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Research Article

Genetic association of single nucleotide polymorphisms in dystrobrevin binding protein 1 gene with schizophrenia in a Malaysian population

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Abstract

Dystrobrevin binding protein 1 (*DTNBP1*) gene is pivotal in regulating the glutamatergic system. Genetic variants of the *DTNBP1* affect cognition and thus may be particularly relevant to schizophrenia. We therefore evaluated the association of six single nucleotide polymorphisms (SNPs) with schizophrenia in a Malaysian population (171 cases; 171 controls). Associations between these six SNPs and schizophrenia were tested in two stages. Association signals with $p < 0.05$ and minor allele frequency > 0.05 in stage 1 were followed by genotyping the SNPs in a replication phase (stage 2). Genotyping was performed with sequenced specific primer (PCR-SSP) and restriction fragment length polymorphism (PCR-RFLP). In our sample, we found significant associations between rs2619522 (allele $p = 0.002$, OR = 1.902, 95%CI = 1.266 - 2.859; genotype $p = 0.002$) and rs2619528 (allele $p = 0.008$, OR = 1.606, 95%CI = 1.130 - 2.281; genotype $p = 6.18 \times 10^{-5}$) and schizophrenia. Given that these two SNPs may be associated with the pathophysiology of schizophrenia, further studies on the other *DTNBP1* variants are warranted.

Keywords: case-control study, *DTNBP1*, schizophrenia, single nucleotide polymorphism.

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Introduction

The dystrobrevin binding protein 1 (*DTNBP1*) gene, also known as dysbindin gene, is located at 6p22.3, a highly susceptible gene region of schizophrenia (Moises *et al.*, 1995; Straub *et al.*, 2002). It spans 140 kb along the chromosome and has 10 exons (Guo *et al.*, 2009). It is one of the genes thought to be pivotal in regulating the glutamatergic system. In the human brain, *DTNBP1* mRNA is predominantly expressed in the dorsolateral prefrontal cortex (DLPFC) and hippocampus (Weickert *et al.*, 2004; Tang *et al.*, 2009). Reduced *DTNBP1* expression (Talbot *et al.*, 2004; Weickert *et al.*, 2004, 2008; Tang *et al.*, 2009) was reported in schizophrenia patients. This reduction was inversely correlated with an increase of vesicular glutamate transporter 1 (VGluT-1) as a result of reduced lysosomal trafficking (Owen *et al.*, 2004; Talbot *et al.*, 2004). VGlut-1 served as the only vesicular glutamate transported in hippocampus (Kaneko *et al.*, 2002). The increased V-Glut-1 could be a counter action of the reduced glutamate neurotransmitter. A low glutamate levels or a deficiency of the glutamate receptors will cause a diminished activation of the glutamate receptors. Individuals of lower glutamate receptor activation have showed schizophrenia like symptoms (Konradi and Heckers, 2003; Reynolds *et al.*, 2005). Therefore, a lower expression of *DTNBP1* may cause glutamate dysfunction and lead to the alteration of glutamatergic neurotransmission.

Dysbindin has been implicated in cognitive performance in healthy (Luciano *et al.*, 2009) and schizophrenia cases (Burdick *et al.*, 2006; Fallgatter *et al.*, 2006). Genetic variants of *DTNBP1* have been associated with working memory (Donohoe *et al.*, 2007), IQ (Zinkstok *et al.*, 2007; Fatjovilas *et al.*, 2011) and execution function (Luciano *et al.*, 2009; Fatjovilas *et al.*, 2011). Along with the above findings, many case-control studies of *DTNBP1* and schizophrenia have been performed. Positive associations have been observed in family studies (Straub *et al.*, 2002; Tang *et al.*, 2003), as well as in case-controls studies (Schwab *et al.*, 2003; Voisey *et al.*, 2010). Nevertheless, many other studies failed to replicate the previous positive associations (Morris *et al.*, 2003; Li *et al.*, 2005; Pae *et al.*, 2009). A meta-analysis identified only weak association of one *DTNBP1* SNP with schizophrenia, which was not significant after multiple testing (Li and He, 2007). These discrepancies may indicate genetic heterogeneity of schizophrenia due to population differences. In Asia, case-control studies were conducted in Japan and Korea to investigate the association of *DTNBP1* with schizophrenia (Numa-

