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ARTIKEL PENELITIAN

Polimorfisme Gen Reseptor Dopamin D2-141C Ins/Del pada Pasien Schizophrenia Paranoid dan Non Schizophrenia Etnis Batak di Sumatera Utara

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Abstrak: Skizofrenia adalah kondisi mental kronis yang ditandai oleh distorsi realitas serta gangguan pikiran, ucapan, dan perilaku. Beberapa faktor yang dapat menyebabkan skizofrenia termasuk kelainan genetik reseptor dopamin. Sejumlah penelitian telah menemukan hubungan antara pasien skizofrenik dan non skizofrenia dalam polimorfisme reseptor dopamin D2 -141C Ins / Del. Tujuan penelitian ini adalah untuk menyelidiki hubungan polimorfisme gen DRD2 -141C Ins / Del pada pasien skizofrenia dan non-skizofrenik etnis Batak di Sumatera Utara, Indonesia. Subyek penelitian adalah 60 pasien skizofrenia paranoid dan 54 non-skizofrenia etnis Batak. Pemeriksaan PCR dilakukan pada isolat DNA untuk memeriksa polimorfisme reseptor D2 -141C Ins / Del *dopamine receptor*. Genotipe I / D tertinggi diidentifikasi (71,7%) dan (63%), dan genotip D / D paling rendah diidentifikasi (6,7%) dan (7,4%) dengan p = 0,726. Berdasarkan distribusi alel, tidak ada perbedaan risiko pada alel D dan alel I pada faktor penyebab skizofrenia (OR: 1,13; 95% CI: 0,66-1,92). Tidak ada hubungan yang signifikan antara distribusi genotipik dan alel gen DRD2 -141C Ins / Del pada pasien skizofrenia dan non skizofrenia.
Kata kunci: gen reseptor dopamin D2 -141C Ins / Del, skizofrenia, *polymerase chain reaction*

Polymorphism of Dopamine D2-141C Ins/Del Receptor Gene in Paranoid Schizophrenia and Non Schizophrenia Patients of Batak Ethnicity in Sumatera Utara

Abstract: *Schizophrenia is a chronic mental condition which is characterized by reality distortion as well as thought, speech and behavior disorders. Several factors that may cause schizophrenia include genetic abnormalities of dopamine receptors and several studies have found an association between schizophrenic and non schizophrenic patients in the dopamine receptor gene polymorphism D2 -141C Ins/Del. The aim of this study is to investigate the relationship of DRD2 -141C Ins/Del gene polymorphism in schizophrenic and non-schizophrenic patients of Batak ethnicity in Sumatera Utara, Indonesia. The subjects of the study were 60 patients of paranoid schizophrenia and 54 non-schizophrenia of Batak*

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ethnicity. PCR examination was performed on the DNA isolates to examine D2 -141C Ins/Del dopamine receptor gene polymorphism. In both schizophrenic and non-schizophrenic patients, the highest I/D genotypes were identified (71.7%) and (63%) , and the least D/D genotypes were identified (6.7%) and (7.4%) with p = 0.726. Based on the allele distribution there was no difference of risk on allele D and allele I on the cause factor of schizophrenia (OR: 1.13; 95% CI: 0.66-1.92). There is no significant association between genotypic distribution and DRD2 -141C Ins/Del gene alleles in schizophrenic and non schizophrenic patients.
Keywords: *dopamine receptor gene D2 -141C Ins/Del, schizophrenia, polymerase chain reaction.*

INTRODUCTION

Schizophrenia is a chronic mental condition characterized by reality distortion and abnormalities of thought, speech and behavior. This condition creates disruption in the work field, education and interpersonal relationships.[1]

The cause of schizophrenia has not been identified for certain, several studies suggested that the pathophysiology of schizophrenia involves heredity, genetics, anatomy, neurotransmitters, neuronal development and neuroimaging.[2] Several genetic and environmental factors contribute to disorders in the brain function and some evidence suggests the presence of structural or chemical disorders in brains of individuals with schizophrenia.[3,4]

