefore, chi-square statistical analysis cannot be carried out.

In summary, we carried out an association study between *TPH2* rs120074175 genetic polymorphism with risk factors of schizophrenia. Our findings indicated that polymorphism of *TPH2* gene rs120074175 is not associated with risk factors of schizophrenia. In further investigation, it is recommended to increase the number of samples from various regions in Indonesia so that the results obtained can be more representative of the population in Indonesia.

Table 1: Allele and genotype frequencies of controls and subjects with schizophrenia at *TPH2* rs120074175 gene

SNP ID	Number	Allele frequency		Genotype frequency			HWE (*P*)*
rs120074175		G	A	G/G	G/A	A/A	
Schizophrenia	46	92 (100%)	-	46 (100%)	-	-	-
Control	51	102 (100%)	-	51 (100%)	-	-	-

*Cannot be measured. HWE = Hardy-Weinberg equilibrium

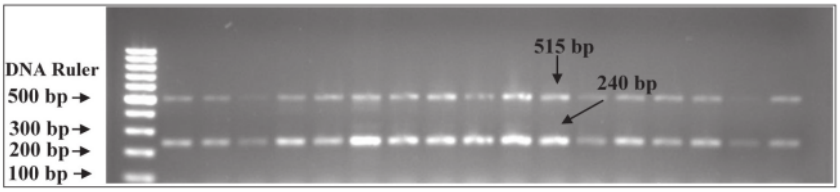

Figure 1: Representative amplification refractory mutation system–polymerase chain reaction results of samples collected from subjects with homozygous GG allele

Table 2: Result of *TPH2* SNP rs120074175 gene study against other psychiatric disorders

Study reference number	Number	G allele	A allele	Subjects
[11]	87	165	9	Unipolar depression
	219	435	3	Control
[18]	1023	2046	-	Unipolar depression
[19]	779	1558	-	Unipolar depression
	1740	3480	-	Major depression
[20]	1071	2142	-	Mental disorder
	246	492	-	Control
[21]	135	270	-	Unipolar depression
	182	364	-	Bipolar disorder
	364	728	-	Control
[15]	123	246	-	Unipolar depression
	122	244	-	Control

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Conflicts of interest

There are no conflicts of interest.

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