J Med Sci 2016;36(2):59-67
DOI: 10.4103/1011-4564.181519

ORIGINAL ARTICLE

The Serotonin Transporter Gene (Triallelic *5-HTTLPR* Polymorphism) May Associate with Male Depression in Han Chinese Population

Pei-Shen Ho[1], Chih-Lun Chen[2], Chuan-Chia Chang[3], Hsin-An Chang[3], Yi-Wei Yeh[3], Chih-Sung Liang[1,4], Che-Hung Yen[4,5], Shin-Chang Kuo[3,4], Chun-Yen Chen[3,4], Chang-Chih Huang[4,6], Chun-Long Lin[7], Ru-Band Lu[8], Mei-Chen Shih[3], San-Yuan Huang[3,4]

[1]Department of Psychiatry, Beitou Branch, Tri-Service General Hospital, National Defense Medical Center, [3]Department of Psychiatry, National Defense Medical Center, Tri-Service General Hospital, [4]Graduate Institute of Medical Sciences, National Defense Medical Center, [5]Department of Neurology, Tri-Service General Hospital, National Defense Medical Center, [6]Department of Psychiatry, Buddhist Tzu Chi General Hospital, Taipei Branch, Taipei, [7]Department of Psychiatry, Taoyuan Armed Forces General Hospital, Taoyuan, [2]Department of Psychiatry, Chiayi Branch, Taichung Veterans General Hospital, Chiayi, [8]Department of Psychiatry, Institute of Behavior Medicine, College of Medicine, National Cheng Kung University, Tainan, Taiwan, ROC

Background: Pharmacological, neurobehavioral, and therapeutic evidence have implicated serotonin in the pathogenesis of depression. There are conflicting reports on the association of genetic variants of serotonin transporter gene (*5-HTTLPR*) with major depressive disorder. The *5-HTTLPR* is thought to have three primary allelic variants (rs25531): LA, LG, and S. The present study examined whether major depression was associated with tri-allelic *5-HTTLPR* polymorphisms in a Han Chinese population. **Materials and Methods:** Bi-allelic and tri-allelic *5-HTTLPR* polymorphisms were assessed in 305 patients with major depressive disorder (MD) and 313 unrelated healthy control subjects. In addition, to reduce clinical heterogeneity, subtype analyses were performed for clinically important variables, including family history of major affective disorder, age at onset, and severity of MD. **Results:** The bi-allelic *5-HTTLPR* polymorphism was not associated with MD and its clinical subgroups. However, the tri-allelic *5-HTTLPR* polymorphism was associated with major depression and with specific subgroups. In particular, in male subjects, patients with a low expressing genotype (*S'/S'*) were at higher risk for MD than those with high expressing genotypes (*S'/L'* and *L'/L'*). This positive association was only observed in the subgroups of late-onset and moderate severity MD. **Conclusions:** The present study suggests that the tri-allelic *5-HTTLPR* polymorphism might be a risk factor for susceptibility to either MD or its clinical subtypes in the Han Chinese male population but not in the female population. However, these results should be validated in a larger patient population that includes different ethnic samples or subdiagnosis groups.

Key words: Major depressive disorder, serotonin transporter gene, subtypes, *triallelic 5-HTTLPR* polymorphism

INTRODUCTION

Major depressive disorder (MD) is a prevalent and disabling clinical condition that reduces patients' productivity and quality of life and also increases patient mortality.[1] Substantial evidence from pharmacological, neurobehavioral, and therapeutic investigations have implicated serotonin (5-HT) as a major contributor to the pathogenesis of depression. The initial evidence suggesting that 5-HT contributed to clinical depression came from reports of elevated cerebrospinal fluid 5-hydroxyindoleacetic acid level in patients with depression.[2,3] Another study of the effect of tryptophan depletion reported a lowering of mood in normal subjects and in patients that had recovered from depression.[4] In addition, serotonin transporter (SERT) binding sites in the brain were found to be

Received: September 29, 2015; Revised: November 18, 2015; Accepted: February 16, 2016
Corresponding Author: Prof. San-Yuan Huang, No. 325, Cheng-Kung Road, Sec. 2, Nei-Hu District, Taipei, 114, Taiwan, ROC. Tel: 011-886-2-8792-7220; Fax: 011-886-2-8792-6715. E-mail: hsy@ndmctsgh.edu.tw

How to cite this article: Ho PS, Chen CL, Chang CC, Chang HA, Yeh YW, Liang CS, *et al.* The serotonin transporter gene (triallelic *5-HTTLPR* polymorphism) may associate with male depression in Han Chinese population. J Med Sci 2016;36:59-67.

decreased in patients with major depression.[5,6] Taken together, these results suggest a decreased 5-HT concentration in depressed individuals, which is consistent with the hypothesis that serotonergic dysfunction in central nervous system is an important contributor in the pathophysiology of MD.