Neurotransmitters are neurochemical compounds that carry messages to different parts of the brain and nervous system, they also function as regulators of the body.[5] Some theories suggest that neurotransmitters play a vital role in mental and behavior disorders. Neurotransmitters that have an effect on behavioral and psychiatric disorders include dopamine, norepinephrine, serotonin, GABA and acetylcholine. Neurotransmitter imbalances, one of which the increase of dopamine levels in the synaptic space, may lead to hyperactivity and aggressive mania in schizophrenia.[6]

Patients with schizophrenia have been found to have increased production of dopamine neurotransmitters.[2] Several studies have identified a gene suspected to be involved in schizophrenia, which are dopamine receptors (DRD2 gene), (DRD3 gene), dopamine

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transporter (DAT gene), neuregulin (NRG1 gene).[7,8]

Dopamine D2 receptor (DRD2) plays a role in the limbic and caudal areas of the brain, and has become the target agent of antipsychosis.[9] Changes in the transmission and receptor of dopamine have been hypothesized as the pathophysiology of schizophrenia.[10] DRD2 is strongly suspected as a candidate gene that affects schizophrenia.[11]

Xiao et al. found that polymorphisms of D2-141C Ins/Del dopamine receptor gene were the target genes that affected Schizophrenia in the Han population in China.[12] Similarly, Lafuante et al, Breen et al, and Arinami et al, also stated that the polymorphism of D2 -141C Ins/Del dopamine receptor gene has an influence in schizorenia.[12,13,14] The allele differences in polymorphisms suggest that polymorphism in the DRD2 -141C Ins/Del gene are highly linked with susceptibility to schizophrenia.[12]

In the study by Arinami et al and Xiao et al, it was found that polymorphism of DRD2 -141C Ins/Del gene, the Del allele being a protective factor in schizophrenia, the Ins allele being a genetic predisposing factor in schizophrenia.[12,14] However the opposite was put forward by Breen et al and Lafuante et al in which their studies found an association between DRD2-141C Ins/Del gene polymorphism with schizophrenia, and that the Del allele was a precipitating factor of susceptibility to schizophrenia.[13,15]

METHODS

This research was done as an unpaired analytical case-control study conducted from September 2014 to February 2015. The research subjects were stored DNA isolate obtained from 60 schizophrenic and 54 non-schizophrenic patients of Batak ethnicity in North Sumatra, Indonesia. The DNA extraction process is carried out using a DNA extraction kit (the Wizard® Genomic DNA purification kit (Promega Corporation, USA)]. The PCR process was done using a thermocycler (Applied

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BiosynthesisTm Veriti 384®) with primers for forward D2-667: 5'-ACT GGC GAG CAG ACG GTG AGG ACC C-3' (Macrogen) and reverse D2-676: 5'-TGC GCG CGT GAG GCT GCC GGT TCG G-3 '(Macrogen) targeted at 356 bp, and PCR f mix [Go Taq® Green Master Mix (Promega, USA)].

The PCR cycle of the DRD2 gene consists of an initial denaturation for 5 minutes at 95°C, denaturation for 60 seconds at 94°C, annealing for 45 seconds at 57°C, elongation for 45 seconds at 72°C and final extension for 10 minutes at 72°C. The electrophoresis process was performed using 8% agarose and documentation was done using Uvitec Essential V4 D-52-20M® tool.

The data collected were then analysed statistically to examine the relationship between DRD2 -141C Ins/Del gene polymorphisms with schizophrenic and non-scizophrenic patients. Genotype distribution and allele frequencies were then tested for significance using X2-test. Distribution of individual genotypes and allele frequencies with schizophrenia and control subjects were then analyzed using Hardy-Weinberg Equilibrium calculator.

