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The frequency of DISC1 $^{Leu}607^{Phe}$ gene polymorphism in schizophrenia patients at Dr. Soetomo General Hospital Surabaya

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ABSTRACT

Schizophrenia is a common health problem in the world, including in Indonesia. Polymorphism of gene disrupted in schizophrenia 1 (DISC1) $^{Leu}607^{Phe}$ is allegedly related to the predisposition to schizophrenia. However, studies on the relationship between polymorphism of DISC1 $^{Leu}607^{Phe}$ and schizophrenia in various ethnics provided different results. The purpose of this study was to determine the frequency of DISC1 $^{Leu}607^{Phe}$gene polymorphism and its association with treatment response in patients with schizophrenia at Department of Psychiatry, Dr. Soetomo General Hospital Surabaya. In this study, the number of male patients with schizophrenia was more than that of the female patients. The mean age of male patients with schizophenia was lower than that of the female patients. Schizophrenia patients were primarily came from Javanese ethnic with positive and negative symptom score (PANSS) lower in male patients than that in the female patients. In conclusion, no DISC1 gene polymorphism at codon 607 is observed in schizophrenia patients at Dr. Soetomo General Hospital Surabaya, but the G nucleotide variation at the number 196.339 in intron regions is found instead.

ABSTRAK

Schizophrenia adalah masalah kesehatan umum di dunia termasuk di Indonesia. Adanya polimorfisme gen schizophrenia 1 (DISC1) $^{Leu}607^{Phe}$ diduga berkaitan dengan predisposisi schizophrenia. Namun demikian, berbagai penelitian yang menghubungkan polimorfisme gen DISC 1 $^{Leu}607^{Phe}$ dan schizophreniapada berbagai etnik memberikan hasil yang berbeda. Penelitian ini bertujuan menentukan frekuensi gen DISC 1 $^{Leu}607^{Phe}$ dan hubungannya dengan respon pengobatan pasien Schizophrenia di Departemen Psikiatri, Rumah Sakit Umum Dr. Soetomo, Surabaya. Dalam penelitian ini jumlah pasien pria lebih banyak dari pada wanita. Rerata umur pasien pria lebih rendah dari pada pasien wanita. Pasien schizophrenia umumnya dari suku Jawa dengan skor skor simptom negatif dan positif lebih rendah pada pria dibandingkan wanita. Dapat disimpulkan, tidak ditemukan polimorfisme gen DISC1 pada kodon 607 pada pasien schizophrenia di Rumah Sakit Umum Dr. Soetomo, Surabaya, namun ditemukan variasi nukleotida G pada nomor 196.339 di daerah intron.

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Keywords: Schizophrenia - DISC1 $^{Leu}607^{Phe}$ gene – polymorphism – PANSS - predisposition

INTRODUCTION

Schizophrenia is a complex psychiatric disorder,which remains a health problem worldwide,[1,2] including Indonesia.[3] According to the DSM-IV-TR (Diagnostic and Statistical Manual of Mental Disorders 4th ed. Text Revision), the annual incidence of schizophrenia is influenced by the geographic variation.[2] It is estimated that there are 24 million people with schizophrenia worldwide, 50% of whom do not get optimal treatment, and 90% of these patients are in developing countries.[4] In Indonesia, the prevalence of schizophrenia ranges from 0.3 to 1% and is especially between the age of 18 - 45 years.[5]

The mechanisms underlying schizophrenia remain unclear, but there are statements of multifactorial etiology.[6] Etiology, which allegedly plays an important role in the predisposition to schizophrenia, is a combination of genetic factors and environmental factors.[2] Forms of genetic variation which allegedly acted as a predisposing factor of schizophrenia is Single Nucleotide Polymorphism (SNP). Polymorphism generally can be analyzed by referencing to the reference sequence (rs) in the database at the National Center of Biotechnology Information (NCBI).[7] Polymorphism that has been studied in various countries is the DISC1 gene.[8-13] The method which can be used to detect polymorphism, among others, are Polymerase Chain Reaction (PCR) followed by Restriction Fragment Length Polymorphism (RFLP),[13,14] or sequencing.[11,15]