The SERT terminates serotonin transmission through reuptake of serotonin from the synaptic cleft. Therefore, inhibiting SERT activity enhances the action of serotonin at the synapse. SERT participation in the modulation of depressive symptomatology is demonstrated by the therapeutic benefit derived from the administration of SERT inhibitors, also referred to as selective serotonin reuptake inhibitors and tricyclic antidepressants.[7,8] Since serotonergic neurotransmission can be regulated by changes in SERT expression, it seems reasonable that genetic variants of SERT may be responsible for the central serotonin system dysregulation and thus may contribute to MD and/or treatment response.

The SERT gene(*5-HTT, SLC6A4*) is located on chromosome 17q11.1-q12, which spans 31 kb and consists of 14 exons.[9,10] A 44 base pair insertion/deletion polymorphism (*5-HTTLPR*) in the promoter region has been described for this gene.[11] A possible association between *5-HTTLPR* gene polymorphisms and MD has been extensively investigated due to the established variances in transporter function resulting from these polymorphisms. The short (S) allele has been associated with lower transcriptional activity and reduced serotonin uptake in lymphoblasts[12] and platelets,[13] and a marked reduction in messenger RNA levels and SERT binding in the brain[14] as compared with the long (L) allele. Some investigators have suggested that the *5-HTTLPR* short (S) allele is associated with MD.[15-18] However, other investigators could not replicate these results.[19-21] One possible reason for the above-mentioned conditions is the presence of higher-expressing (L) alleles, which contains a different functional polymorphism. A third functional allele (tri-allelic *5-HTTLPR* polymorphism, rs25531) has been described in which an A > G (L_A to L_G) polymorphism is present at position 6 of the first of two 22-bp imperfect repeats that define the 16-repeat L allele.[22] Previous functional study showed that the L_A allele expressed at higher level than the L_G allele and that L_G is equivalent in expression to the S allele.[23] Zalsman *et al*.[24] also reported that the lower-expressing alleles (i.e., L_G and S) independently predicted the greater severity of depression and predicted the greater severity of MD with moderate-to-severe life events compared with the higher-expressing L_A allele. Of interest, these association results may be strengthened by the use of tri-allelic *5-HTTLPR* polymorphism methods.

In the present study, we investigated the possible association of tri-allelic *5-HTTLPR* polymorphisms and MD by comparing the frequency of this polymorphism in both Han Chinese patients with MD and healthy controls. In addition, it has been postulated that investigation focused on more homogenous subclinical phenotypes might increase the power to detect genes possibly involved in major psychiatric disorders.[25,26] Thus, in order to reduce clinical heterogeneity, this study further examined specific patient subgroups according to clinical variables including family history of major affective disorder, the age at onset of MD, and the severity of depression.

METHODS

Subjects

This study was conducted in the inpatient and outpatient units of Tri-Service General Hospital, a medical teaching hospital belonging to the National Defense Medical Center in Taipei, Taiwan. The Institutional Review Board approved the protocol for the Protection of Human Subjects at Tri-Service General Hospital. Written informed consent was obtained from all participants and all study procedures were fully explained to the participants. To minimize the effect of ethnic differences in gene frequencies, the study participants were recruited from the Han Chinese population in Northern Taiwan. All the participants were unrelated, born and living in Taiwan, and had biological grandparents of Han Chinese ancestry. A total of 618 individuals were recruited.

