

ORIGINAL ARTICLE

Lack of association between TPH2 gene polymorphisms with major depressive disorder in multiethnic Malaysian population

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major depressive disorder, polymorphism, serotonin, tryptophan hydroxylase gene 2

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Abstract**Introduction:** Numerous association studies of candidate genes studies with major depressive disorder (MDD) have been conducted for many years; however, the evidence of association between genes and the risk of developing MDD still remains inconclusive. In this study, we aimed to investigate the association between the tryptophan hydroxylase 2 (TPH2) gene and MDD in three ethnic groups (Malay, Chinese and Indian) within the Malaysian population.**Methods:** Two hundred and sixty five MDD patients who fulfilled the Diagnostic and Statistical Manual of Mental Disorders-IV criteria for MDD and 332 healthy controls were recruited for the study. All cases and controls were then genotyped for TPH2 polymorphisms rs1386494, rs1386495 and rs7305115.**Results:** Single locus analysis in pooled and ethnically stratified subjects revealed no association between each of the three variants of the TPH2 gene with susceptibility to MDD. Strong linkage disequilibrium was detected between rs1386495 and rs1386494 in pooled subjects; however, no significant association was found in the haplotype analysis.**Discussions:** In this study, we suggest that in both the Chinese and Indian populations, gender distribution differ significantly between cases and controls, showing that women are more at risk of developing MDD compared with men. Therefore, we suggest that the occurrence of MDD in both Chinese and Indians in the Malaysian population may be influenced by gender.**Introduction**

Major depressive disorder (MDD) is a common psychiatric disorder that is associated with high morbidity (due to number of years of lost productivity and burden of disease) (Craddock and Forty, 2006) and high mortality (due to suicides) (Fairweather-Schmidt *et al.*, 2009). MDD is of major concern because it promotes a pervasive negative impact on the quality of a life, and has been projected to become the second leading cause of death and disability by year 2020 (Murray and Lopez, 1996). The lifetime prevalence of MDD is said to be about 15%, with women being more prone to MDD than men (Weissman *et al.*, 1993; Wilhelm *et al.*, 2003).

The underlying factors of what triggers MDD, however, remain unclear. It is estimated that the

heritability of MDD is about 42% (Kendler *et al.*, 2006). Strong evidence for the heritability of MDD has been demonstrated by previous studies (Tsuang *et al.*, 1980; Gershon *et al.*, 1982; Weissman *et al.*, 1984; Maier *et al.*, 1993). These findings have changed the direction of MDD research toward identifying the candidate genes that may contribute to the susceptibility to MDD.

Serotonin plays an important role in various psychiatry conditions, including MDD, as demonstrated by therapeutic efficiency of the serotonin modulators, such as the selective serotonin reuptake inhibitors. Tryptophan hydroxylase (TPH) is the rate-limiting enzyme in the synthesis of serotonin and is a major focus for much research. It has been suggested that this gene is a major candidate gene responsible for the

underlying mechanism of various psychiatric disorders, especially depression (Mann *et al.*, 1990; Lucki, 1998). Two isoforms of the TPH gene, TPH1 and TPH2 genes have been discovered (Walther *et al.*, 2003), and numerous studies have been carried out on the association of the TPH gene with schizophrenia, depression, alcoholism and other neuropsychiatric disorders (Nielsen *et al.*, 1998; Serretti *et al.*, 2001; Lalovic and Turecki, 2002; Bellivier *et al.*, 2004). However, this result remains inconclusive. A study on the association of two polymorphisms, A218C and A779C in the intron 7 of the TPH1 gene with susceptibility to MDD in three main ethnic groups of the Malaysian population, has failed to detect any association between the two polymorphisms and MDD (Lian *et al.*, 2013). TPH2 gene has been reported to be widely expressed in the brain stem. It is located on chromosome 12q15, which consists of 11 exons spanning approximately 93.5 kb. Here, however, a study on association between MDD and the TPH2 polymorphisms in a Caucasian population found positive associations between rs1386494 and a haplotype of TPH2 gene with MDD (Zill *et al.*, 2004). This is also supported by findings of Harvey *et al.* (2004). Another polymorphism located in exons 7 of TPH2, rs7305115, is also reported to be associated with susceptibility to MDD and its treatment response (Zhou *et al.*, 2005). A study in the Chinese Han population reported no significant relationship between rs7305115 with susceptibility to MDD (Shen *et al.*, 2011). There has so far been no other published study determining the association of the TPH2 gene with the risk of MDD in the Asian populations.