DISC1 protein resulted from DISC1 gene expression is a scaffold protein that plays a role in the regulatory process of neural progenitor cells proliferation, neurite outgrowth, neuronal migration, and c-AMP signals.[6] DISC1 protein will interact with a variety of signaling molecules and affect the occurrence of impaired cognitive function and working memory in patients with schizophrenia.[16] Signaling molecules interacting with DISC1 protein plays an important role for developing antipsychotic drugs.[2,6]

The main therapy for schizophrenia patients is an antipsychotic (typical or atypical or combination of both). Identification and analysis of polymorphisms as biological biomarkers in patients with schizophrenia are expected to be very useful for the clinician to predict the effectiveness of antipsychotic, side effects of antipsychotic drugs and to track gene hereditary disease in families of patients.[17,18] Considering that there has never been any research on the role of genetic factors implicated in schizophrenia, the study aimed to detect polymorphisms of DISC1 $^{Leu}607^{Phe}$ gene in patients with schizophrenia at the Department of Psychiatry, Dr. Soetomo General Hospital, Surabaya.

MATERIALS AND METHODS

Subjects

This was an observational descriptive study with cross-sectional design to evaluate the polymorphism of DISC1 $^{Leu}607^{Phe}$ gene in patients with schizophrenia and its association with treatment response in patients with schizophrenia at Department of Psychiatry, Dr. Soetomo General Hospital Surabaya. All patients with schizophrenia who came/were treated at the hospital for 4 months and met the inclusion-exclusion criteria were involved in this study. The study was performed after an ethical clearance was obtained from the Research Ethics Committee of Dr. Soetomo General Hospital Surabaya.

Procedure

Prior to research performed, patients's families were explained concerning the background, objectives and benefit of the study and then were given an informed consent to be signed. The diagnosis of schizophrenia of patients was based on a psychiatric history and mental status examination using Code Classification and Diagnosis of Mental Disorders in Indonesia III (PPDGJ issue 3)[19] and the criteria of Positive and Negative symptom Scale (PANSS).[20]

Blood samples of patients were taken and put into venoject 5 ml tubes with EDTA anticoagulant. Mononuclear Peripheral Blood Cells (PBMCs) were then separated and put in eppendorf tubes. Furthermore, PBMC was stored at a temperature of minus 80^0 C at the Institute of Tropical Disease, Airlangga University until laboratory examination. Deoxyribonucleic Acids (DNA) was extracted from PBMC, followed by the PCR with the primary: 5 - GAT GGC AAT GGA TTC ACC AC - 3 '(forward) and 5'-CAG ACA GTT GGG GAG AAC AG - 3' (reverse).[11] After that, electrophoresis was performed. PCR products in the form of DNA fragments with a length of 689 bp were done by sequencing using ABI 310 genetic analyzer sequencer from applied biosystems, inc. Multiple alignment with genetyx version 10 was then conducted to the result of sequencing.

Statistical analysis

Data of the characteristics of patients were presented as percentage.

RESULTS

Characteristics of patients with schizophrenia at Department of Psychiatry, Dr. Soetomo General Hospital are shown in TABLE 1. The number of patients having a history of mental illness in the family was higher than patients who did not have the history. Patients with schizophrenia receiving typical antipsychotic therapy was considerably less (28.28%) compared to those getting atypical antipsychotics (36.36%) and combination (36.36%). Female patients, who received antipsychotic therapy, had the least combination of numbers (22.22%), followed by a typical antipsychotic (33.33%) and the highest was an atypical antipsychotic (44.45%).

TABLE 1. Characteristics of patients with schizophrenia at SMF Dr. Soetomo General Hospital

Sex	Number of patients		Mean age range	Ethnic						Family History			
	N	%	Year	Javanese		Madurese		Mixed		Yes		No	
				n	%	n	%	n	%	n	%	n	%
Male	11	55	38.82 (25 – 62)	9	45	1	5	1	5	4	20	7	35
Female	9	45	43.11 (31 – 58)	8	40	1	5	0	0	7	35	2	10
Total	20	100	40.97 (25 – 62)	17	85	2	10	1	5	11	55	9	45

PCR product in this study was DNA fragment with a length of 689 bp. The electrophoresis, when compared with 100 bp DNA marker band, would appear as a band placed marker bands between 600 and 700 bp.

