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اصول تنظیم قراردادها

آموزش مهارت های کاربردی در تدوین و چاپ مقاله
Genetic Association Analysis of Dopamine DRD3 Ser9Gly Polymorphism and Schizophrenia in Malay Population

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Abstract
Background: Molecular components of the dopamine receptor (DRD3) play an important role in the pathophysiology of schizophrenia (SCZ). Previous studies have demonstrated an association between the DRD3 Ser9Gly polymorphism and SCZ but the results have been inconclusive.

Method: In this study, we investigated this controversial association between the Ser9Gly (A/G) polymorphism and SCZ using Malay cases-control (261 cases/157 controls) samples. PCR-RFLP was performed to genotype the distribution of the DRD3 Ser9Gly polymorphism.

Results: Both healthy control and SCHZ patient groups were in of Hardy-Weinberg equilibrium for the analyzed genetic variability. There was a significant association between the genotype distribution DRD3 polymorphisms and SCZ ($\chi^2 = 9.359; \text{df} = 2; P = 0.009$).

Conclusion: We believe that further studies are required to examine the association between others dopamine-related genes and the behavioral phenotypes of SCZ.

Keywords: Schizophrenia, Dopamine receptor, Single nucleotide polymorphism, Restriction fragment length polymorphism

Introduction
Schizophrenia (SCZ) is common but complex disease with worldwide lifetime risk of 1% (1). The Burden of Disease Study in 2004 showed that mental disorders contributed 8.6% of total disability-adjusted life year (DALY), ranking as the 4th leading cause of disease burden in Malaysia (2). From 2003 to 2005, a total number of 7351 cases of SCZ have been registered in Malaysia. The registered cases increased about 216 cases, from 2292 cases to 2508 cases between these 3 yrs. With the Malaysian population of 25 million, these numbers represent a very small proportion of the population, indicating that under-reporting cases may occur especially in East Malaysia (2).

Dysregulation in the dopaminergic system has been suggested to play an important role in the pathophysiology of SCZ (3). The dopamine D3 receptor (DRD3) is localized to limbic areas of the brain and involved in the reinforcing effects of emotional, cognitive and endocrine functions. It is thought to be implicated in SCZ and other neuropsychiatric disorders (4). It is well known that DRD3 may mediate the therapeutic actions of antipsychotic drugs (5). The DRD3 gene maps to chromosome 3q13.3 (6) and there is a polymorphic site in its first exon that leads to a serine to glycine amino acid substitution at position 9 (Ser9Gly) in the extracellular N-terminal domain of the receptor (7). Several case-control studies between the DRD3 Ser9Gly polymorphism and SCZ have been conducted and these studies have yielded variable results. Some studies suggested a significant association between the
DRD3 Ser9Gly polymorphism and SCZ (8-11), while others did not find any evidence of either excess of homozygosity, allelic or genotypic association (12-14). Even several meta-analyses reported that the DRD3 Ser9Gly polymorphism might not confer susceptibility to SCZ (3, 15-16). With the increased of variable results obtained from studies in both European and Asian populations, there is a need to examine the association between the DRD3 Ser9Gly polymorphism with SCZ in Malaysia. In the present study, we were interested in the genotyping the Malay population which is the major ethnic in Malaysia.

Materials and Methods
This case-control study involved 261 in-patients with SCZ (137 males; 124 females) recruited from Hospital bahagia, Ulu Kinta, Perak, Malaysia. The patients had a mean age of 46.5 (S.D. 13.6). All patients were evaluated using the Mini International Neuropsychiatric Interview (MINI). Patients with co-morbidity were excluded. A total of 157 volunteer control subjects (86 males; 71 females), with a mean age of 38.4 (S.D. 14.2) were recruited from blood donation centers at Universiti Tunku Abdul Rahman and Kuala Lumpur. All controls were free of any psychiatric illness, drug abuse and family history of psychiatric disorders. All participating subjects were unrelated, born in Malaysia and self-identified as being of Malay descent. This study was approved by the Medical Research Ethics Committee, Ministry of Health, Malaysia. Peripheral blood sample was obtained from each subject. Genomic DNA was prepared using the Promega Wizard DNA Isolation Kit (USA). The distribution of the DRD3 Ser9Gly polymorphism was determined by PCR-RFLP analysis. Amplification was performed with the following primers: 5’-GCTCATCTCAACTCTCACA-3’ and 5’-AAGTCTACTCACCTCGTA-3’ (17). The PCR products were then digested with 5 U MscI. DNA fragments were visualized by 2.5% agarose gel electrophoresis and stained with ethidium bromide. The fitness of genotype frequency distribution to the Hardy-Weinberg equilibrium (HWE) was tested using Arlequin version 3.11 (http://anthro.unige.ch/arlequin). Allelic and genotype frequency differences between patients and controls were analyzed using the Chi-square ($\chi^2$) test of the Statistical Package for the Social Sciences, version 12.0.

