

Original article

Polymorphism of DTNBP1 Gene P 1635 (Rs3213207) In Schizophrenic Javanese, Indonesia

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Abstract:

Background: Many putative genes were predicted as risk factors of schizophrenia. One of these genes is DTNBP1 gene. One of single nucleotide polymorphism (SNPs) of DTNBP1 gene is P1635 (rs3213207). **Objective:** This study aimed to find out the association between DTNBP1 gene, P1635 (rs3213207), and risk of schizophrenia. **Methods:** DNA was isolated from blood of 25 schizophrenia patients and 25 healthy controls. DNA was applied to restriction fragment length polymorphism polymerase chain reaction (RFLP PCR). PCR products were digested by BsrI enzyme. **Results:** The results show that there are 24 AA genotype (wild type) from 25 Javanese schizophrenia patients and 1 GG homozygote, while all 25 of controls are AA genotype (wild type). The p value of Fisher's exact test is 0,500. **Conclusion:** There is no significant differences of genotype distribution of SNP P 1635(rs3213207) between schizophrenia patients and healthy controls in Javanese population

Keywords: DTNBP1 gene; Schizophrenia; Javanese

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Introduction

Schizophrenia is a disease with mental deterioration. The symptoms of this disease include hallucinations, delusion, speech disorder, catatonic reflexes and others¹. The prevalence of this disease approximately 1%^{2,3}. The disorder causes a reduction in affective and social abilities. The most age with schizophrenia is 15-35 years old⁴. In 1995, the prevalence of schizophrenia in Indonesia (>15 years old) was approximately 3 in 1000 household⁵.

Genetic factor thought to be one of predisposing factors of schizophrenia. Several genes were predicted as a risk factor for schizophrenia, including Catechol O methyltransferase (COMPT) gene⁶ and Dystrobrevin binding protein 1 (DTNBP1) gene in schizophrenia Iranian population⁷. Dystrobrevin binding protein 1 is genes that function in the formation of dysbindin (Dystrobrevin-binding protein 1)⁸. DTNBP1 gene is located on chromosome 6p22.3. Several studies state that this gene increases the risk of schizophrenia. Dysbindin has molecular weight of 40 kD. This protein is bound to dystrobrevin (a complex glycoprotein dystrophin)⁹.

The DTNBP1 gene was allegedly affecting the incidence of schizophrenia. Variation in this gene affects the expression and processes in mRNA¹⁰. Research conducted by Weikert *et al.*, 2004 showed that schizophrenics have low levels dysbindin¹¹. The polymorphisms of DTNBP1 gene at p 1635 (located in intron 4) are predicted to affect the incidence of schizophrenia. An allele at p1635 increases the risk of schizophrenia, while G allele lowers the risk of schizophrenia in Iranian schizophrenia patients¹².

Material and Methods

A total of 25 schizophrenia patients and 25 healthy controls were used as research subjects. DNA was isolated from blood of the subjects. DNA isolation was performed as follows: 20 mL of Proteinase K was put in a 1.5 mL tube then 200 µL of blood sample was added into the tube and mixed. 200 mL of buffer was added to the mixture and mix by vortexing for 15 seconds. The mixtures were incubated in a water bath at temperature of 56°C for 10 minutes followed by centrifugation. Supernatant was removed and 200 mL of absolute ethanol was added to the pellet followed by vortexing for 15 seconds. Sample was mixed and

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placed in a column and centrifuged at 8000 rpm for 1 minute. Column was removed and transferred to a new tube and 500 μ L of buffer AW 1 (Qiagen kit) was added to the column. Sample was centrifuged at 8000 rpm for 1 minute and column was transferred into a new tube. 500 μ L of buffer AW 2 (Qiagen kit) was added and then centrifuged at 13,000 rpm for 3 minutes. Column was transferred in a 1.5 mL new tube and 200 μ L of buffer AE (Qiagen kit) was added to the column. This mixture was maintained for 1 minute at room temperature and then centrifuged at 8000 rpm for 1 minute.