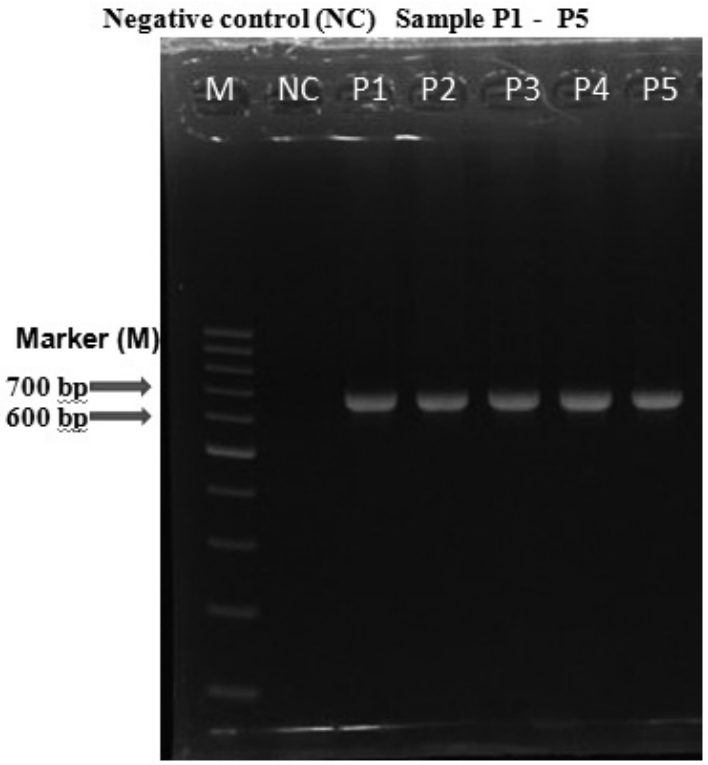

FIGURE 1. Examples of some of the electrophoresis results of PCR products DISC1 $^{Leu}607^{Phe}$ gene with positive results

If the PCR results were positive, the sequencing would be conducted by using ABI 310 sequencer and forward primary (sense). The results of sequencing were used for Multiple alignment of the nucleotide sequence of the sample compared to rs 6675281, using a computer program genetyx version.10. Results of Multiple alignment pieces rs 6675281 sequencing results of 20 samples of patients with schizophrenia at Department of Psychiatry, Dr. Soetomo General Hospital is shown in FIGURE 2.