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kawa *et al.*, 2004; Joo *et al.*, 2006; Tochigi *et al.*, 2006; Pae *et al.*, 2009). Two of the studied SNPs (rs2619522 and rs2619528) were associated in the Japanese population (Numakawa *et al.*, 2004); however a replication of the two SNPs failed (Tochigi *et al.*, 2006). On the contrary, studies in Korea (Joo *et al.*, 2006; Pae *et al.*, 2009) showed consistent results for rs2619522, in which no association of rs2619522 with the Korean schizophrenia patients was reported. Information on other Asian ethnics appear to be lacking.

Malaysia is a multiethnic country where the population is comprised of three major ethnic groups, Malays, Chinese and Indians. Ethnic distribution of total prevalence schizophrenia cases from 2003 to 2007 showed that Malays accounted for the highest percentage (53%), followed by Chinese (30%) and Indians (9%) (Aziz *et al.*, 2008). There have not been any published reports on the DTNBP1 polymorphisms in Malaysians. We thereby investigated the genetic association between *DTNBP1* and schizophrenia in Malaysian subjects.

Materials and Methods

Subjects

The Malaysian sample consisted of 171 cases. The subjects were of mixed origin. Ethnicity and gender of the schizophrenia patients and control group are shown in Table 1. All schizophrenia cases are composed of in-patients recruited from the Hospital Bahagia Ulu Kinta (HBUK), Perak. The patients were hospitalized for more than three years. All of them met the Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition (DSM-IV) diagnostic criteria. They were selected and interviewed by trained psychiatrists using the Mini International Neuropsychiatric Interview (MINI). MINI is a brief psychiatric structured diagnostic interview for Axis Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition (DSM-IV) and International Classification of Diseases-Tenth Edition (ICD 10) psychiatric disorders. MINI has been validated by senior psychiatrists and compared against Structured Clinical Interview for DSM-III-R (SCID) and Composite In-ternational Diagnostic Interview (CIDI) (Sheehan *et al.*, 1998). Comorbidity of the patients was recorded during interview. Patients with comorbidity with other psychiatry disorders were excluded. The healthy controls were recruited from blood donation campaigns in various areas in Klang Valley. All controls were required to provide their medical history. The main inclusion criteria for controls were: free of any psychiatric illness, non drug abuser and without family history of psychiatric disorders. Written informed consent was obtained from all participants. This project was approved by the National Institutes of Health (NIH), Ministry of Health (MOH) Malaysia (KKM/NIHSEC/08/0804/P07-42, NMRR-11-189-8478).

Genotyping

Genotyping was performed in two stages. The subjects are comprised of 50 random patients and 50 controls in stage 1; stage 2 involved the addition of 121 patients and 121 controls on the existing participants in stage 1. Increasing the total sample size in stage 2 was done in an attempt to reduce the false-positive associations from the results of stage 1. Association signals with $p < 0.050$ and minor allele frequency > 0.050 in stage 1 were followed-up by genotyping the SNPs in the replication phase (stage 2).

Genomic DNA was isolated from 20 cc peripheral blood samples according to a standard phenol-chloroform method. Six single nucleotide polymorphisms (SNPs) were chosen in this study. Two genotyping approaches were used. Rs2619522, rs2619528 and rs1011313 were genotyped using the PCR-RFLP (restriction fragment length polymorphism) method (Buttner, 2009). Primer sequences are listed in Table 2. The PCR products were digested with restriction enzymes (Table 2) and incubated at the specific optimum temperature (Table 2) for 3 h. The digested products were analyzed on 3% agarose gel.

As there is no appropriate restriction site near the polymorphic sites of rs760761, rs3213207 and rs4236167, these three SNPs were genotyped using the PCR-SSP (sequence specific primer) method. Primer sequences (Table 2) were designed using Clone Manager Professional 9 (Scientific & Educational Software, USA), with the polymorphic sites located at the 3' end of the primers. A primer pair was incorporated in each PCR reaction to ensure reliable amplification. The primer sequences for this positive control were 5'-TCGTGGACGCCGTGATTCAGG-3' and 5'-AGGTCTGACAACGGGTCAGGCATG-3' (Nock *et al.*, 2006). PCR products were resolved on 2% agarose gel. Three PCR products were randomly selected from each genotype for direct sequencing to validate the reliability of each genotyping method.