The patient group consisted of 305 patients with MD (118 males and 187 females; mean age 38.49 ± 14.10 years, range: 18–65 years) who were consecutively recruited from the psychiatric department of Tri-Service General Hospital. Each patient was initially evaluated by an attending psychiatrist and then interviewed by a well-trained psychologist using the Chinese Version of the Modified Schedule of Affective Disorder and Schizophrenia-Lifetime (SADS-L)[27,28] to reach the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV)[29]. The inter-rater reliability (Kappa value) of the Chinese version of SADS-L were considered good to excellent for MD (0.79), bipolar disorder (0.71), anxiety disorder (0.86), schizophrenia (0.95), alcohol abuse and alcohol dependence (1.00), and substance abuse and dependence (0.82). All patients in this study met the DSM-IV criteria for MD on the basis of interviews and a best-estimated procedure that used all available information, including clinical observations, hospital records, and family information. Individuals with a history of substance dependence, severe medical illness, organic brain disease, or any concomitant major psychiatric disorders such as schizophrenia and bipolar affective disorders were excluded from this study. Patients were further classified into more homogeneous clinical subgroups as shown in Table 1: Moderate *versus* severe MD,

Table 1: Comparison of the mean age, gender, *and* genotype distributions of bi-allelic and tri-allelic *(rs25531) 5-HTTLPR* polymorphisms between patients with MD and healthy control subjects

Group	*n*	Age (years)* (mean±SD)	Sex* (%)		Bi-allelic *5-HTTLPR* (%)			*P**	Tri-allelic *5-HTTLPR* (%)			
			Male	Female	*S/S*	*S/L*	*L/L*		*S'/S'*	*S'/L'*	*L'/L'*	*P**
Major depression	305	38.49±14.10	118 (38.7)	187 (61.3)	163 (53.4)	111 (36.4)	31 (10.2)	0.111	236 (77.4)	63 (20.7)	6 (2.0)	0.111
MD, positive FH	81	36.69±13.80	27 (33.3)	54 (66.7)	45 (55.6)	26 (32.1)	10 (12.3)	0.092	61 (75.3)	19 (23.5)	1 (1.2)	0.612†
MD, negative FH	224	39.50±14.10	91 (40.6)	133 (59.4)	118 (52.7)	85 (37.9)	21 (9.4)	0.320	175 (78.1)	44 (19.6)	5 (2.2)	0.108†
MD, early-onset	46	24.76±6.84‡	28 (60.9)	18 (39.1)	23 (50.0)	19 (41.3)	4 (8.7)	0.884†	31 (67.4)	13 (28.3)	2 (4.3)	0.773†
MD, late-onset	259	40.87±13.67‡	90 (34.7)	169 (65.3)	140 (53.8)	92 (35.4)	27 (10.4)	0.084	205 (79.2)	50 (19.3)	4 (1.5)	0.037†
MD, moderate	84	37.98±13.73	36 (42.9)	48 (57.1)	46 (54.8)	33 (39.3)	5 (6.0)	0.592†	66 (78.6)	17 (20.2)	1 (1.2)	0.361†
MD, severe	221	38.69±14.26	82 (37.1)	139 (62.9)	117 (52.9)	78 (35.3)	26 (11.8)	0.058	170 (76.9)	46 (20.8)	5 (2.3)	0.205†
Healthy control	313	37.82±12.04	201 (64.2)	112 (35.8)	152 (48.6)	138 (44.1)	23 (7.3)		219 (70.0)	85 (27.2)	9 (2.9)	

Significant differences were found in gender between controls and patients with MD except in the subgroup of early-onset MD. *Compared with the control group; †Statistical analysis was performed by Fisher's exact test; ‡*P*=0.01. FH=Family history; MD=Major depression; SD=Standard deviation

early versus late onset MD, and MD with or without a family history of major affective disorders. The severity of the episode was assessed with the 17-item version of Hamilton Depression Rating Scale (HAM-D). Only subjects with a minimum score of 18 on the HAM-D entered the study. Eighty-four patients had moderate MD (HAM-D scores 18–24),[30] and the rest of the patients met the severe major depression (HAM-D scores >24).[30] The age at onset of the first depressive episode before 18 was defined as early-onset. Age 18 or older was defined as late-onset. Family history was defined as having one or more first-degree relatives that were affected with bipolar disorder or MD. Family history was collected from interviews, hospital record, and patient information.

The control group included 313 healthy volunteers (201 males and 112 females; mean age 37.82 ± 12.04 years, range: 18–65 years), recruited from the community. The Chinese version of the SADS-L[27,28] was used to screen out psychiatric conditions in the control group. Control subjects and their first-degree relatives were free of past or present, major or minor psychiatric disorders, including affective disorder, schizophrenia, anxiety disorder, personality disorder, and substance use disorders.