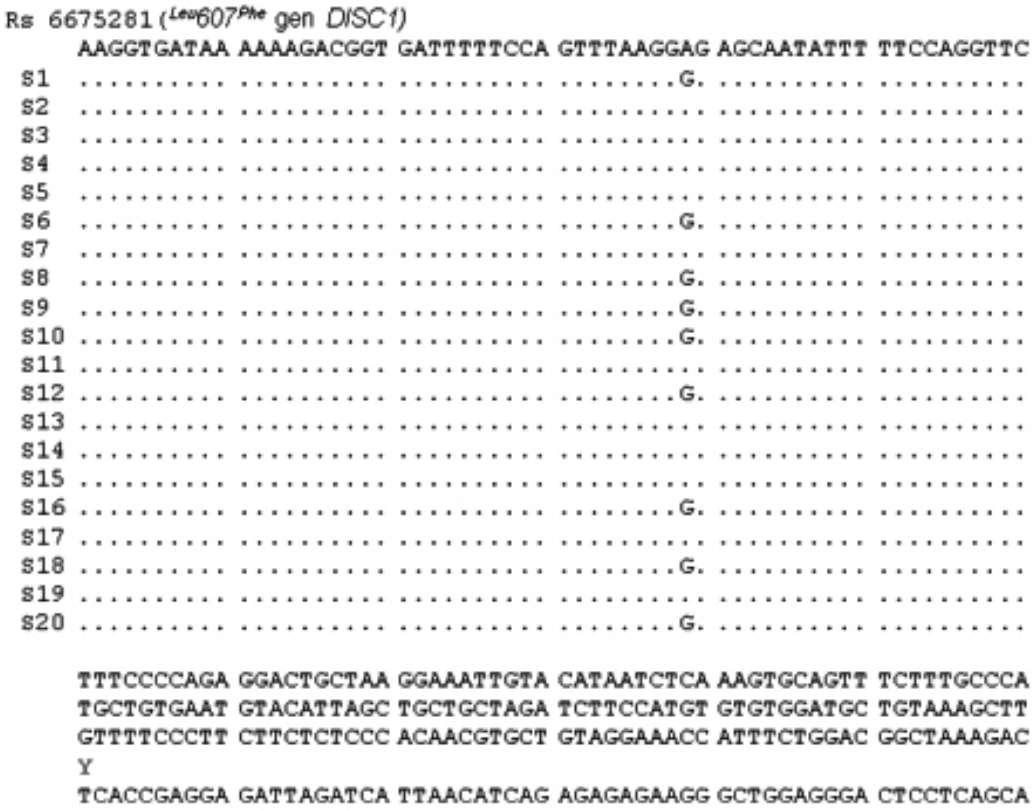

FIGURE 2. Results of multiple alignment pieces *rs* 6675281 (DISC1 SNP $^{Leu}607^{Phe}$ gene) from the sequencing result of 20 samples of schizophrenia patients at Department of Psychiatry of Dr. Soetomo General Hospital Surabaya.

In this study there was no DISC1 $^{Leu}607^{Phe}$ gene polymorphism found with the code of rs 6675281 located in exon 9 at codon number 607, because all samples show C nucleotides in the nucleotide sequence of numbers 196.541 (y), according to the basic data DISC1 gene from NCBI. On further analysis of the sequencing results, it was obtained that electropherogram of samples 1, 6, 8, 9, 10, 12, 16, 18 and 20 had the variation of nucleotide G, while at the the other sample numbers nucleotide A were obtained in accordance with the basic data of DISC1 genes from NCBI (rs 6675281). Example of electropherogram sequencing result DISC1$^{Leu}607^{Phe}$gene with the nucleotide A at number 196.339 in patients with schizophrenia is shown in FIGURE 3.196.339

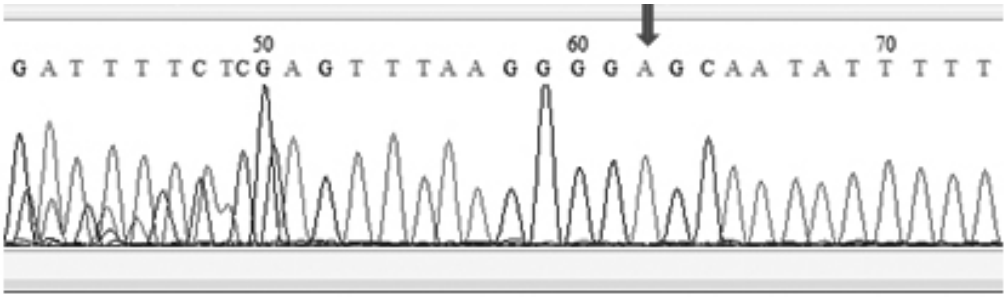

FIGURE 3. Example of electropherogram intron sequencing result DISC1 $^{Leu}607^{Phe}$ gene with the nucleotide A at number 196.339.

