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Brief communication (Original)

Association study of the tryptophan hydroxylase 1 gene with major depressive disorder in three ethnic groups of the Malaysian population

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Background: Alterations of the serotonergic neurotransmission system have been suspected to be involved in the pathogenesis of neuropsychiatric disorders including major depressive disorders (MDD). Tryptophan hydroxylase (TPH) 1 gene has been proposed as a candidate gene for MDD in Caucasian and Chinese populations from different countries. However, there is no comprehensive study of TPH1 gene in the MDD samples from Malaysia.
Objective: We examined the possible association between the two intronic polymorphisms, A218C (rs1800532) and A779C (rs1799913), and MDD in the three main ethnic groups of the Malaysian population.
Methods: We enrolled and genotyped 265 unrelated patients and 332 unrelated healthy subjects using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and amplification refractory mutation system-polymerase chain reaction (ARMS-PCR).
Results: We observed no significant association in the genotype and allele frequencies between the MDD patients and control subjects for either of the tested polymorphisms, whereas the linkage disequilibrium (LD) among the three ethnicities and combined samples were strong ($D' > 0.8$).
Conclusion: In summary, our preliminary study suggests that the TPH1 gene may be associated with the MDD in the Indian population. However, further studies using larger sample sizes are necessary in order to verify this result. The ability of obtaining the MDD patient's genotypic data, in addition to the diagnosis of the myriad of symptoms, will most definitely be able to assist clinicians to better care, manage, and treat their patients.

Keywords: Genetic polymorphism, Malaysia, MDD, PCR-RFLP, TPH1

Tryptophan hydroxylase (TPH) is a rate-limiting enzyme, which is involved in the biosynthesis of the serotonin [1]. Considerable evidences have shown that the TPH gene, which encodes for TPH, is a possible candidate involved in the etiology of major depressive disorder (MDD) [2, 3]. Two isoforms of the TPH gene have been identified thus far, i.e., TPH1 and TPH2 [4]. However, contradictory results have been observed when determining which TPH genes are predisposes to MDD [5, 6].

The TPH1 gene, mapped on chromosome 11p15.3-p14, is about 29 kb long and contains 11 exons [7]. Several investigators have pointed out that the TPH1 gene may be associated to a number of psychiatric disorders and behavior traits such as alcoholism [8], impulsive-aggression [9], self-harm [10], generalized anxiety disorder [11], schizophrenia [12], MDD [13, 6], bipolar disorder [14] and suicidal behavior [15, 16]. However, the findings between the TPH1 gene and these psychiatric disorders are largely inconsistent.

The aim of this study was to investigate whether the two best known polymorphisms, A218C (rs1800532) and A779C (rs1799913), located on intron seven of the TPH1 gene, were associated with MDD in the three main ethnic groups of the Malaysian population.

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Materials and methods

Subjects

This study was approved by the Ethics Committee of the University of Malaya Medical Centre (UMMC) reference no. 690.22. The patient group composing of 265 unrelated subjects (59 Malay, 129 Chinese, and 77 Indian) with MDD, who met the Diagnostic and Statistical Manual of Mental Disorder criteria, fourth edition, was recruited from the UMMC Psychiatry Clinic, within a period of 16 months, between September 2007 and December 2008. Patients with other coexisting psychiatric disorders such as schizophrenia, epilepsy, etc., were excluded. Three hundred thirty two ethnically matched healthy subjects (110 Malay, 129 Chinese, and 93 Indian) were drawn from the general population of Malaysia. Most of the healthy participants were students, who studied in the University of Malaya, while the rest were recruited among local blood donors. None of the individuals in the control group suffered from any psychiatric symptoms during the time of blood collection. All of them were questioned for personal and family history of neuropsychiatric disorders by trained medical doctors before blood was sampled. Demographic characteristics of the MDD patients and control subjects are summarized in **Table 1**.

The ethnicity of each patient and control was confirmed through the skin color and morphological features. Individuals, whose parents or grandparents were of mixed marriage, were excluded from the study. All individuals and their parents were Malaysian. Informed consent was obtained from all MDD patients and healthy subjects after the procedures of the study had been fully explained.

Genotyping

Blood was drawn from each subject in ethylenediamine tetraacetic acid tubes. Genomic DNA was extracted from whole blood according to the manufacturer's protocol of QIAamp® DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany). Genotyping of the A218C polymorphism was carried out using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) as previously described [14] with minor modifications.

Meanwhile, the A779C polymorphism was screened via amplification refractory mutation system-polymerase chain reaction (ARMS-PCR), based on a study by Liu and colleagues (2006) [16]. All samples were reanalyzed to ensure the accuracy of the genotyping results, so that no discrepancies were detected.

Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 16.0 for Windows. The mean ages for the patient and control groups of the three ethnic groups were compared using Levene's Test for Equality of Variances. The chi-squared (2) test was used to compare the gender distribution, determine the allele and genotype frequencies between patient and control groups, as well as the presence of Hardy-Weinberg equilibrium (HWE). Differences were considered significant at $p < 0.05$. The linkage disequilibrium (LD) pattern was assessed using Haploview software version 4.1 [17]. In addition, haplotype frequency estimation and comparisons between the MDD patients and healthy controls were performed using

Table 1. Demographic data for all subjects according to their ethnicities and overall grouping

Ethnic group	Female	Male	Age (mean standard deviation)	Total
Malay				
Healthy Controls	55 (50.0%)	55 (50.0%)	22.64±4.40	110
MDD Patients	34 (57.6%)	25 (42.4%)	39.45±12.76	59
Chinese				
Healthy Controls	45 (34.9%)	84 (65.1%)	29.41±9.75	129
MDD Patients	81 (62.8%)	48 (37.2%)	43.11±13.11	129
Indian				
Healthy Controls	34 (36.6%)	59 (63.4%)	33.59±13.11	93
MDD Patients	46 (59.7%)	31 (40.3%)	46.86±12.26	77
Combined samples				
Healthy Controls	134 (40.4%)	198 (59.6%)	27.79±9.71	332
MDD Patients	161 (60.6%)	104 (39.2%)	43.31±13.01	265

SHEsis program [18]. Haplotype frequencies of less than 3% in the MDD patients and healthy controls were excluded.

Results

The genotype distributions of the TPH1 A218C and A779C polymorphisms were noted to be in HWE in the healthy subjects of the three ethnicities and combined samples, except for the A779C polymorphism in the Chinese ($p = 0.041$). This might be due to the selection bias as most of the control subjects were young university students [19].

No statistical significance was observed between the MDD patients and control subjects in the genotype and allele frequencies of the three ethnicities and combined samples, with regards to the two intronic polymorphisms (**Table 2**). Our group also performed gender analysis in the combined samples, but no significance was found either (data not shown). However, our analyses demonstrated that the A allele was slightly higher in depressed females, with frequencies of A218 and A779 recorded at 0.404 and 0.407, respectively. Conversely, the 218C (0.606) and 779C (0.591) alleles were more common in the depressed males compared with the healthy subjects.

The A218C and A779C polymorphisms were in strong LD in the three ethnicities. The LD of Malays ($D' = 0.860$) was much weaker if compared with the Chinese ($D' = 0.916$) and Indians ($D' = 0.912$), which were almost in complete LD. Furthermore, D' value of 0.866 indicated that the two intronic polymorphisms were also in strong LD in the combined samples. Our findings are consistent with Nielsen and coworkers' study based on seven populations who reported D' values ranging from 0.838 to 1.00 [20].

Table 3 shows the estimated haplotype frequencies of the MDD patients and control subjects of three ethnicities and combined samples. The overall frequency comparisons revealed a borderline significance ($p = 0.0495$) in the Indians. As for the individual haplotype association test, four common haplotypes (AA, AC, CA and CC) were constructed and their frequencies were compared between the two samples. Significant differences were observed for the AC and CA haplotypes in the Malays ($p = 0.019$) and Indians ($p = 0.007$), respectively.

Table 2. Distribution of genotype and allele frequencies of A218C and A779C polymorphisms of the TPH1 gene between the MDD patients and healthy controls of three ethnicities and combined samples

Markers	Malay		Chinese		Indian		Combined	
	Patients	Controls	Patients	Controls	Patients	Controls	Patients	Controls
A218C								
Genotype								
A/A	0.169	0.173	0.171	0.233	0.104	0.118	0.151	0.181
A/C	0.424	0.482	0.597	0.535	0.390	0.441	0.498	0.491
C/C	0.407	0.345	0.233	0.233	0.506	0.441	0.351	0.328
P-value	0.713		0.434		0.695		0.601	
Allele								
A	0.381	0.414	0.469	0.500	0.299	0.339	0.400	0.426
C	0.619	0.586	0.531	0.500	0.701	0.661	0.600	0.574
P-value	0.564		0.481		0.431		0.361	
A779C								
Genotype								
A/A	0.203	0.145	0.163	0.186	0.091	0.097	0.151	0.148
A/C	0.458	0.582	0.581	0.589	0.442	0.409	0.513	0.533
C/C	0.339	0.273	0.256	0.225	0.468	0.495	0.336	0.319
P-value	0.294		0.793		0.911		0.884	
Allele								
A	0.432	0.436	0.453	0.481	0.312	0.301	0.408	0.414
C	0.568	0.564	0.547	0.519	0.688	0.699	0.592	0.586
P-value	0.941		0.537		0.833		0.818	