Table 1 - Demographic characteristics of the schizophrenia patients and controls.

Gender	Male			Total	Female			Total
Ethnicity	Malay	Chinese	Indian		Malay	Chinese	Indian	
Patients	32	36	19	87	26	42	16	84
Controls	37	29	44	110	13	23	25	61

Table 2 - The SNP primer sequences, detected alleles for PCR-SSP, restriction enzyme, and PCR product sizes.

Genotyping method	SNP/Primer sequences 5'-3'	Detected Maj & Min alleles	RE	PCR product size (bp)	T_a (°C)
PCR-SSP	rs760761			189	61.0
	F:GTGTCTAATTTTTATCTTGTT**G**	G			
	R:GTACGGCTTCTTTATTAC				
	F:GTGTCTAATTTTTATCTTGTT**A**	(A)			
	R:GTACGGCTTCTTTATTAC				
PCR-SSP	rs3213207			157	56.5
	F:CTTCCTTTCGTAAAGCCA**A**	A			
	R:GTCACTTCTAAGTATACCCTG				
	F:CTTCCTTTCGTAAAGCCA**G**	(G)			
	R:GTCACTTCTAAGTATACCCTG				
PCR-SSP	rs4236167			378	64.0
	F:GACCTTCTGGGCGTGCTCTG				
	R:CACTGGAGTTAGTAGAAGGAC**G**	C			
	F:GACCTTCTGGGCGTGCTCTG				
	R:CACTGGAGTTAGTAGAAGGAC**A**	(T)			
PCR-RFLP	rs2619522		*BseGI*		55.8
	F:CTGTAACAGAGTCCACAG	C(A)		363	
	R:CCTGATACTTCTGACGTAG				
PCR-RFLP	rs2619528		*HpyCHIV*	306	45.0
	F:GGAACTAAAATTGAATGT	G(A)			
	R:GCACTTATATGATGTTCCTAG				
PCR-RFLP	rs1011313		*HpyCHIV*	140	61.0
	F:CAGGCTACAGAATGGATGTTAC	C(T)			
	R:GGCTGTATGAACAGAGTATCG				

Maj-Major, Min-Minor. RE-Restriction enzyme. Ta-annealing temperature. Bold letters represent the 3' end nucleotides corresponding to the major and minor alleles.

Statistical analysis

Hardy-Weinberg Equilibrium (HWE) testing was performed using Arlequin version 3.11 (Excoffier *et al.*, 2005). Allele and genotype frequencies were calculated and compared between patients and controls using a standard chi-square test (Statistical Package for the Social Science (SPSS) version 20, SPSS Inc., Chicago IL.). For subgroup analyses, patients were sub-divided according to subtypes of schizophrenia, gender and ethnicity. Residual and catatonic subtypes were not included due to limited case number (residual, n = 10, catatonic, n = 1). Three patients with unknown subtype were excluded for subgroup analysis according to schizophrenia subtype.

SNP data were converted into Haploview format using Microsoft-Excel (Chen *et al.*, 2009). The linkage disequilibrium (LD) and haplotype map were obtained using Haploview version 4.2 (Broad Institute of MIT and Harvard, Cambridge) (Barrett *et al.*, 2005).

Results

Allele and genotype frequencies of the respective SNPs in stage 1 are given in Table 3. Only two SNPs, rs1011313 (genotype p = 0.047) and rs2619528 (allele, p = 0.038; genotype, p = 0.004) showed significant association with schizophrenia among the six SNPs studied. Association analysis of rs2619522 showed a marginal allele difference between patients and controls (p = 0.060). Therefore, further screening was performed for rs2619522 in addition with rs2619528 and rs1011313 to eliminate the close proximity to a significant result.

In stage 2, genotype frequencies of both controls and patients were in HWE for rs1011313; whereas genotype frequencies in patients for rs2619528 and rs2619522 deviated from HWE (Table 4). The CC genotype of rs2619522 (p = 0.002) and the GG genotype of rs2619528 ($p = 6.18 \times 10^{-5}$) were significantly more abundant in patients, whereas positive association failed to be replicated for rs1011313.

