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BDNF and *DARPP-32* genes are not risk factors for schizophrenia in the Malay population

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ABSTRACT. A number of studies have pointed to the association of *BDNF* (brain-derived neurotrophic factor) and *DARPP-32* (dopamine- and cAMP-regulated phosphoprotein, 32 kDa) with schizophrenia. The purpose of this study was to determine whether these two genes are involved in the pathogenesis of schizophrenia in the Malay population. Two single nucleotide polymorphisms Val66Met of *BDNF*, -2036C>G and g.1238delG of *DARPP-32* were genotyped in the Malay population in 200 patients with schizophrenia and 256 healthy controls. Analysis of allele and genotype frequencies in these two groups revealed no significant association of *BDNF* or *DARPP-32* polymorphisms with schizophrenia in Malays. This is the first such association study in the Malay population.

Key words: Schizophrenia; *BDNF*; *DARPP*; SNP; Restriction fragment length polymorphism

INTRODUCTION

A number of studies have proposed that disruption of monoaminergic pathways contributes to schizophrenia, particularly in the dopaminergic pathway (Murray et al., 2004). Therefore, genes involved in the dopamine pathway such as brain-derived neurotrophic factor (*BDNF*) and dopamine- and cAMP-regulated phosphoprotein, 32 kDa (*DARPP-32*) are biologically plausible candidates in schizophrenia susceptibility.

The *BDNF* gene is a mediator involved in neuronal survival and plasticity of dopaminergic, cholinergic, and serotonergic neurons in the central nervous system (CNS) (Angelucci et al., 2005). The most extensively studied SNP of this gene is rs6265, which produces a G/A amino acid substitution (valine to methionine) at codon 66 (Val66Met; Chen et al., 2006). This functional polymorphism may affect human memory and hippocampal function (Egan et al., 2003). Association studies between the functional SNP Val66Met and schizophrenia have generated conflicting results. A positive association was found in a family study in Italian subjects (Muglia et al., 2003). Modest associations were found in Caucasian (Neves-Pereira et al., 2005) and Chinese (Hong et al., 2003) populations, while three other studies reported negative findings (Kanazawa et al., 2007; Gratacòs et al., 2007; Xu et al., 2007).

DARPP-32 is a critical molecule in striatal neurons. It regulates the dopaminergic signaling pathway through phosphorylation of protein phosphatase-1 and protein kinase A (Fienberg et al., 1998). *DARPP-32* also plays an important role in the regulation of the glutamatergic signaling pathway, which is also thought to contribute to the development of schizophrenia (Nishi et al., 2005). Reduced expression of *DARPP-32* has been observed in the postmortem brain of schizophrenic patients (Albert et al., 2002). That is suggested to be related to the abnormalities in patients with schizophrenia, e.g., activation, neostriatal volume, and functional connectivity in the prefrontal cortex (Meyer-Lindenberg et al., 2007).

In view of increase in variable results obtained from studies in world populations, there is a need to examine the association between the *BDNF* Val66Met, *DARPP-32* -2036C>G and g.1238delG polymorphisms with schizophrenia in Malays, the major ethnic group in Malaysia. To the best of our knowledge, this is the first association study between the three above genes and schizophrenia in Malays.

MATERIAL AND METHODS

This case-control study involved all 200 Malay in-patients with schizophrenia (109 males; 91 females) recruited from Hospital Bahagia Ulu Kinta, Perak, Malaysia. The patients had a mean age of 44.7 years (SD = 11.3). All patients were evaluated using the Mini International Neuropsychiatric Interview (MINI). MINI is a brief structured interview for Axis I diagnosis of major psychiatric disorders in Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition (DSM-IV) and International Classification of Diseases-Tenth Edition (ICD 10). MINI has been validated by senior psychiatrists and compared against Structured Clinical Interview for DSM-III-R (SCID) and Composite International Diagnostic Interview (CIDI) (Sheehan et al., 1998). In addition, the interview has been validated in our hospital, and all of the interviewers are psychiatrists who have

good experience using MINI in clinical trials. Patients with co-morbidity were excluded. A total of 256 healthy control subjects (139 males; 117 females), with a mean age of 41.9 years (SD = 12.1) were recruited from blood donation centers at Universiti Tunku Abdul Rahman and around Kuala Lumpur. All controls were required to provide their medical history; only those who were free of any psychiatric illness, drug abuse and family history of psychiatric disorders were recruited. All participating subjects were unrelated, born in Malaysia and self-identified as being of Malay descent. Written informed consent was given by all participants. This study was approved by the Medical Research Ethics Committee, Ministry of Health, Malaysia.

A peripheral blood sample was obtained from each subject. Preparation of Genomic DNA was previously reported (Tee et al., 2010, 2011, 2012). Amplifications of *BDNF* Val66Met, *DARPP-32* -2036C>G and DARPP-32 g.1238delG were performed on a G-Storm (UK) thermal cycler in a total volume of 25 µL. Reaction mixtures contained 1X PCR buffer (i-DNA, Malaysia), 1.5 mM $MgCl_2$, 200 mM of each dNTP, 0.2 mM of each primer (i-DNA) (Table 1), 1 U *Taq* polymerase (i-DNA) and 20 ng genomic DNA. Amplifications of these three SNPs were carried out by an initial denaturation for 5 min followed by 35 cycles for 30 s of denaturation at 94°C, 30 s annealing at the temperature detailed in Table 1, and 30 s extension at 72°C, with a final extension for 5 min at 72°C. The distribution of the *BDNF* Val66Met and *DARPP-32* -2036C>G polymorphisms were determined by PCR-RFLP analysis. For genotyping, PCR products of *BDNF* Val66Met and *DARPP-32* -2036C>G were then digested with 5 U *Nla*III (Fermentas, Germany) and 5 U *Sty*I (Fermentas), respectively, at 37°C for 3 h. DNA fragments were subjected to electrophoresis on a 2.5% agarose gel. PCR products of *DARPP-32* g.1238delG were run on a 10% polyacrylamide gel and the variants were further determined by direct sequencing. Allelic and genotype frequency differences between patients and controls were analyzed using the chi-square (χ^2) test of the Statistical Package for the Social Sciences, version 12.0.