Previous findings concerning the influence of genetic polymorphisms with MDD in the Asian population have been inconsistent (Tsai *et al.*, 2009). The rationale for choosing TPH2 single-nucleotide polymorphisms (SNPs) rs1386494 and rs1386495 is because both have been reported to be associated with depression in case-control studies (Zill *et al.*, 2004; Anttila *et al.*, 2009). Furthermore, the two SNPs were found to correlate with anxiety and MDD in the Caucasian and African American populations (Zhou *et al.*, 2005). One other SNP of the TPH2 gene (rs7305115) was previously reported to be associated with bipolar depression (Harvey *et al.*, 2004, 2007), and with both MDD and anxiety disorder patients with a history of suicide attempts (Zhou *et al.*, 2005). Therefore, the purpose of our study is to investigate the association between the three polymorphisms in the TPH2 gene (rs1386495, rs1386494 and rs7305115) with susceptibility to MDD in the multiethnic Malaysian population (Malay, Chinese and Indian). Our study is the first

genetic association study conducted in the Malaysian population on polymorphisms of TPH2 gene with susceptibility to MDD, focusing only on the three major ethnic groups in the country.

Methods

Subjects

Two hundred and sixty-five unrelated MDD patients who met Diagnostic and Statistical Manual of Mental Disorders-IV criteria were recruited from the University Malaya Medical Centre (UMMC), Kuala Lumpur, Malaysia. Three hundred and thirty-two healthy controls were recruited from the local community. Controls with a history of any psychiatric disorders were excluded and both case and control groups were matched for age. Race of each cases and control subjects was determined from birth record data, by a short interview with the subjects, and by absence of mixed marriages for at least three generations. This study was approved by the Ethics Committee of UMMC, and informed consent was obtained from all participants prior to sample collection.

Genotyping

Genomic DNA was extracted from whole blood using QIAamp® DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany). Polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP) and amplification refractory mutation system-PCR (ARMS-PCR) assays were performed to genotype three SNPs of the TPH2 gene in our study (rs13864494, rs1386495 using PCR-RFLP, and rs7305115 using ARMS-PCR). The forward and reverse primers of the three SNPs are as follows:

F 5' GACACTGCAAACCTGTTTCTCGC 3' and
R 5' GCTCACCCAAATTGAATGTGCCT 3'
(rs1386494).

F 5' TGGCATTGTGAAAAGTTATTCTCC 3' and
R 5' ACACATCCCTGGCAATTGATTT 3'
(rs1386495).

For rs7305115, which used the ARMS-PCR technique, the primers are as follows:

F 5' TTAGAAAGGTCTGGCTTCACGGTGAG 3'
and R 5' AGGAGTCTGATCCTTCAGTGAGCCC 3'
(outer primers) and

F 5' GGCTCAGATCCCCTCTACACCACA 3' (A-allele) and R 5' GGCTTTAATGTAGGTAATCAGGTGCC 3' (G-allele) for inner primers.

The amplification mixture consisted of 15 μ L of DNA (50–100 ng/reaction), 1 \times Buffer, 1.5 mM MgCl₂, 200 μ M dNTPs, 0.4 μ M of each primer and 0.4 units of Taq DNA polymerase (Vivantis Technologies Sdn. Bhd, Shah Alam, Malaysia) for both rs1386494 and rs1386495, with the same PCR reaction procedure of 94°C for 5 minutes (initial denaturation) followed by 35 cycles of 94°C for 30 seconds (denaturation), 55°C for 30 seconds (annealing), 72°C for 30 seconds (extension) and a final extension step of 72°C for 10 minutes. For rs7305115, the PCR reaction procedure was altered slightly, starting from 94°C for 5 minutes, followed by 35 cycles each at 94°C for 30 seconds, 67°C for 30 seconds, 72°C for 30 seconds and a final extension step of 72°C for 10 minutes. The PCR product of rs1386494 and rs1386495 were digested with *Msp* I (Vivantis Technologies Sdn. Bhd) and Tsp451 (New England Biolab, Ipswich, MA, USA) at 37°C and 65°C. The restricted products were separated using 2.5% agarose gels (Vivantis Technologies Sdn Bhd), yielding three genotypes: A/A, A/G and G/G (rs1386494) and C/C, C/T and T/T genotypes (rs1486495). For rs7305115, the PCR products were resolved via 2% agarose gels (Vivantis Technologies Sdn. Bhd) thereby producing two PCR fragments.