The patients were further sub-classified into groups according to gender, ethnicity and subtypes. All these groups were compared with controls. Significant associations for rs2619522 and rs2619528 were observed in two subgroups, *i.e.* male patients and Malay patients. We detected interesting associations between *DTNBP1* variants and subtypes of schizophrenia. Positive associations (allele p = 0.019; genotype p = 0.036) were only demonstrated be-

Table 3 - Allele and genotype frequencies for the 6 SNPs of the *DTNBP1* gene in stage 1 consisting of 50 patients and 50 controls.

	Allele		Genotype			HWE		Allele		Genotype			HWE
rs760761	A	G	AA	AG	GG		rs2619522	A	C	AA	AC	CC	
SCHZ	0.07	0.93	0.00	0.14	0.86	1.00	SCHZ	0.12	0.88	0.00	0.24	0.76	1.00
CTR	0.07	0.93	0.02	0.10	0.88	0.20	CTR	0.22	0.78	0.04	0.36	0.60	1.00
x^2	0.000		1.345				x^2	3.544		4.141			
p-value	1.000	0.510					p-value	0.06		0.126			
OR (95%CI)	1.000 (0.337-2.963)						OR (95%CI)	0.483 (0.225-1.041)					
rs3213207	G	A	GG	GA	AA		rs2619528	A	G	AA	AG	GG	
SCHZ	0.17	0.83	0.00	0.34	0.66	0.32	SCHZ	0.42	0.58	0.24	0.36	0.40	0.08
CTR	0.14	0.86	0.00	0.26	0.74	0.58	CTR	0.28	0.72	0.02	0.52	0.46	0.08
x^2	0.627		0.762				x^2	4.308		10.970			
p-value	0.428		0.383				p-value	**0.038**		**0.004**			
OR (95%CI)	0.730 (0.334-1.595)						OR (95%CI)	1.862 (1.032-3.360)					
rs4236167	T	C	TT	CT	CC		rs1011313	C	T	CC	CT	TT	
SCHZ	0.23	0.77	0.08	0.30	0.62	0.25	SCHZ	0.19	0.81	0.00	0.38	0.62	0.18
CTR	0.14	0.86	0.00	0.28	0.72	0.57	CTR	0.10	0.90	0.00	0.20	0.80	1.00
x^2	2.686	4.408					x^2	3.267		4.934			
p-value	0.101	0.110					p-value	0.071		**0.047**			
OR (95%CI)	0.545 (0.262-1.133)						OR (95%CI)	2.111 (0.928-4.805)					

Significant p-values are shown in bold fonts. SCHZ-schizophrenia. CTR-control. HWE-Hardy-Weinberg Equilibrium.

tween rs2619522 and the patients of paranoid subtype, whereas significant associations between rs2619528 were shown in patients with the disorganized (allele p = 0.004; genotype p = 0.001) and the undifferentiated (genotype p = 0.013) subtypes. None of the subtypes were associated with rs1011313 (Table 5).

Intermediate to strong linkage was observed between rs2619528 and rs2619522 (D' = 0.667 and $r^2 = 0.284$) (Table 6). Two haplotypes, the G-C ($p = 6.0 \times 10^{-4}$) and A-A (p = 0.021) haplotypes (Table 7), were reported to be significantly associated with schizophrenia. The rare G-A haplotype was not investigated due to its low frequency (< 0.05). These two haplotypes were also found to be risk haplotypes in males, while the G-C haplotype (p = 0.001) was significantly associated with schizophrenia in the Malays.

Discussion

After the stage 1 genotyping, three SNPs, rs760761, rs3213207 and rs4236167, were eliminated. Rs760761 is located in between rs2619522 and rs2619528 and strong linkage between rs2619522-rs760761-rs2619528 has been reported (Stefanis *et al.*, 2007; Luciano *et al.*, 2009; Pae *et al.*, 2009). Although rs2619522 and rs2619528 were significant associated with schizophrenia in the Malaysians, rs760761 did not show any association. To date, the majority of case-control studies failed to show an association of rs3213207 with schizophrenia (Fallgatter *et al.*, 2006; Sanders *et al.*, 2008; Pae *et al.*, 2009). The present results also concur with the meta-analysis performed by Li and He (2007), reporting that rs3213207 is not associated with schizophrenia. Rs4236167 was not widely studied so far. The negative association found in the current preliminary study is supported by Sanders *et al.* (2008) and Strohmaier *et al.* (2010). To date, only a weak association was reported with schizophrenia (Voisey *et al.*, 2010). This indicates a low susceptibility of rs4236167 with schizophrenia.