Genotyping

The whole blood was drawn from a peripheral vein using vacutainer tubes containing an ethylene-diaminetetracetic acid solution. Genomic DNA was extracted from the blood leukocytes using standard methods. Bi-allelic *5-HTTLPR* (both S and L alleles) and tri-allelic *5-HTTLPR* (S, L_A, and L_G) polymorphisms in the *5-HTT* gene were detected using the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods. The PCR primer design and the methods of PCR-RFLP were modified from Wendland *et al*.,[31] and the PCR-RFLP protocol was performed in a PerkinElmer 9700 thermal cycler (Boston, MA, USA). The forward primer 5'-CTCCGCTTTGGCGCCTCTTC-3' and reverse primer 5'-GATCCTGGGAGAGGTGCAGG-3' were used for the genotyping of the *5-HTT* promoter polymorphism. The bi-allelic *5-HTTLPR*-L allele was 490 bp long, whereas the S allele was 447 bp in PCR products. Subsequently, the PCR products of bi-allelic *5-HTTLPR* were digested by HpaII restriction enzymes for 2 h at 37°C. The L_A allele remained uncut (490 bp), whereas the L_G allele was cut into two DNA fragments of 395 bp and 95 bp.

In our study, the low expressing S and L_G alleles were designated as S' and the higher expressing L_A allele was designated as L' according to the described of Zalsman *et al*.[24] Therefore, for our data analyses (described below), genotypes were classified into six groupings: *S/S, S/L, L/L,* S'/S', *S'/L',* and *L'//L'*.

Statistical analysis

The independent-samples *t*-test and Pearson's Chi-square test were employed to compare the clinical and demographical parameters between patients with major depression and normal controls. The Hardy–Weinberg equilibrium was assessed for each group. Genotype and allele frequencies were also compared between patients and controls using Pearson's Chi-square analysis. Fisher's exact test was substituted for Pearson's Chi-square test when sample sizes were smaller than expected (<5 subjects). Multiple logistic regression analysis was also applied to correct for any possible effect of the covariates of age and gender on the risk of MD. Power analysis was performed with the use of G-power.[32] All tests were two-tailed. Throughout the study, there are 63 hypothesis testing for the differences in genotype frequencies between patients and controls. To reduce the possibility of false positive findings due to multiple comparisons, the alpha was set at a more stringent level of 0.01 instead of 0.05.

RESULTS

There was no significant difference in mean age between patients with MD and healthy controls, with the exception of the clinical subgroup of early and late-onset. However, significant differences were found in gender between these two groups [Table 1]. Genotype distributions of bi-allelic and tri-allelic *5-HTTLPR* (rs25531) polymorphisms were in Hardy–Weinberg equilibrium, both in patients with MD and in control subjects ($P > 0.1$). The results of the genotype distributions for these bi-allelic and tri-allelic *5-HTTLPR* polymorphisms in patients and control subjects are summarized in Table 1. Regarding the bi-allelic *5-HTTLPR* polymorphism, no statistically significant differences were evident for the genotype and allele frequencies between patients with MD and the control subjects or between different clinical subtypes of MD [Tables 1-3]. In the analyses of tri-allelic *5-HTTLPR* polymorphism, we also found no significant association between genotype frequencies and patients with MD [Table 1].

Because gender differences were found between patients with MD and control subjects, we analyzed males and females separately. Among females neither the bi-allelic *5-HTTLPR* polymorphism nor the tri-allelic *5-HTTLPR* polymorphisms were significantly different in the genotype or allele frequency between patients with MD and control subjects, even when they were divided into the different subgroups of MD ($P > 0.1$, Table 2). Among male subjects, the bi-allelic *5-HTTLPR* genotype frequencies also showed no associations between control subjects versus total MD subjects and between the clinical subgroups [Table 3]. Regarding genotype frequencies of the tri-allelic *5-HTTLPR* polymorphism in male subjects, we found that there was only a weak association between total MD versus controls ($P = 0.032$), between MD without family history versus controls ($P = 0.039$), and between moderate MD

Table 2: Genotype distributions of the bi-allelic *and* tri-allelic *(rs25531) 5-HTTLPR* polymorphisms in female patients with major depression and healthy control subjects