Statistical data analysis

Comparisons of the genotypic and allelic frequencies between cases and controls were performed using the chi-square test, whenever appropriate. The odd ratios and their 95% confidence intervals were used for estimating the risk or effects of alleles. Hardy–Weinberg equilibrium (HWE) test, linkage disequilibrium (LD) test, haplotype estimation frequency, genotype and allelic frequencies were calculated using the Haploview program (MIT Broad Institute Harvard University, Cambridge, USA).

Results

Demographic data

The demographic data of both cases and controls groups are shown in Table 1. The 265 cases consists of 59 Malays, 129 Chinese and 77 Indians. For the 332 controls, 110 of them were Malays, 129 Chinese and 93 of them were Indians. There was no significant difference in the gender distribution for the Malays ($\chi^2 = 0.896$, $P = 0.344$) between case and control groups. However, significant differences were observed between the two groups in terms of Chinese

Table 1. Demographic data on subjects in the Malaysian population and its three ethnic groups

Ethnic groups	Male (n)	Females (n)	Age (mean \pm SD)	Total (n)
Malay				
Cases	25	34	38.86 \pm 12.13	59
Controls	55	55	22.64 \pm 4.40	110
Chinese				
Cases	48	81	40.86 \pm 12.53	129
Controls	84	45	56.42 \pm 307.86	129
Indian				
Cases	31	46	44.91 \pm 12.71	77
Controls	59	34	33.69 \pm 11.71	93

SD, standard deviation.

and Indian males and females (Chinese, $\chi^2 = 20.104$, $P = 0.000$, Indian, $\chi^2 = 9.086$, $P = 0.003$).

SNP association analyses

Two SNPs (rs1386494 and rs1386495) were found to be in HWE in both case and control samples in the three ethnic groups of Malays, Chinese and Indians ($P > 0.05$ by the chi-square test). The rs730511 was found to be in HWE in Malay and Indian ethnic groups but not in the Chinese ethnic group ($P = 0.000$). The rs730511 in all three ethnic groups of the controls was not in HWE ($P < 0.05$). We decided to exclude the rs730511 from the association analyses in this study because according to Lunetta (2008), SNPs with HWE test values of $P < 0.01$ or 0.001 should be omitted (Lunetta, 2008).

When we compared the genotype and allele frequencies of cases and controls in the two SNPs, we failed to detect any significant difference in either allele or genotype frequency between both groups in the two polymorphisms (rs1386494 and rs1386495) (As shown in Table 2).

LD and haplotype analysis of TPH2 SNPs (rs1386495 and rs1385494)

LD analysis revealed a very strong LD between rs1386495 and rs1386494 ($D' = 0.98$) in the overall subjects. Analysis of LD by ethnicity was not done because of the deviation from HWE following ethnic stratification. The same reason was applied for the exclusion of rs730511 from the analysis.

Two diplotypes with frequencies of above 5% are presented in Table 3. Diplotype TG is more frequent than diplotype CA (93.4%). However, we found no significant effect of these diplotypes on susceptibility to MDD.

Table 2. Allele and genotype frequencies of SNPs and association analysis of each SNP between the case and control samples

Ethnic groups	Genotype frequency			P-value	Allele frequency		P-value	OR	95% CI	HWE (P)
	AA	AG	GG		A	G				
<i>rs1386494</i>										
Malay										
Cases	0 (0%)	4 (7%)	55 (93%)	0.905	4 (3%)	114 (97%)	0.848	0.930	0.274–3.155	0.788
Controls	0 (0%)	8 (7%)	102 (93%)		8 (4%)	212 (96%)				
Chinese										
Cases	1 (1%)	22 (17%)	106 (82%)	0.566	24 (10%)	234 (90%)	0.636	1.221	0.656NA2.269	0.903
Controls	0 (0%)	20 (15%)	109 (85%)		20 (8%)	238 (92%)				
Indians										
Cases	1 (1%)	14 (18%)	62 (81%)	0.746	16 (10%)	138 (90%)	0.461	1.380	0.692NA2.751	0.836
Controls	1 (1%)	13 (14%)	79 (85%)		20 (8%)	238 (92%)				
Total cases (n = 265)	2 (1%)	40 (15%)	223 (84%)	0.450	44 (8%)	486 (92%)	0.274	1.307	0.845NA2.024	0.503
Total controls (n = 332)	1 (1%)	41 (12%)	290 (87%)		43 (6%)	621 (94%)	NA	NA	NA	0.801
<i>rs1386495</i>										
Malay										
Cases	0 (0%)	4 (7%)	55 (93%)	0.728	4 (3%)	114 (97%)	0.995	1.251	0.346–4.526	0.786
Controls	0 (0%)	6 (5%)	104 (95%)		6 (3%)	214 (97%)				
Chinese										
Cases	1 (1%)	15 (11%)	113 (88%)	0.470	17 (6%)	241 (94%)	0.767	0.850	0.431NA1.676	0.529
Controls	0 (0%)	19 (15%)	110 (85%)		19 (8%)	229 (92%)				
Indians										
Cases	1 (1%)	14 (18%)	62 (81%)	0.850	16 (10%)	138 (90%)	0.707	1.232	0.595NA2.552	0.836
Controls	1 (1%)	14 (15%)	78 (84%)		16 (8%)	170 (92%)				
Total cases (n = 265)	2 (1%)	33 (12%)	230 (87%)	0.709	37 (7%)	493 (93%)	0.943	0.979	0.557NA1.722	0.777
Total controls (n = 332)	1 (1%)	39 (11%)	292 (88%)		20 (5.6%)	261 (51.1%)	NA	NA	NA	0.761