DISCUSSION

The mean age range of the male schizophrenia patients was younger than the average age of the female patients. It is believed to be related to the estrogen hormone thought to have a protective effect against schizophrenia, so the manifestation of schizophrenia in men tend to occur at a younger age than women.[2,21] In female schizophrenia, psychotic symptom fluctuation is obtained during the menstrual cycle. The estrogen hormone has a pleiotrophic effect to variations of brain developmental processes in adults.[21]

The data showed that genetic factors play a quite significant role as predisposition to schizophrenia. This is supported by previous research on families, twins and adopted children, which demonstrated that genetic factors play a major role for schizophrenia.[22] Genetic predisposition to schizophrenia is a complex factor for their interaction with environmental factors.[2,22] In Scottish family DISC1 gene is identified as potential genes associated with susceptibility to the onset of psychiatric disorders, because the Scottish family obtained schizophrenia and other psychiatric disorders with a high frequency.[15,22]

DISC1 gene plays a key role during the process of brain development, especially on the thickness of the cerebral cortex, which is highly heritable. This is supported by evidence from MRI examination, which shows a reduction of the thickness of the cerebral cortex in schizophrenia patients.[23] From the results of sequencing on samples of patients with schizophrenia at SMF Psychiatry of Dr. Soetomo Hospital, there was no DISC1 $^{Leu}607^{Phe}$ gene polymorphism. Study of DISC1 $^{Leu}607^{Phe}$ gene polymorphism in patients with schizophrenia in some ethnic population provides inconsistent results. The earlier study in patients with schizophrenia by Brauns in 2011 showed an interference of neural activity in the dorsolateral prefrontal cortex (DLPFC) during the process of working memory and the reduction of the thickness of the cortex cerebri in the left part of gyrus supramarginal of the carrier allele Phe from DISC1 $^{Leu}607^{Phe}$ gene when compared to the homozygote Leu/Leu.[23] Hodgkinson's study in 2004 also found an increased risk of schizophrenia in the DISC1 $^{Leu}607^{Phe}$ gene polymorphism.[24] Results of research DISC1 $^{Leu}607^{Phe}$ gene are various, because the predisposition to schizophrenia is influenced by multigenic and multifactorial factors. Thus, in different demographic areas and ethnicities we can find some different variation polymorphism from the DISC1 gene or polymorphisms in other genes that are also allegedly associated with predisposition to schizophrenia.[2,13-15]

In the group sample of patients with schizophrenia, it was found that the number of male patients who come from Javanese ethnicity are far more than any other ethnicities or the mixed ones; it also happened in the case of the female patients. This is presumably caused by the fact that the sampling was performed in Dr. Soetomo General Hospital which is a referral hospital in the east region of Java island, so that patients with schizophrenia are mainly dominated by the Javanese. Data from Indonesia Basic Health Research in 2013 reported that the prevalence of severe mental disorders in East Java was 0.22%, and the highest prevalence was found in Yogyakarta Special Region area that was equal to 0.27%, while the national prevalence was 0.17%.

The overall sample of patients with schizophrenia receiving various antipsychotic therapy. Olincy *et al*.[25] reported that the DISC1 protein is allegedly associated with the response to antipsychotic drug therapy and plays an important role to identify the lowest effective dose for the patient as well as the lowest side effects. Another previous studies conducted on rats showed that the administration of atypical antipsychotics will increase the DISC1 gene expression in the frontal cortex, while the provision of typical antipsychotic effect is not found in the DISC1 gene expression.[26]

This study did not find any DISC1 gene polymorphism at codon 607, but found variations of G nucleotide at the number 196.339 in intron regions. These nucleotide differences in this study with the SNPrs 6675281 (NCBI) could occur because schizophrenia is a disease that has a multifactorial etiology, so it is possible that the DISC1 $^{Leu}607^{Phe}$gene polymorphism is not a dominant genetic factor. The impact of the differences in the nucleotide has been known, yet.

The results of this study were not in accordance with the research conducted previously on the Finnish population[15,27] which found the presence of DISC1 $^{Leu}607^{Phe}$gene. Presumably, this is because the predisposition to schizophrenia is associated with a multifactorial and multigenetic etiology.[2,28]

CONCLUSION

It can be concluded that the male patients with schizophrenia is higher in number, with a lower average age, and lower PANSS scores than those of the female patients. Patients with schizophrenia at the Department of Psychiatry, Dr. Soetomo General Hospital mainly come from Javanese ethnic. This study does not find any DISC1 gene polymorphism at codon 607, but find variations of G nucleotide at the number 196.339 in intron regions.

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