RESULT

Based on the study conducted on 60 samples of schizophrenic and 54 samples of non-schizophrenic subjects, it was obtained that the frequency of genotypes distribution of I/D is a variation of DRD2 -141C Ins/Del gene polymorphisms that is mostly identified in schizophrenic and non-schizophrenic patients, whereas it was found that the lowest frequency of genotypic distribution is D/D (Table 1) The result of statistical test using chi square analysis showed that there was no association between the frequency of genotypic distribution in schizophrenic with non-schizophrenia subjects.

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Table 1 The distribution of genotype polimorphysme dopamine D2 -141C Ins/Del receptor in schizophrenia and non schizophrenia

		Genotype distribution			Total	*p**
		I/D	D/D	I/I		
Schizophrenia	Genotype	43	3	14	60	
	Percentage (%)	72	7	22	100	
Non Schizophrenia	Genotype	35	3	16	54	0,73
	Percentage	63	7	30	100	
Total	Genotype	78	6	30	114	
	Percentage	68	7	25	100	

* Pearson Chi Square

Based on the distribution of allele frequency, it was found that schizophrenic subjects had D allele and I allele that exceeds those found in non-shizophrenic subjects. Statistical analysis using chi square test revealed that there was no significant association between frequency distribution of the dopamine receptor gene allele D2 -141C Ins / Del in schizophrenic subjects with non-schizophrenic subjects. (Table 2)

Table 2 The distribution of alele

Sample	D (%)	I (%)	Total (%)	p*
Schizophrenia	49 (54,4%)	71 (51,4%)	120 (100%)	0,69
Non Schizophrenia	41 (45,6%)	67 (48,6%)	108 (100%)	
Total	90 (100%)	138 (100%)	228 (100%)	

* Pearson Chi Square

Based on the odds ratio on genotypes distribution of I/D, D/D and I/I (1.38), it did not reveal the prevalence of risk of susceptibility to schizophrenia. Based on the odds ratio of the distribution of allele frequency D or allele frequency I (1.13), it did not reveal the prevalence of risk of susceptibility to schizophrenia.

The Hardy-Weinberg Equilibrium (HWE) law states that in one population, the allele frequency and genotype frequency must remain constant, and in this study, Hardy-Weinberg Equilibration analysis was performed based on the genotypic

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distribution of schizophrenia and non-schizophrenic subjects (Table 3). Based on the analysis using HWE calculator for genotype distribution in schizophrenic and non-schizophrenia subjects, the total H-W freq of both groups amounted to 100%, which indicates that this study does not deviate from the Hardy-Weinberg Equilibrium law. Based on the HWE analysis there was a significant association on the genotype frequency distribution expressed with P <0.05 (Table 3) (Table 4).

Table 3 The Odds ratio based on genotype distribution in schizophrenia and non schizophrenia

Genotype	Schizophrenia	Non Schizophrenia	Total	OR*	95% CI
I/D+ D/D	46	38	84	1,38	0,59-3,19
I/I	14	16	30		
Total	60	54	114		

** Odds Ratio*

Table 4 The Odds ratio based on allele distribution in schizophrenia and non Schizophrenia

Allele	Schizophrenia	Non Schizophrenia	Total	OR*	95% CI
D	49	41	90	1,13	0,66 - 1,92
I	71	67	138		
Total	120	108	228		

**Odds Ratio*

Table 5 The analyse of Hardy-Weinberg Equilibrium law in schizofrenia

Genotype	D/D	I/D	I/I	*P*
Observed	3	43	14	0,0002
Expected	10	28,99	21	
H-W Freq	17%	48%	35%	
Alelle Freq	D=49 (40,83%)		I=71 (59%)	

Table 6 The Analyse of Hardy-Weinberg Equilibrium law in non schizophrenia

Genotype	D/D	I/D	I/I	*P*
Observed	3	35	16	0,0057
Expected	7,78	25,44	20,78	
H-W Freq	14%	47%	39%	
Allele Freq	D=41 (38%)		I=67 (62%)	