Table 3. Estimated haplotype frequencies for A218C and A779C alleles in the patient and control groups of the three ethnicities

Ethnic group	Haplotype[a]		Frequency (patients)		Frequency (controls)		p^b	O.R (95% CI)
	A	A	0.381		0.368		0.811	1.058 (0.667-1.678)
Malay	A	C	0.000		0.046		0.019	0.000 (0.000-0.000)
	C	A	0.051		0.068		0.529	0.732 (0.276-1.940)
	C	C	0.568		0.518		0.383	1.222 (0.779-1.916)
	All haplotypes			d.f. = 3		χ^2 = 6.124		0.106
	A	A	0.434		0.461		0.532	0.895 (0.630-1.269)
Chinese	A	C	0.035		0.039		0.815	0.896 (0.358-2.244)
	C	A	-		-		-	-
	C	C	0.512		0.481		0.477	0.840 (0.801-1.601)
	All haplotypes			d.f. = 2		χ^2 = 0.515		0.773
	A	A	0.273		0.301		0.566	0.871 (0.542-1.397)
Indian	A	C	0.026		0.038		0.545	0.682 (0.196-2.374)
	C	A	0.039		0.000		0.007	0.000 (0.000-0.000)
	C	C	0.662		0.661		0.984	1.005 (0.640-1.578)
	All haplotypes			d.f. = 3		χ^2 = 7.836		0.0495
	A	A	0.376		0.394		0.762	0.964 (0.762-1.220)
Combined	A	C	0.025		0.042		0.096	0.571 (0.293-1.114)
samples	C	A	0.032		0.030		0.846	1.067 (0.553-2.058)
	C	C	0.568		0.702		0.402	1.108 (0.877-1.388)
	All haplotypes			d.f. = 3		χ^2 = 3.077		0.380

[a]Haplotypes with a frequency <3% in patient and control groups are not shown. [b]Significant p <0.05 are in italics bold

Discussion

To the best of our knowledge, this is the first study examining the association of MDD and the TPH1 gene in the Malaysian population. In the present study, we failed to detect any association between the A218C and A779C polymorphisms with MDD in the three ethnicities and combined samples. Our findings are in accordance with several studies based upon the Caucasian populations [2, 21-23]. Nielsen and coworkers showed that the A218C polymorphism is located on the GATA transcription factor binding site. Therefore, it is possible it will alter the processing of the premessenger RNA, and subsequently modify the gene expression [20]. The A779C polymorphism, on the other hand, is located on a polypyrimidine stretch, upstream of the 3' acceptor splice site, which forms a consensus sequence [20]. Transversions of pyrimidines (C and T nucleotides) to purines (A and G nucleotides) within this sequence may decrease the splicing efficiency of particular gene [24]. Nonetheless, the substitution of the C nucleotide to A of the two intronic polymorphisms does not result in the change in amino acid sequence, nor affect the transcription efficiency [20]. Thus, our study suggests that these two polymorphisms may not be the true susceptibility locus that increases vulnerability to MDD in the Malaysian population, but might be in LD with the actual causative variants. For instance, some studies have reported that the two polymorphisms within the intron 7 are in LD with the variants located on the promoter regions of the TPH1 gene [25, 26].

A recent study reported that stress-induced depression is associated with A779C polymorphism in a Caucasian population [6]. Moreover, a trend towards significance (p = 0.068) is observed for the A218C polymorphism of the same population [6]. In addition, the TPH1 A218C polymorphism is related to the somatic anxiety symptoms in MDD [27]. Tsai and co-researchers indicated that the TPH1 A218C polymorphism is linked to MDD patients, particularly with suicidal behavior in Taiwanese population [28]. Therefore, we suggest that the two intronic polymorphisms of the TPH1 gene might not correlate to the phenotype of MDD itself, but rather with

the symptoms or suicidal behaviors as proposed by another two studies [29, 30]. Furthermore, Serretti et al. suggested that the analysis of clusters that narrow down the phenotype is more suitable in the genetic studies of MDD because it is a heterogeneous illness.

Tan et al. found a possible association between the genotype frequency of the A218C polymorphism and MDD in the Singaporean Chinese [13]. Similarly, a recent study indicated that the TPH1 218A/C genotype and allele frequencies differed between the Taiwanese healthy controls and MDD patients [31]. Our study however, revealed the genotype and allele frequencies of both the polymorphisms did not differ between the MDD patients and control subjects in the Malaysian Chinese (as shown in Table 2). Hence, it is possible that geographical variation, cultural and environment factors could be another possible explanation leading to the differences in the genetic components of the same population. This can be seen in the varied frequencies of the A779C polymorphism for MDD samples in the Chinese population of different countries in **Table 4**. The Malaysian Chinese are descendants from the Han, and this is true of the Singaporean Chinese. A majority of the descendants of the Malaysian Chinese migrated from the Fujian, Guangdong, and Hainan districts of mainland China during the fifteenth and mid-twentieth century [32]. The history of Singaporean Chinese migrants had also followed a similar ancestral origin [13].