Results
PCR product of DRD3 and MscI restriction enzyme-digested PCR products are shown in Fig. 1. The distribution allelic and genotypic frequencies of DRD3 Ser9Gly in patients and controls are summarized in Table 1. The genotype distribution of the polymorphism was in HWE for both patients ($P$= 0.210) and controls ($P$= 0.179). There was no significant difference between the two groups regarding the allelic distribution ($\chi^2$ = 0.004; df = 1; $P$= 0.925; OR = 0.982 (95% CI = 0.545–1.769)). A significant genotype distribution between patients and controls was observed ($\chi^2$ = 9.359; df = 2; $P$= 0.009). However, no significant trend for an excess of Gly-containing genotype (Ser/Gly and Gly/Gly) was observed in patients compared with controls ($\chi^2$ = 0.325; df= 1; $P$ = 0.569; OR = 0.850 (95% CI = 0.486–1.487)). Besides, there was no evidence that Ser9Gly homozygosity served as a risk factor for SCZ ($\chi^2$ = 1.527; df = 1; $P$ = 0.217; OR = 1.422 (95% CI = 0.813–2.487)).

Table 1: Allelic and genotype distribution of DRD3 Ser9Gly polymorphism in patients and controls

|       | n  | Allele (%) | Genotype     |
|-------|----|------------|--------------|
|       |    | Ser     | Gly     | Ser/Ser | Ser/Gly | Gly/Gly |
| Schizophrenia | 261 | 347 (66.5) | 175 (33.5) | 120 (46.0) | 107 (41.0) | 34 (13.0) |
| Control       | 157 | 210 (66.9) | 104 (33.1) | 66 (42.0)  | 78 (49.7)  | 13 (8.3)  |
Fig. 1: PCR product of DRD3 and MscI restriction enzyme-digested PCR products

Lane 1: Low Range DNA Ladder (MassRuler™), Lane 2: PCR product of DRD3, Lane 3: Gly/Gly genotype (control)
Lane 4: Gly/Gly genotype (patient), Lane 5 and 6: Ser/Gly genotype (control), Lane 7: Ser/Gly genotype (patient)
Lane 8: Ser/Ser genotype (control), Lane 9: Ser/Ser genotype (patient)

Discussion

The results of the current case-control study suggested a significant difference in genotype distribution between patients and controls. This is in partial accordance with several case-control studies conducted among Asians and Caucasians. Hoogenboom et al. (18), Chang et al. (19) and Fathalli et al. (14) suggested negative association between allelic and genotype distributions with SCZ, while Utsunomiya et al. (3) found statistically significant evidence of such association.

Our inability to detect association between homozygosity with SCZ, as demonstrated in more homogenous Caucasian populations (8, 15) may due to genetic heterogeneity of the Malay population. Fathalli et al. (14) reported a lack of association between homozygosity with SCZ in Caucasians recruited from different countries. The issue of population stratification cannot be excluded even in the Japanese, which is considered a more homogenous ethnic (3). In Malaysia, ethnicity measures identity rather than origin and ancestry. Immigrants from Indonesian Archipelago have been absorbed into the Malay community (20). Thus, the measurement by self-identification suggested the possibility of population which may contribute to false positive and false negative (21). Despite of the limitation of ethnicity, this has merits in terms of clinical diagnosis. We can rule out other confounding factors, such as different diagnostic tools, or the use of inpatients or outpatients. All of the schizophrenic subjects were inpatients from one psychiatric hospital (Hospital Bahagia Ulu Kinta) and clinical records were complete.

Although our results are inconclusive to associate the DRD3 Ser9Gly polymorphism with SCZ, this gene cannot be excluded as an implication in SCZ. Talkowski et al. (16) reported that the Ser9 Gly polymorphism was associated with SCZ only when it was present in a common haplotype spanning intron 1 to the 3’ region of DRD3 gene but not when it was present on other haplotypes. Thus, further studies with linked variants for this region may contribute to SCZ susceptibility.

Ethical Considerations

Ethical issues including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc. have been completely observed by the authors.

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