HWE, HardyNAWeinberg equilibrium; NA, not applicable.

Table 3. Estimated haplotype frequencies of the TPH2 *rs1386495* and *rs1386494* between the depressed patients and control subjects in pooled subjects

Haplotype†	Case (%)	Control (%)	P-value	OR (95% CI)
Total				
TG	93	94	0.234	0.805 (0.504–1.285)
CA	7	6	0.512	1.243 (0.778–1.984)
Total	100	100	NA	NA

†Haplotypes with a frequency <3% in patient and control groups are not shown, SNPs with HWE < 0.05 is not shown.

CI, confidence interval; NA, not applicable; OR, odds ratio.

Discussions

TPH 2 gene is a potentially important candidate gene for susceptibility to MDD, as indicated by its high expression in the brain regions such as the brain stem, the major locus of the serotonin-producing neurons. Several studies have successfully demonstrated the association between TPH2 gene polymorphisms with MDD in Caucasian populations (Zill *et al.*, 2004; Zhou *et al.*, 2005) and in African Americans as well as Southwestern American Indians (Zhou *et al.*, 2005).

In the present study, our data indicate that two SNPs of the TPH2 gene (*rs1386495* and *rs1386494*)

were not associated with susceptibility to MDD in the Malaysian population. This finding is in agreement with previous studies carried out by Illi *et al.* (2009) and Gizatullin *et al.* (2008), in which they reported that there is no significant association between *rs1386494* with susceptibility to MDD (Gizatullin *et al.*, 2008; Illi *et al.*, 2009). However, our result is in contrast to the study by Zill *et al.* (2004), the first study to report an association between *rs1386494* and MDD in a Southern German population and another study carried out by Anttila *et al.* (2009) in a Finnish population. The discrepancy in the findings may be explained by genetic differences between the populations studied. The allelic frequency of *rs1386494* in our study is 0.96 in the controls, while in the Caucasian population, the allelic frequency reported was 0.79 in the controls (Zill *et al.*, 2004).

Our study showed a significant difference in gender distribution between the cases and controls in the Chinese and Indians. We found that in the population studied, women were more at risk of MDD compared with men, which is supported by a study by Shen *et al.* (2011) in the Chinese Han population. The TPH2 *rs1386495* and *rs1386494* were also found to be correlated with anxiety and MDD in US Caucasians and African Americans (Zhou *et al.*, 2005).

In our finding, we reported a strong LD for the SNP pair TPH2 (rs1386494 and rs1386495) in the Malaysian population, which is similar to previous studies carried out in different populations (Zill *et al.*, 2004; Zhou *et al.*, 2005; Gizatullin *et al.*, 2008). Zill *et al.* (2004) reported significant differences between cases and control groups in three haplotypes, which is in contrast with our finding. We found no significant differences of the diplotypes between the case and control groups.

Nevertheless, there are some limitations to our study. Firstly, the small sample size results in smaller numbers following subgroup analysis, and further contributes to the negative association of the three SNPs with susceptibility to MDD. Secondly, the age and gender of both cases and controls were not matched, thus possibly contributing to the lack of association. Therefore, TPH2 gene might be a protective gene against MDD and so it is worth looking at in future, albeit with larger sample sizes.

In conclusion, we suggest that the TPH2 gene may have a gender-dependent effect on the risk of MDD in the Chinese and Indian ethnicities within the Malaysian population. However, this study should be replicated in future using a larger sample size to further confirm our findings.

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Conflict of interest

All authors do not have any conflict of interest to disclose.

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