Group	Female	Genotype											
		Bi-allelic *5-HTTLPR* (%)			χ^2	df	*P**	Tri-allelic *5-HTTLPR* (%)			χ^2	df	*P**
	n	*S/S*	*S/L*	*L/L*				*S'/S'*	*S'/L'*	*L'/L'*			
Major depression	187	97 (51.9)	69 (36.9)	21 (11.2)	2.648	2	0.266	138 (73.8)	45 (24.1)	4 (2.1)	1.785	2	0.402†
MD, positive FH	54	29 (53.7)	17 (31.5)	8 (14.8)	3.520	2	0.172	39 (72.2)	14 (25.9)	1 (1.9)	0.577	2	0.816†
MD, negative FH	133	68 (51.1)	52 (39.1)	13 (9.8)	1.463	2	0.481	99 (74.4)	31 (23.3)	3 (2.3)	1.495	2	0.482†
MD, early-onset	18	6 (33.3)	9 (50.0)	3 (16.7)	1.348	2	0.538†	12 (66.7)	5 (27.8)	1 (5.6)	0.416	2	0.999†
MD, late-onset	169	91 (53.8)	60 (35.5)	18 (10.7)	3.44	2	0.179	126 (74.6)	40 (23.7)	3 (1.8)	2.298	2	0.323†
MD, moderate	48	22 (45.8)	22 (45.8)	4 (8.3)	0.117	2	1.00†	33 (68.8)	14 (29.2)	1 (2.1)	0.439	2	0.808†
MD, severe	139	75 (54.0)	47 (33.8)	17 (12.2)	4.133	2	0.127	105 (75.5)	31 (22.3)	3 (2.2)	1.960	2	0.363†
Healthy control	112	49 (43.8)	52 (46.4)	11 (9.8)				77 (68.8)	30 (26.8)	5 (4.5)			

*Compared with the control group; †Statistical analysis was performed by Fisher's exact test. FH=Family history; MD=Major depression

Table 3: Genotype distributions of the bi-allelic *and* tri-allelic *(rs25531) 5-HTTLPR* polymorphisms in male patients with major depression and healthy control subjects

Group	Male	Genotype											
		Bi-allelic *5-HTTLPR* (%)			χ^2	df	*P**	Tri-allelic *5-HTTLPR* (%)			χ^2	df	*P**
	n	*S/S*	*S/L*	*L/L*				*S'/S'*	*S'/L'*	*L'/L'*			
Major depression	118	66 (55.9)	42 (35.6)	10 (8.5)	1.943	2	0.378	98 (83.1)	18 (15.3)	2 (1.7)	6.467	2	0.032†
MD, positive FH	27	16 (59.3)	9 (33.3)	2 (7.4)	0.884	2	0.630†	22 (81.5)	5 (18.5)	0 (0.0)	1.027	2	0.616†
MD, negative FH	91	50 (54.9)	33 (36.3)	8 (8.8)	1.545	2	0.462	76 (83.5)	13 (14.3)	2 (2.2)	6.285	2	0.039†
MD, early-onset	28	17 (60.7)	10 (35.7)	1 (3.6)	0.748	2	0.750†	19 (67.9)	8 (28.6)	1 (3.5)	0.878	2	0.609†
MD, late-onset	90	49 (54.4)	32 (35.6)	9 (10.0)	2.322	2	0.313	79 (87.8)	10 (11.1)	1 (1.1)	10.480	2	0.004†
MD, moderate	36	24 (66.7)	11 (30.6)	1 (2.8)	2.664	2	0.257†	33 (91.7)	3 (8.3)	0 (0.0)	6.942	2	0.033†
MD, severe	82	42 (51.2)	31 (37.8)	9 (11.0)	2.316	2	0.314	65 (79.3)	15 (18.3)	2 (2.4)	2.723	2	0.249†
Healthy Control	201	103 (51.2)	86 (42.8)	12 (6.0)				142 (70.6)	55 (27.4)	4 (2.0)			

*Compared with the control group; †Statistical analysis was performed by Fisher's exact test. FH=Family history; MD=Major depression

versus controls (P = 0.033) [Table 3]. However, there was a highly significant difference in the genotype distribution and in the allele frequency between patients with major depression of late-onset and control subjects (P = 0.004).