A significant association of rs1011313 was observed in stage 1 genotyping, but the positive result could not be replicated in stage 2. Although rs1011313 was investigated in different populations (Funke *et al.*, 2004; Pae *et al.*, 2009; Strohmaier *et al.*, 2010), most studies showed a negative association. Our observation further indicated that rs1011313 is unlikely to confer risk of schizophrenia.

For rs2619522, our results conferred strong associations with schizophrenia. Specifically, the CC variant of rs2619522 was associated with increased schizophrenia risk in the Malaysians. The positive results are in line with previous reports (Funke *et al.*, 2004; Numakawa *et al.*, 2004), where the C allele conferred schizophrenia susceptibility. Moreover, association evidence of the C allele with lower memory and processing speed (Luciano *et al.*, 2009) and intelligence were reported (Zhang *et al.*, 2010). Due to a high rate (80%) of neurocognitive impairment in Malaysian schizophrenia patients (Ibrahim *et al.*, 2009), there is a possibility that schizophrenia patients with cognitive defects were included in this study. Our current study also revealed the significant association of rs2619522 in patients with the paranoid subtype. The result is supported by clini-

Table 4 - Allele and genotype frequencies for the 3 SNPs of the *DTNBP1* gene in stage 2 consisting of 171 patients and 171 controls.

	N	rs2619522						rs2619628						rs1011313					
		Allele		Genotype			HWE	Allele		Genotype			HWE	Allele		Genotype			HWE
All		C	A	CC	AC	AA		G	A	GG	AG	AA		C	T	CC	CT	TT	
SCHZ	171	0.87	0.13	0.78	0.19	0.03	**0.04**	0.80	0.20	0.69	0.21	0.10	**4.0 x 10^{-5}**	0.09	0.91	0.00	0.19	0.81	0.37
CTR	171	0.78	0.22	0.60	0.36	0.04	0.66	0.71	0.29	0.49	0.43	0.08	0.71	0.13	0.87	0.01	0.24	0.75	0.48
p-value		**0.002**		**0.002**				**0.008**		**6.18 x 10^{-5}**				0.178		0.289			
OR (95%CI)		1.902 (1.266-2.859)						1.606 (1.130-2.281)						0.718 (0.442-1.165)					
Male																			
SCHZ	87	0.92	0.08	0.84	0.16	0.00	1.00	0.82	0.18	0.72	0.18	0.10	**0.001**	0.09	0.91	0.00	0.18	0.82	1.00
CTR	110	0.76	0.24	0.56	0.40	0.04	0.43	0.71	0.29	0.48	0.46	0.06	0.24	0.14	0.86	0.01	0.26	0.73	0.69
p-value		**3.9 x 10^{-5}**		**1.25 x 10^{-4}**				**0.018**		**2.06 x 10^{-4}**				0.137		0.267			
OR (95%CI)		3.537 (1.887-6.633)						1.781 (1.099-2.884)						0.617 (0.326-1.170)					
Malay																			
SCHZ	58	0.91	0.09	0.81	0.19	0.00	1.00	0.77	0.23	0.67	0.19	0.14	**0.0001**	0.09	0.91	0.00	0.19	0.81	1.00
CTR	50	0.74	0.26	0.54	0.40	0.06	1.00	0.63	0.37	0.38	0.50	0.12	0.77	0.07	0.93	0.00	0.14	0.86	1.00
p-value		**0.001**		**0.005**				**0.028**		**0.002**				0.510		0.49			
OR (95%CI)		3.354 (1.560-7.208)						1.936 (1.071-3.499)						1.392 (0.518-3.738)					

Only significant results were shown. Significant p-values are shown in bold fonts. SCHZ-schizophrenia. CTR-control. HWE-Hardy-Weinberg Equilibrium.

Table 5 - Allele and genotype frequencies for the 3 SNPs of the *DTNBP1* gene in stage 2 consisting of 157 patients of 3 subtypes and 171 controls.