Unfortunately, as there is only limited data available on the A779C polymorphism for the MDD samples in the Chinese population from different countries, no in-depth comparison can be made.

The actual risk-alleles, A and C, being predisposed to MDD, are still in debate. Our group found an excess of the 218C allele in the depressed patients of the three ethnicities and combined samples. Similar results were observed for the Malay, Chinese, and combined samples in terms of the A779C polymorphism. Our findings are in agreement with another two studies, which demonstrated that the C allele is more frequent in the MDD patients [13, 33]. However, the 779A allele was found to be more common in the Indians. A study conducted by Jokela et al. noted that an increase in the depressive symptoms are observed in individuals carrying the A allele with the lack of social support [34]. In addition, considerable evidences have been shown that the A allele might have significant impact on men [30, 34]. Our results showed that the A allele may be a protective factor against depressive symptoms in depressed males, which is supported by Serretti et al.'s study [30]. Moreover, the A allele was found to be more frequent in the depressed females in our data. Conversely, Porter and colleagues reported that the 218C polymorphism is associated with low tryptophan concentrations in depressed women [35]. Sex hormones may be a probable factor involved as well [35, 36]. Furthermore, population stratification could be another parameter of contention in our study. Hence, our group suggests that the two variants might have different effects on MDD and may be gender-dependent, as well as ethnicity-specific. Unfortunately, we cannot rule out whether the two variants involved in the development of MDD are affected by gender or ethnicity, or both because the gender sizes in each ethnicity are relatively small. Further studies exploring larger sample sizes are needed.

The haplotype analysis suggests that the TPH1 gene may act as a risk factor for MDD in the Indian ethnicity. This finding is in line with a study of the prevalence of depressive disorders in Malaysian primary care clinics, whereby the highest figures were observed among Indian patients (3.4%) [37]. The CA haplotype have been shown to be a vulnerable haplotype in the Indian ethnicity, which may carry one or more predisposing variants. This finding is supported by two studies (with different diseases). They reported that haplotypes containing the 218C allele are more frequent in patient-subjects than in healthy controls [15, 38]. However, this significant finding in our study

Table 4. Comparison of the A allele frequency of the A218C and A779C polymorphism in the control groups of Chinese ancestry in Malaysia, Singapore, China, and Taiwan

Country	N	References	Allele frequency (A218/A779)
Malaysia	129	This study	0.50/ 0.48
Singapore	139	Tan et al. (2003)	0.49/ NA
China	184	Liu et al. (2006)	0.48/ NA
Taiwan	105	Wang et al. (2011)	0.38/ NA

could be due to false-positive finding(s) because the 'small' sample sizes of the Indian subjects. Now, similar results were not observed in the analysis of the combined samples. In addition, there is no comparative data available for the haplotype analysis of the two intronic polymorphisms on the MDD samples from other different populations.

Undoubtedly, there are several limitations in our study. First, the mean age of the healthy controls was relatively younger than the MDD patients, especially in the Malay and Chinese ethnicities. This difference would have affected the results since some of the individuals in the control group may develop a depressive disorder later in life and weaken the difference between the two groups. Secondly, the control volunteers did not answer any detailed psychiatric questionnaire(s), hence, it is possible that they did not realize that they had mild depressive symptoms and were included in this study. Finally, we only examined the two best-known intronic polymorphisms instead of other variants located along the TPH1 gene. Thus, we cannot exclude the possibility that there could be other variants on the TPH1 gene, which may confer susceptibility to MDD in the Malaysian population.

We postulate that the genotyping method that is presently utilized is relatively cheap and widely available in many molecular laboratories. Hence, this could be relevant for wider population groups to increase the general application of the findings since the incidence of MDD in a population is relatively high. Peripheral tryptophan levels have frequently been found to be lower in depressed compared with healthy subjects and this, combined with the finding of an association between TPH1 gene and MDD can provide a clearer picture of the patient's condition [6]. This kind of information may, in future, allow clinicians to manage and treat moderate or severe MDD patients with the appropriate anti-depressant(s), which is tailored to the individual patient's genetic make-up, although the literature thus far have been inconsistent with reports of studies showing positive correlations [39-41], or no significance[42-44].

In summary, the single locus analysis showed that no association between the two intronic polymorphisms and MDD in Malaysian population. The preliminary results of the haplotype analysis, however, suggest that the TPH1 gene may confer susceptibility to the MDD in the Indian ethnicity. Further analysis sampling a larger number of patients and control samples is essential to replicate this finding.

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