Table 1. SNPs *BDNF* and *DARPP-32* and their corresponding primers and annealing temperatures.

SNP	Primers	Annealing temperature
BDNF Val66Met	Forward: 5'-ACTCTGGAGAGCGTGAATGG-3' Reverse: 5'-CCGAACTTTCTGGTCCTCAT-3' (Takahashi et al., 2007)	50°C
DARPP-32 -2036C>G	Forward: 5'-GCTCCTATGGGCTCTGAGGT-3' Reverse: 5'-TCTTCTGGAATTGGGGTCAG-3' (Li et al., 2006)	60°C
DARPP-32 g.1238delG	Forward: 5'-CTTTGTGCATTTCCCTGGAG-3' Reverse: 5'-GCCCTTCCTCTCTCCAGTTT-3' (Li et al., 2006)	62°C

RESULTS

The distribution of allelic and genotypic frequencies of *BDNF* Val66Met and *DARPP-32* -2036C>G in controls and patients are summarized in Table 2. *DARPP-32* g.1238delG was monomorphic and thus was not further analyzed. There was no significantly different distribution of either allele or genotype frequencies for *BDNF* Val66Met between controls and patients, as well as for *DARPP-32* -2036C>G (Table 2).

Table 2. Genotype and allele frequencies for the SNPs in *BDNF* and *DARPP-32* genes.

SNPs	Allele (%)		Genotype (%)		
BDNF	G	A	GG	GA	AA
Patients	126 (31.5)	274 (68.5)	9 (4.5)	108 (54.0)	83 (41.5)
Controls	158 (30.9)	354 (69.1)	12 (4.7)	134 (52.3)	110 (55.0)
chi square (d.f.)	0.011 (1)		1.22 (2)		
P value	0.917		0.545		
OR (95%CI)	0.97 (0.53-1.76)				
DARPP-32 -2036C>G	C	G	CC	GC	GG
Patients	88 (22.0)	312 (78.0)	4 (2.0)	80 (40.0)	116 (58.0)
Controls	104 (20.3)	408 (79.7)	0 (0.0)	104 (40.6)	152 (59.4)
chi square (d.f.)	0.121 (1)		2.02 (2)		
P value	0.728		0.364		
OR (95%CI)	1.13 (0.57-2.23)				
DARPP-32 Exon 2	G	del	GG	G/del	del/del
Patients	400 (100.)	0 (0.0)	200	0 (0.0)	0 (0.0)
Controls	512 (100.0)	0 (0.0)	256	0 (0.0)	0 (0.0)

d.f. = degrees of freedom.

DISCUSSION

We found no association between the genes tested and schizophrenia. Previous association studies between *BDNF* Val66Met and schizophrenia have so far produced equivocal results. Hong et al. (2003) reported a significantly higher frequency of the GG genotype (18.2% for the GG genotype and 47.7% for the G allele) of the *BDNF* Val66Met in schizophrenic patients than in controls. However, in other association studies involving Polish (Skibinska et al., 2004) and Italian (Squassina et al., 2010) individuals, failed to find an association between this SNP and schizophrenia.

The meta-analysis based on Asian and Caucasian subjects further supported the non-association of this SNP with schizophrenia (Qian et al., 2007). Although we failed to detect any association of this SNP, we cannot exclude the possibility that there are still unidentified causal variants in or near the *BDNF* gene. Nanko et al. (2003) detected a significant association between SNP C270T in the 5'-non-coding region with schizophrenia in Japanese subjects. A recent meta-analysis indicated that the T allele of C270T and the *BDNF* Val66Met homozygous state were associated with this mental disorder (Jonsson et al., 2006).

Our results showed that *DARPP-32* -2036C>G was unlikely to be associated with the development of schizophrenia. These results are consistent with the study that examined Chinese patients (Li et al., 2006). Thus, *DARPP-32* -2036C>G is unlikely a major susceptible SNP for schizophrenia. However, our results for *DARPP-32* g.1238delG variants are contradictory. Li and co-workers (2006) found a significant difference in allele and genotype distributions between patients with schizophrenia and controls, whereas we could not detect any variants in both patients and controls. Although the present study showed no association of these two SNPs in the *DARPP-32* gene with schizophrenia, it is worthwhile to test this association in other SNPs, due to the multiple phosphorylation sites of *DARPP-32*, which are targets for many protein kinases and phosphatases (Svenningsson et al., 2002a,b).

Several limitations of the current study should be noted with regard to interpretation of our results. Our study did not recruit big samples and lacked haplotype analysis, which limited the power to detect an association with the disease. To conclude, our results indicate

that SNPs in the *BDNF* and *DARPP-32* genes do not play a major role in patients with schizophrenia. Further genotyping of other attractive candidate variants will be required for a more conclusive result.

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