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DISCUSSION

The dopamine hypothesis in schizophrenia remains a theory that is continued to be developed. Excessive dopaminergic activity on the mesolimbic pathway may lead to positive symptoms in schizophrenia and this phenomenon can occur due to several factors, such as; supersensitivity of dopamine receptors causing more dopamine levels to be drawn and accumulated in the synaptic space. Dopamine degradation in the mesocortical pathway is affected by the subsensitivity of dopamine receptor activity that may cause negative symptoms in schizophrenia. Genetic abnormalities are one of the factors affecting the translation process of dopamine receptor proteins.

Variations of polymorphism are thought to be one of the factors associated with the type of sensitivity of dopamine receptor function and may affect dopaminergic activity in the synaptic space. According to the HWE law, the results of this study found that individuals of Batak ethnicity are in a state of imbalance or disequilibrium with a significance of p value of HWE <0.05. HWE equilibrium can be disrupted by certain factors such as non-random mating, mutation and migration that ensues in large populations. Marriages between close relatives may result in an allele that carries certain characteristics to result in more frequent traits in one population, resulting in abnormal genes and recessive genes more likely to occur. Batak ethnic is known as a society that highly keeps its cultural indigenousity. Most of the Batak individuals would keep the family lineage by marrying between families. This is likely to result in the HWE analysis being in disequilibrium condition.

Based on statistical test results using X2-test it was found that there was no association between genotype and allele frequency distribution of DRD2 -141C Ins / Del gene polymorphism in schizophrenic and non schizophrenia subjects of Batak ethnicity (p> 0.05). This study is in line with Xiao et al.'s study of the Han population in China which states that there was no significant

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difference in the frequency of genotypic distribution among schizophrenic and non schizophrenic subjects.[12] However this is in contrast to a study by Breen et al. and Lafuante et al. in which they revealed a significant association in DRD2 -141C Ins/Del gene polymorphism between schizophrenic and non schizophrenic subjects in the British Caucasian population and Spanish Population.[13,14]

Several studies have been conducted to prove the dopamine hypothesis associated with DRD2 -141C Ins/Del gene polymorphism. In a study by Inada et al, it was found that there was a significant relationship between polymorphism to DRD2 -141C Ins/Del genes in schizophrenic and non-schizophrenic subjects.16 However, Xiao et al. found no significant association in variation in the DRD2 -141C Ins/Del gene polymorphism between schizophrenic and non schizophrenic subjects. This difference is thought to be due to several factors such as differences in sample size, differences in sample characteristics and genetic disorders associated with other dopamine receptors.[12]

Several studies have found a number of factors causing schizophrenia based on genetic disorders that are associated with the hypothesis of dopamine DRD2 gene. The Dubertret 2003 study looked at the relationship of DRD2 Taq1 A2/A2 gene polymorphism in which there were significant differences in the A2 allele between schizophrenic and non-schizophrenic subjects.17 A study by Ohara et al. on other dopamine receptors such as DRD1 gene obtained from the postmortem Canadian Brain Tissue Bank (Toronto, Canada) found that the DNA sequence in schizophrenia was normal, however polymorphism was found in the gene.18 Meanwhile in a study conducted by Saiz et al., it was found that the ratio of the DRD3 gene polymorphism to the DAT SLC6A3 gene was a protective factor against schizophrenia.[7]

CONCLUSION

There was no significant association between genotype and allele distribution of DRD2 -141C

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Ins/Del gene polymorphism in subjects with paranoid schizophrenia with non-schizophrenia of Batak ethnicity in North Sumatera. Based on the odds ratio there was no difference in the prevalence of risk to schizophrenia in genotype distribution of heterozygous I/D and homozygous D/D as well as homozygous I/I variant and there was no difference in the prevalence of risk to susceptibility to schizophrenia in the frequency distribution of allele D or allele I.

