Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

Linkage of schizophrenia with *TPH2* and *5-HTR2A* gene polymorphisms in the Malay population

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Genet. Mol. Res. 9 (3): 1274-1278 (2010)
Received March 10, 2010
Accepted May 10, 2010
Published July 6, 2010
DOI 10.4238/vol9-3gmr789

ABSTRACT. The serotoninergic system has been implicated in the etiology of schizophrenia and other behavioral disorders. Association studies have focused on the tryptophan hydroxylase 2 gene (*TPH2*) and the 5-hydroxytryptamine receptor 2A gene (*5-HTR2A*). We genotyped two single-nucleotide polymorphisms, A1438G of *5-HTR2A* and intronic rs1386494 of *TPH2* in the Malay population, using a sample size of 289 schizophrenic patients and 130 healthy controls. We found a significant association of A1438G of *5-HTR2A* with schizophrenia in Malays. On the other hand, *TPH2* polymorphism was not associated with schizophrenia. This is the first genetic association study concerning schizophrenia in the Malay population.

Key words: Schizophrenia; Serotonin; *5-HTR2A*; *TPH2*; Single-nucleotide polymorphism

INTRODUCTION

Schizophrenia (SCZ) is a prevalent psychiatric disorder affecting approximately 1% of the worldwide population (Schwab and Wildenauer, 2008). From 2003 to 2005, a total of 7351 cases of SCZ, with 3714 incident cases have been registered in Malaysia. The registered cases increased from 2292 cases in 2003 to 2508 cases in 2005. With a Malaysian population of 25 million, these numbers represent a very small proportion of the population, indicating that under-reporting cases may occur (Anonymous, 2003-2005; National Mental Health Registry Report).

Serotonin (5-hydroxytryptamine, or 5-HT) dysfunction has been implicated in the pathogenesis of SCZ (Chen et al., 1992). The serotonin system has a central role in the circuitry of cognition and emotions, where psychobiological domains are usually altered in SCZ (Riedel et al., 2003). 5-Hydroxytryptamine receptor 2A (5-*HTR2A*), one of the serotonin receptors, has been reported to be upregulated in the brain or platelets of schizophrenic patients (Arango et al., 1997). Tryptophan hydroxylase 2 (*TPH2*), also known as neuronal tryptophan hydroxylase, is the key rate-limiting enzyme in the synthesis of serotonin (Fitzpatrick, 1999). Low turnover rate of serotonin is associated with impaired impulse control (Nielsen et al., 1998).

The involvement of a genetic risk factor in SCZ is supported by family, twin and adoption studies (Prasad et al., 2009). The human *TPH2* gene is located on chromosome 12, comprises 11 exons and covers a region of about 93.5 kb (Zill et al., 2004a). Several genetic studies showed an association between *TPH2* and suicidal behavior (Zill et al., 2004a; Li et al., 2006) and major depression (Zill et al., 2004b, 2007) and panic disorder (Maron et al., 2007). However, contradicting results were found for mood disorder (Mann et al., 2008). *5-HTR2A* is located on chromosome 13 and contains three exons and two introns spanning 20 kb (Sparkes et al., 1991; Chen et al., 1992). Polymorphisms such as T102C (Virgos et al., 2001; Abdolmaleky et al., 2004) and A1438G (Sáiz et al., 2007) of the *5-HTR2A* gene have been proposed as candidate markers in SCZ.

An association study of SCZ in relation to *TPH2* and *5-HTR2A* has not been conducted in Malays from Malaysia. Anthropologically, Malaysia is a melting-pot of various ethnic groups due to its geographical location at the crossroads of maritime trade between the West and the East (Andaya and Andaya, 1982). The registered schizophrenic patients in Malaysia were predominantly Malays (54%) followed by Chinese (28%) and Indians (9%), which are reflective of the Malaysian population. Thus, we decided to conduct an association study on intronic single-nucleotide polymorphism (SNP) rs1386494 A/G (*TPH2*) and A1438G *5-HTR2A* using samples of Malays, the major ethnic group in Malaysia.

MATERIAL AND METHODS

This case-control study involved 289 inpatients with SCZ (188 males; 101 females) recruited from the Ulu Kinta Bahagia Hospital, Perak, Malaysia. The patients had a mean age of 45.5 years (SD = 13.6). They were diagnosed as having SCZ using the Mini-International Neuropsychiatric Interview (MINI). Patients with co-mobidity were excluded. The 130 volunteer control subjects (73 males; 57 females) were recruited from the blood donor centers at the Universiti Tunku Abdul Rahman and Kuala Lumpur with a mean age of 36.8 years (SD = 11.2). All controls were free of any psychiatric illness. The other exclusion criteria included drug abuse and family history of psychiatric disorders. All 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, and informed consent was obtained from all subjects. A peripheral blood sample was then obtained from each subject for extraction of genomic DNA.

Genomic DNA extraction was performed using a commercial DNA isolation kit (Wizard, Promega). Two SNPs (*TPH2* and *5-HTR2A*) were genotyped with polymerase chain reaction-based restriction fragment length polymorphism analysis, and the detailed information is described in Table 1. Allele and genotype frequencies for the two SNPs in the study groups were assessed for deviation from Hardy-Weinberg equilibrium (HWE) using Arlequin 3.11 (http://anthro.unige.ch/arlequin). The chi-square test was carried out to compare the allelic and genotypic SNP frequencies between patients and controls using the SPSS V.12.0 statistical package.