	N	rs2619522						rs2619628						rs1011313					
		Allele		Genotype			HWE	Allele		Genotype			HWE	Allele		Genotype			HWE
Disorganized																			
SCHZ	38	0.87	0.13	0.79	0.16	0.05	**0.41**	0.87	0.13	0.82	0.10	0.08	**0.01**	0.11	0.89	0.00	0.21	0.79	1.00
CTR	171	0.78	0.22	0.60	0.36	0.04	0.66	0.71	0.29	0.49	0.43	0.08	0.71	0.13	0.87	0.01	0.24	0.75	0.47
p-value		0.086		0.059				**0.004**		**0.001**				0.622		0.825			
OR (95%CI)		1.854 (0.909-3.781)						2.727 (1.348-5.518)						0.818 (0.368-1.819)					
Paranoid																			
SCHZ	71	0.87	0.13	0.77	0.20	0.03	**0.294**	0.77	0.23	0.63	0.27	0.10	**0.044**	0.09	0.91	0.00	0.18	0.82	1.000
CTR	171	0.78	0.22	0.60	0.36	0.04	0.660	0.71	0.29	0.49	0.43	0.08	0.711	0.13	0.87	0.01	0.24	0.75	0.474
p-value		**0.019**		**0.036**				0.178		0.056				0.284		0.499			
OR (95%CI)		1.935 (1.109-3.377)						1.365 (0.867-2.149)						0.701 (0.364-1.347)					
Undifferentiated																			
SCHZ	48	0.86	0.14	0.77	0.19	0.04	**0.18**	0.75	0.25	0.65	0.20	0.15	**0.003**	0.11	0.89	0.00	0.23	0.77	1.0
CTR	171	0.78	0.22	0.60	0.36	0.04	0.66	0.71	0.29	0.49	0.43	0.08	0.71	0.13	0.87	0.01	0.24	0.75	0.48
p-value		0.070	0.081			0.415	**0.013**			0.769	0.856								
OR (95%CI)		1.793 (0.947-3.395)						1.240 (0.739-2.080)						0.900 (0.445-1.821)					

Significant p-values are shown in bold fonts. SCHZ-schizophrenia. CTR-control. HWE-Hardy-Weinberg Equilibrium.

Table 6 - Pairwise linkage disequilibrium (LD) results.

	rs1011313	rs2618928	rs2619522
rs1011313	-	0.128	0.052
rs2618928	0.006	-	0.667
rs2619522	0.002	0.284	-

D' values are shown in the right upper diagonal. r^2 values are shown in the left lower diagonal.

cal findings showing that the patients with the paranoid subtype had significantly worse results in memory, language, and execution functions compared to controls (Dillon *et al.*, 2007).

For rs2619528, this is the first study in Asia that reported the G carriers showing higher risk of schizophrenia. The prefrontal cortex region connects and facilitates the implementation of cognition control and intelligence (Cole *et al.*, 2012), and the GG genotype has been associated with prefrontal brain function disturbance (Fallgatter *et al.*, 2010). Our study also showed significant association between the GG variant and schizophrenia in patients of disorganized and undifferentiated subtypes. This significant result may have been due to the cross-sectional relationships between the disorganized symptoms and cognitive deficits. A meta-analysis reported that disorganized symptoms were associated with majority cognitive domains, such as executive control, verbal fluency, attention, verbal learning and memory (Dominguez *et al.*, 2009). The significant evidence in undifferentiated subtype patients was probably due to the partial disorganized symptoms observed in these patients. Patients with undifferentiated subtype may display partial disorganized symptoms, but the intensity and duration of the disorganized symptoms may not be sufficient that they are classified as of the disorganized subtype (American Psychiatric Association, 1994). Notwithstanding, the findings might be biased due to the instability in the diagnosis of subtypes, where the patients of different subtypes have not been showing distinctive pattern of treatment responses or longitudinal courses.