Polymerase chain reaction (PCR): PCR was performed by restriction fragment length polymorphism (RFLP) PCR. A total of 12,5 μ L master mix, 6,5 μ L dH₂O, 2 μ L forward primer: 5-GCAGACCATG-TATTTGAAAAGC-3, 2 μ L reverse primer: 5-GCCAGTTTC-CTCAAATTCC-3) and 2 μ L DNA were run by PCR. The thermo cycling condition as follows: the initial denaturation was 94°C for 5 minutes, followed by 35 cycles at 95°C for 30 seconds, 54°C for 30 seconds and 72°C for 30 seconds. The final step was 72°C for 5 minutes and 12°C for 10 minutes. PCR products were digested by BsrI enzyme. The digestion products were analyzed by gel electrophoresis using 12% agarose gel.

Analyzes

The differences of genotype distribution of SNP P1635 between schizophrenia patients and healthy controls were analyzed by Fisher's exact test.

Ethical clearance: This protocol of research was approved by Health research ethics committee of Faculty of medicine of Universitas Muhammadiyah Surakarta, Indonesia, with no. 081/B1/KEPK/-FKUMS/V/2015.

Results

In order to explore the effects of DTNBP1 gene (P1635 (rs3213207)), DNA from 25 schizophrenia patients and 25 healthy controls were isolated. Polymerase chain reaction was performed by RFLP PCR. The figure of BsrI-digested PCR product is shown in figure 1

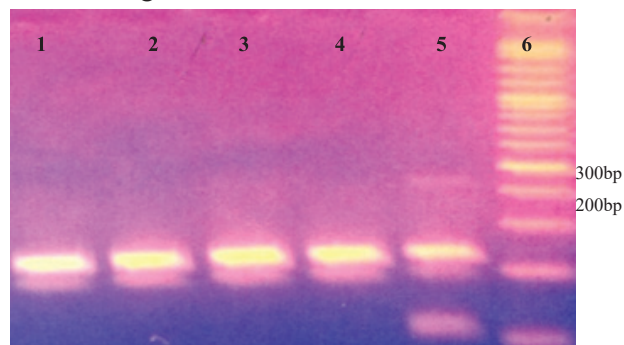


Table 1. Distribution of genotype of DTNBP1 gene (P1635)

Group	Genotype		
	AA	AG	GG
Control (n=25)	25(1.0)	0	0 (0.00)
Schizophrenia (n=25)	24 (0.96)	0	1 (0.04)

Based on Table 1, it can be seen that homozygous GG was found in one patient with schizophrenia and not found in the control (n = 25). The p value on Fisher's exact test is 0,500.

Discussion

DTNBP1 gene encodes dysbindin (dystrobrevin binding protein 1). This gene is widely expressed in the brain. This gene is thought to play an important role in cognitive function and memory⁸. The gene is located on 6p22.3 and thought to correlate with the incidence of schizophrenia¹³. The study involving 200 schizophrenics patients and 200 controls Iranian subjects found that the A and C alleles in the DTNBP1 gene P1635 increases the risk of schizophrenia. This research found 156 AA genotype and 44 genotype of AG in schizophrenia patients, while 117 of AA genotype and 83 of AG genotype in the control. There was not homozygote GG on both groups¹². Research conducted by Alizadeh *et al.*, 2012, on 115 patients with schizophrenia in population of Iran and 117 control showed 99 of AA genotype (0.86) and AG 16 (0:14) in schizophrenics patients, while 102 (0.87) of AA genotype and 15 of AG genotype (0:13) in control subjects. It was concluded that there was no significant difference of genotype distribution between patients with schizophrenia and control⁷. Research by Numakawa *et al.*, showed polymorphism of P1635 A/G of DTNBP1 was associated with Japanese schizophrenia with odds ratio of 2.71 (1.46-5.79)¹⁴. A contrary result was found in a research conducted by Joo *et al.*, 2005. There was not association of DTNBP1 P1635 polymorphism with incidence of schizophrenia in Korean population¹⁵. This study is linier with Joo *et al.* research. The genotype of AG was not found in the schizophrenics and control group. Researchers found one (1) GG genotype in schizophrenics¹⁶.

Conclusion

There is no significant difference in genotype distribution of SNP 1635 in schizophrenia patients and healthy controls in Javanese population ($P > 0.05$)

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Conflict of interest: None

Authors's contribution:

Data gathering and idea owner of this study: EM Sutrisna, Ratna Yuliani

Study design: EM Sutrisna, Ratna Yuliani

Data gathering: EM Sutrisna, Ratna Yuliani

Writing and submitting manuscript: EM Sutrisna, Ratna Yuliani

Editing and approval of final draft: EM Sutrisna, Ratna Yuliani

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