The *L'/L'* genotype of the tri-allelic *5-HTTLPR* polymorphism was rare in our population (<5%), and the *L'/L'* genotype was not found in two male subgroups of a positive family history of MD and moderate MD [Table 3]. Therefore, in the multiple logistic regression analyses, we combined the S'/*L'* and *L'/L'* genotypes for data analyses, and the S'/S' genotype as a risk factor (as a reference group) for MD after correction for age and gender. We found that the S'/*L'* and *L'/L'* genotypes in the tri-allelic *5-HTTLPR* polymorphism were associated with lower risk for total MD. In the clinical subtypes of MD, we also found positive associations between S'/*L'* and *L'/L'* genotypes and late-onset MD [Table 4]. Once gender is taken into account, we found a positive association between major depression and the tri-allelic *5-HTTLPR* polymorphism only existed in male subjects. In the clinical subtypes of male subjects, multiple logistic regression analyses revealed associations between the tri-allelic *5-HTTLPR* polymorphism and the groups of moderate degree and late-onset [Table 5].

Table 4: Multiple logistic regression analysis of the tri-allelic *5-HTTLPR* polymorphism *(rs25531)* for risk of major depression and its clinical subtypes

Group / Variable	Major depression (n=305)		
	OR	95% CI	P
S'/L' + *L'/L'*	0.619	0.425-0.901	0.012
Gender	2.983	2.121-4.195	<0.001
Age	0.983	0.971-0.996	0.011

Group / Variable	MD, positive FH (n=81)			MD, negative FH (n=224)		
	OR	95% CI	P	OR	95% CI	P
S'/L' + *L'/L'*	0.692	0.385-1.241	0.216	0.605	0.401-0.912	0.016
Gender	3.805	2.240-6.464	<0.001	2.644	1.832-3.817	<0.001
Age	0.967	0.947-0.987	0.001	0.990	0.976-1.004	0.144

Group / Variable	MD, early-onset (n=45)			MD, late-onset (n=260)		
	OR	95% CI	P	OR	95% CI	P
S'/L' + *L'/L'*	0.843	0.385-1.844	0.669	0.565	0.379-0.844	0.005
Gender	1.130	0.533-2.398	0.749	3.227	2.263-4.601	<0.001
Age	0.818	0.767-0.872	<0.001	0.998	0.984-1.011	0.735

Group / Variable	MD, moderate (n=84)			MD, severe (n=221)		
	OR	95% CI	P	OR	95% CI	P
S'/L' + *L'/L'*	0.573	0.318-1.031	0.063	0.659	0.437-0.994	0.067
Gender	2.493	1.506-4.127	<0.001	3.103	2.142-4.494	<0.001
Age	0.982	0.962-1.001	0.068	0.984	0.971-0.998	0.029

The reference groups are *S'/S'* and female, respectively. FH=Family history; MD=Major depression; OR=Odds ratio; CI=Confidence interval

DISCUSSION

In terms of susceptibility to major depression and/or response to antidepressant therapy, the SERT gene is a plausible candidate gene for this disorder. The present study did not find any associations between MD and the bi-allelic *5-HTTLPR* polymorphism, even when patients were divided according to gender [Tables 1 and 3]. However, we observed a positive association between MD and the tri-allelic *5-HTTLPR* polymorphism after correction for age and gender in a Han Chinese population, especially in male subjects [Tables 3 and 5]. Our results are in contrast with previous reports of a significant association between MD and the bi-allelic *5-HTTLPR* polymorphism.[15-17] However, our results are also in agreement with many studies that did not find an association between MD and the bi-allelic *5-HTTLPR* polymorphism.[19-21] There are several possible reasons for these divergent results. First, several bi-allelic *5HTTLPR* and tri-allelic *5-HTTLPR* variants have been identified in recent years that vary according to ethnicity. In the bi-allelic *5-HTTLPR* polymorphism, our control population had a lower frequency of the *L*/*L* genotype (7.3%, Table 1) than Caucasian populations (approximately 30–35%).[15,17] With the tri-allelic *5-HTTLPR* polymorphism, the lower-expressing L_G alleles accounted for 44.0% of the L alleles (n = 81 of 184) in the study and were functionally grouped with the S allele and designated as S'. The frequencies of L'/L', L'/S', and S'/S' variants of tri-allelic *5-HTTLPR* polymorphism in our Han Chinese control subjects (2.9%, 27.2%, and 70.0%, respectively) were different from the frequencies for Caucasian population (26.4%, 44.8%, and 28.8%, respectively) by Zalsman *et al*.[24] Consequently, ethnic variations in the frequencies of tri-allelic *5-HTTLPR* polymorphism may affect the association observed. The higher S' allele frequency in healthy control subjects may also mask the effect of the S' allele on mood disorders.[33] Second, the control groups in the various studies have not been consistently defined. In genetic association studies, the use of suitable controls is very important.[34] Thus, we excluded substance use disorders and other major or minor mental disorders in our controls because the lack of a carefully matched control group may cause spurious positive or negative results. Third, the diagnosis of MD and MD subtypes encompasses a high degree of heterogeneity; therefore, genetic studies may provide different results based on differences in definitions and composition of subgroup populations.[35,36] Investigation of the various subtypes in this disease could reduce contradictory