We also observed a gender dimorphism, where the C allele (rs2619522) and G allele (rs2619528) increased the risk of schizophrenia in male patients. Up till date it is not yet clear, however, how rs2619528 and rs2619522 may increase the risk of schizophrenia in males. The association in male patients may probably be due to differences in brain structure for the two genders, as well as differences in neural lateralization (Antonova *et al.*, 2004) that caused the male patients to exhibit deficits in clinical and cognitive domains (Han *et al.*, 2012). Studies revealed that male patients showed more cognitive impairments than females (Seidman *et al.*, 1997; Goldstein *et al.*, 1998). As discussed, the DTNBP1 variants may be associated with cognitive ability (Zhang *et al.*, 2010). We therefore infer that the male patients in this study could have been cognitively more impaired.

In addition to the above findings, our results showed positive associations of rs2679522 and rs2619528 with schizophrenia in Malays only. It is worthy of note that two significant SNPs, rs2619522 and -1438 G/A were associated with short term verbal memory in schizophrenia patients (Alfimova *et al.*, 2010). Moreover, in a previous study, -1438 G/A of the serotonin 2A receptor gene (5-

Table 7 - Haplotype analysis for rs2619528-rs2619522.

rs2619528 - rs2619522	Haplotype frequency	Frequency		x^2	p-value
		Patient	Control		
All patients					
G-C	0.708	0.767	0.649	11.640	**6.000×10^{-4}**
A-A	0.131	0.101	0.160	5.373	**0.021**
A-C	0.118	0.104	0.132	1.289	0.256
G-A	0.043	0.028	0.059	-	-
Male					
G-C	0.718	0.812	0.644	13.64	**2.000×10^{-4}**
A-A	0.127	0.076	0.166	7.077	**0.008**
A-C	0.114	0.107	0.120	0.155	0.694
G-A	0.041	0.004	0.007	-	-
Malay					
G-C	0.664	0.760	0.552	10.45	**0.001**
A-A	0.164	0.145	0.188	0.727	0.394
A-C	0.132	0.089	0.182	4.156	0.042
G-A	0.039	0.007	0.078	-	-

Bold fonts represent significance at $p < 0.050$. x^2 - chi square.

HTR2A) was significantly associated with schizophrenia in the Malays (Tee *et al.*, 2010). These evidences raise the possibility that the Malay patients in this study were cognitively more impaired than patients of other ethnic groups.

The strong linkage between rs2619528 and rs2619522 denoted in this study is well supported by previous reports (Numakawa *et al.*, 2004; Li *et al.*, 2005; Tochigi *et al.*, 2006; Luciano *et al.*, 2009). Our results indicate that the location of these two SNPs is likely to be a region for high schizophrenia susceptibility, as rs2619522 has been associated with schizophrenia (Funke *et al.*, 2004; Numakawa *et al.*, 2004). These two intronic SNPs could be in linkage disequilibrium with a functional SNP that has yet to be identified.

Based on the results of the Hardy-Weinberg Equilibrium test, the genotype distributions were in HWE for both patients and controls for rs1011313. On the other hand, deviation of HWE was observed in patients for rs2619528 and rs2619522. Frequently, deviation from HWE is an indication of possible errors in genotyping (Xu *et al.*, 2002; Hosking *et al.*, 2004), population stratification (Cardon and Palmer, 2003; Freedman *et al.*, 2004) and biasness (Schaid and Jacobsen, 1999). In this study, however, there was no genotyping error, as our RFLP results were validated through sequencing. Population stratification is a form of confounding that arises when cases and controls are sampled from genetically distinct populations (Schork *et al.*, 2001). Genotype distributions in our controls were in HWE, suggesting minimal stratification. Population stratification might exist in patients. Our patients, who had an average 8.67 years of hospitalization, were recruited from a small pool of inpatients in one hospital. Lastly, HWE testing is typically conducted in patients, and deviation in cases may indicate an association between the genotype and case-control status (Feder *et al.*, 1996; Nielsen *et al.*, 1999). Therefore, this may explain the deviation from HWE in patients for rs2619522 and rs2619528.

Limitations of this study are due to the relatively small sample size, and schizophrenia symptoms and cognitive ability that were not assessed during sample recruitment. A larger sample size would likely increase the statistical power of our results. Nonetheless, all patients were hospitalized for at least eight years. Their inpatient status minimizes discrepancy in diagnosis and clinical measures. Since schizophrenia is a multigenic disorder, further studies are needed to investigate the impact of other variants of *DTNBP1* and other genes.

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