Table 1. Single-nucleotide polymorphisms (SNPs) and their corresponding restriction enzymes.

SNP	Gene	Primers	Restriction enzyme	Allele
rs1386494	*TPH2*	Forward: GTGACAGAACTAAGTGACTTG G Reverse: GATATGCTAGTCCTCTGTTGG	*Hpa*II	A/G
A1438G	*5-HTR2A*	Forward: ACTGCGAAACCAACTTATTTCC Reverse: TTGTGCAGATTCCCATTAAGG	*Hpa*II	G/A

RESULTS

We analyzed the rs1386494 and A1438G polymorphisms in 289 patients and 130 controls. The distributions of the genotype and allele frequencies of these two SNPs in patients and controls are summarized in Table 2. The genotype distributions of these two loci were in HWE for both patients and controls. The results of the chi-square analysis showed that there were no significantly different distributions of either allele (P = 0.235, OR = 0.62, 95%CI = 0.28-1.37) and genotype (P = 0.116) frequencies for rs1386494 between the two groups. On the other hand, there were statistical differences in allele (P = 0.008, OR = 0.46, 95%CI = 0.25-0.82) and genotype (P = 0.004) frequencies between patients and controls for A1438G. These significant differences were due to a higher G allele frequency in patients (46.4%) than controls (27.7%). Thus, the genotype frequencies of AG and GG were higher in patients than controls (Table 2).

Table 2. Genotype and allele frequencies for the single-nucleotide polymorphisms (SNPs) rs1386494 of *TPH2* gene and A1438G of *5-HTR2A* gene.

SNP	Allele (%)		Genotype (%)			HWE
	A	G	AA	AG	GG	P
rs1386494 (*TPH2*)						
SCZ	70 (12.1)	508 (87.9)	7 (2.4)	56 (19.4)	226 (78.2)	0.0928
Control	46 (17.7)	214 (82.3)	2 (1.5)	42 (32.3)	86 (66.2)	0.3949
Chi-square (d.f.)	1.41 (1)		4.31 (2)			
P	0.235		0.116			
OR (95%CI)	0.62 (0.28-1.37)					
A1438G (*5-HTR2A*)						
SCZ	310 (53.6)	268 (46.4)	103 (35.6)	104 (36.0)	82 (28.4)	0.0000
Control	188 (72.3)	72 (27.7)	76 (58.5)	36 (27.7)	18 (13.8)	0.0000
Chi-square (d.f.)	6.95 (1)		11.23 (2)			
P	0.008		0.004			
OR (95%CI)	0.46 (0.25-0.82)					

SCZ: patients with schizophrenia. d.f. = degrees of freedom.

DISCUSSION

TPH2 has become a major candidate for association studies in psychiatry due to its predominant expression in the brain. Rs1386494 of *TPH2* has been associated with major depression (Zill et al., 2004a), treatment-resistant depression (Anttila et al., 2009) and suicidal behavior (Zill et al., 2004b). However, we found a negative association between rs1386494 of *TPH2* and SCZ in this study. Our finding showed an increased frequency of the G allele in schizophrenic patients. This finding is similar to the results of the study by Zill et al. (2004a,b) which showed a greater prevalence of the G allele in major depression patients and suicide victims. However, we can rule out the role of suicidal behavior in obscuring our finding because only 1% of the patients showed a suicidal tendency.

In the present study, we successfully identified significant associations at the allele and genotype levels in the Malay population. A similar genotype distribution has been observed in Asturian (Northern Spain) patients (Sáiz et al., 2007). A significant association of the A1438G allele and SCZ was found in Malays in our study, as in Spanish (Peñas-Lledó et al., 2007) and North Indians (Semwal et al., 2002). However, our results show some discrepancies with the finding of Sáiz et al. (2007). First, the -1438G allele was significantly less frequent in the Asturian patients. Second, the -1438A allele and AA genotype were more frequent in Malay controls. These discrepancies indicate heterogeneity between different ethnic groups.

This association study had limitations. In Malaysia, ethnicity is based on identity rather than origin and ancestry. Immigrants from the Indonesian Archipelago have been absorbed into the Malay community (Nagaraj et al., 2008). Thus, the assessment by self-identification suggested the possibility of population stratification, which may contribute to false positive and false negatives (Schulze and McMahon, 2002). Nevertheless, genotype distributions in our control and patients were in HWE, suggesting minimal stratification. Despite the limitation of ethnicity, this study has merits in terms of clinical diagnosis. All schizophrenic subjects were inpatients from one psychiatric hospital (Bahagia Ulu Kinta Hospital). There was no discrepancy in phenotype definition or assessment of SCZ, and documentation of clinical records was complete.

ACKNOWLEDGMENTS

Research supported by Universiti Tunku Abdul Rahman (UTAR, Research Grants #6200/L02, #6202/T02 and #6202/C01). We are very grateful to Dato' Dr. Suarn Singh Jasmit Singh, Director and senior psychiatrist of Ulu Kinta Hospital Bahagia and also all other psychiatrists (Yee Chuang Cheah, Rabaiah Mohd Salleh, Tak Wah Loo, Raziffah Abdul Rahman, Satnam Kaur Harbhajan Singh, Zulkifri Ghaus, Ahmad Syukri Chew Abdullah, Bilbir Kaur Chigara Singh) who participated in patient diagnosis and blood sample collection. We would like to thank the UTAR undergraduate research team for the extraction of genomic DNA.

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