Table 5: Multiple logistic regression analysis of the tri-allelic *5-HTTLPR* polymorphism *(rs25531)* for risk of major depression, and its clinical subtypes

Male subjects						
Group	Major depression (*n*=118)					
Variable	OR	95% CI			*P*	
S'/L' + *L'/L'*	0.465	0.260-0.831			0.010	
Age	0.966	0.946-0.987			0.002	
Group	MD, positive FH (*n*=27)			MD, negative FH (*n*=91)		
Variable	OR	95% CI	*P*	OR	95% CI	*P*
S'/L' + *L'/L'*	0.495	0.175-1.396	0.184	0.454	0.239-0.863	0.016
Age	0.932	0.888-0.979	0.005	0.970	0.948-0.993	0.011
Group	MD, early-onset (*n*=28)			MD, late-onset (*n*=90)		
Variable	OR	95% CI	*P*	OR	95% CI	*P*
S'/L' + *L'/L'*	0.596	0.200-1.772	0.351	0.364	0.184-0.721	0.004
Age	0.745	0.667-0.833	<0.001	0.989	0.967-1.011	0.328
Group	MD, moderate (*n*=36)			MD, severe (*n*=82)		
Variable	OR	95% CI	*P*	OR	95% CI	*P*
S'/L' + *L'/L'*	0.200	0.058-0.684	0.010	0.605	0.324-1.131	0.115
Age	0.954	0.918-0.992	0.018	0.966	0.943-0.990	0.006
Female subjects						
Group	Major depression (*n*=187)					
Variable	OR	95% CI			*P*	
S'/L' + *L'/L'*	0.772	0.465-1.284			0.319	
Age	0.995	0.978-1.011			0.521	
Group	MD, positive FH (*n*=54)			MD, negative FH (*n*=133)		
Variable	OR	95% CI	*P*	OR	95% CI	*P*
S'/L' + *L'/L'*	0.816	0.397-1.675	0.579	0.755	0.435-1.310	0.317
Age	0.976	0.954-0.999	0.042	1.003	0.985-1.021	0.781
Group	MD, early-onset (*n*=18)			MD, late-onset (*n*=169)		
Variable	OR	95% CI	*P*	OR	95% CI	*P*
S'/L' + *L'/L'*	1.086	0.334-3.529	0.890	0.750	0.445-1.263	0.279
Age	0.877	0.819-0.940	<0.001	1.003	0.986-1.021	0.717
Group	MD, moderate (*n*=48)			MD, severe (*n*=139)		
Variable	OR	95% CI	*P*	OR	95% CI	*P*
S'/L' + *L'/L'*	0.983	0.476-2.031	0.963	0.704	0.406-1.219	0.210
Age	0.994	0.970-1.018	0.603	0.995	0.977-1.013	0.569

The reference group is *S'/S'*. FH=Family history; MD=Major depression; OR=Odds ratio; CI=Confidence interval

factors of this condition and uncover an association between genes and specific subtypes.