REFERENCE

1. Falvo.D. Medical and Psychosocial Aspect of Chronics Illness and Disability, Jones Bartlett Publisher, Third Edition 2005, p:182-185
2. Weyandt.L, The pysiological bases of cognitive and behavioral disorders, LEA London [Internet]. 2006. [cited 2014 Juni 25];107-111.
3. Tsuang, M. Schizophrenia: Genes and environment. Biological Psychiatry. [Internet]. 2000. [cited 2014 Juni 25]: 47, 210–220 Available from:
4. Freedman, R. Schizophrenia. New England Journal of Medicine 2003, 349(18), 1738–1749
5. Baehr.M., Frotscher.M., Diagnosis Topik Neurologis Duus, Edisi 4 Penerbit Buku Kedokteran EGC Jakarta, 2007, 3-11.
6. Steiger.H, Bruce.K.R, Groleau.p., Neural circuits, neurotransmitters, and behavior: serotinine and temperament in bulimic syndromes. Curr Top Behav Neurosci 2011; 6: 125-38.
7. Saiz.P.A., Garcı´a-Portilla MP and Arango C. Genetic polymorphisms in the dopamine-2 receptor (DRD2), dopamine-3 receptor (DRD3), and dopamine transporter (SLC6A3) genes in schizophrenia: data from an association study. Prog Neuropsychopharmacol Biol Psychiatry 2010; 34: 26–31
8. Effendi.E., Loebis.B., Amir.N., Siregar.Y., Imunoreaktivitas neuregulin1 serum dan SNP8NNRG433E1006 gen neuregulin1 pada suku Batak

E-ISSN: 2528-410X

yang menderita Skizofrenia Paranoid, 2014, USU Institutional Repository.

9. Gejman PV, Sanders A and Duan J. The role of genetics in the etiology of schizophrenia. Psychiatr Clin North Am 2010; 33: 35–66.
10. Murray RM., Lappin J and Di Forti M. Schizophrenia: from developmental deviance to dopamine dysregulation. Eur Neuropsychopharmacol 2008; 18: S129-S134.
11. Bulayeva KB, Glatt SJ, Bulayev OA, et al. Genome-wide linkage scan of schizophrenia: a cross-isolate study. Genomics 2007; 89: 167-177.
12. Xiao.L, Functional -141C Ins/Del Polymorphism in The Dopamine Receptor Gene Promoter and Schizophrenia in a Chinese Han Population, Journal of International Medical Research. 2013 1171-1178
13. Breen G, Brown J, Maude S, et al. 141C ins/del polymorphism of the dopamine receptor 2 gene is associated with schizophrenia in a British population. Am J Med Genet 1999; 88: 407–410.
14. Arinami T, Gao M and Hamaguchi H. A functional polymorphism in the promoter region of the dopamine D2 receptor gene is associated with schizophrenia. Hum Mol Genet 1997; 6: 577–582.
15. Lafuente A, Bernardo M, Mas S, et al. 141C Ins/Del polymorphism of the dopamine D2 receptor gene is associated with schizophrenia in a Spanish population. Psychiatr Genet 2008; 18: 122–127.
16. Inada, T, Arinami, T, Yagi, G. Association between a polymorphism in the promoter region of the dopamine D2 receptor gene and schizophrenia in Japanese subjects: replication and evaluation for antipsychotic-related features. Int. J. Neuropsychopharmacol. 1999; 2: 181 – 186
17. Dubertret C et al, The 3Vregion of the DRD2 gene is involved in genetic susceptibility to schizophrenia. Schizophrenia research. 2004; 7: 75-85

E-ISSN: 2528-410X

18. Ohara K, Carla Ulpian, Philip Seeman, Roger K. Sunahara, Hubert H. M. Van Tol, Hyman B. Niznik. Schizophrenia: Dopamine Dl Receptor Sequence Is Normal, But Has DNA Polymorphisms. Neuropsychopharmacology 8:131-135, 199