Regarding clinical subtypes in patients with MD, we hypothesized that the tri-allelic *5-HTTLPR* polymorphism might be associated with early-onset or more severe MD or MD with a family history in female subjects. This hypothesis was based on previous reports that individuals with a family history and/or early-onset may have a genetic predisposition to develop MD suggested that the lower-expressing allele was associated with genotype-environmental risk in Caucasian females with MD.[24,37] However, the present study found the opposite results in that the lower-expressing allele predicted late-onset MD in male patients [Tables 3 and 5]. Distinct ethnic populations have different cultures, different lifestyles, and different environmental stresses. These factors may affect the development of MD in different populations and genders and may affect the association between MD and the tri-allelic *5-HTTLPR* polymorphism. To our knowledge, patients with early-onset MD have more opportunities to develop manic episodes, which mean some patients might be cases of bipolar disorders with the initial depressive episode. Thus, patients with late-onset MD might be representative of the general population of patients with MD. Moreover, male patients with MD without a family history were often recruited from the late-onset MD population. As a result, we found a weak association between those patients and controls. In addition, there were only 36 male patients with moderate MD in this study, so the weak association of this population with the tri-allelic *5-HTTLPR* polymorphism may be a chance effect.

Ethnic stratification may be an important issue to reset population gene frequencies. It may produce a false positive or false negative result by chance when investigating the association between an allele and a disease.[38] However, all of our subjects were unrelated Han Chinese subjects that came from a population in the Northern part of Taiwan that is known to be genetically homogeneous.[39-41] All of the biological grandparents of our recruited subjects were of Han Chinese ancestry. Therefore, it was unlikely that stratification produced a false-positive result in our study. Moreover, the healthy control subjects were interviewed with the Chinese version of the modified schedule of SADS-L[27,28] to rule out psychiatric disorders. Thus, it was unlikely that cases of affective disorder were included in our control group.

With the sample size of 618 participants, we had a power of 0.60 to detect a small effect, 1.00 to detect a medium effect, and 1.00 to detect a large effect on the *5HTTLPR* genotype distributions. With a power of 0.80, we were able to detect an effect size of 0.125 for a significant difference in genotype distributions. Using the allele frequencies of these two polymorphisms (n = 1236), this study had a power of 0.94 to detect a small effect, 1.00 to detect a medium

effect, and 1.00 to detect a large effect. With regard to the patient subgroups, the statistical power was considerably relatively lower due to the small sample sizes. In this power analyses, effect size conventions were determined according to the method of Buchner *et al*.[32] as follows: Small effect size = 0.10, medium effect size = 0.30, and large effect size = 0.50 ($\alpha = 0.05$).

There are several potential limitations to this study. First, our sample size was large enough to detect an effect of SERT polymorphism, but after dividing subjects into different gender groups or/and MD subgroups, the sample size of subgroups became considerably smaller. Thus, these results should be interpreted with caution and further research regarding MD subgroups may be required. Second, our study design utilized a cross-sectional approach, and the demographic data and clinical diagnosis during the time of interview may be not the same as those assessed several years later. For example, some of the cases included in the unipolar disorder category may ultimately develop manic episode and the diagnosis will be revised as bipolar disorder, especially for those with a single depressive episode[42] or early-onset MD.[43,44] These possibilities may also lead to bias in the results, so patients across subtypes should receive follow-up in the future. Third, literature reviews revealed that certain gene-environment interactions have been associated with MD.[24,37,45] In this study, we investigated the bi-allelic *5-HTTLPR* and tri-allelic *5-HTTLPR* polymorphisms of the 5-HTT gene only. This study did not evaluate the degree of psychosocial stressors quantitatively or gene-environment interactions, but these reasons might explain our contradictory results when compared with the report on Caucasian populations. In spite of the above-mentioned limitations, our results revealed a positive association between the tri-allelic *5-HTTLPR* polymorphism and MD after correction for age and gender, especially in male subjects in a Han Chinese population.

CONCLUSIONS

Our results showed no association between MD and the bi-allelic *5-HTTLPR* polymorphism in a Han Chinese population after correction for age and gender. However, we did find a positive association between MD and the tri-allelic *5-HTTLPR* polymorphism when correcting for these covariates, especially in male subjects. Therefore, we suggest that the S'/S' genotype of the tri-allelic *5-HTTLPR* polymorphism may be a risk factors that increases the susceptibility to MD in male Han Chinese population. Continued investigations utilizing a larger patient population and different ethnic groups or subdiagnosis groups may be warranted.

Acknowledgment

The authors thank Miss. Fan-Yi Lin and Chen-Chang Huang for their assistance in the preparation of this manuscript.

Financial support and sponsorship

This work was supported by National Science Council NSC101-2325-B-016-003 (SYH), Tri-Service General Hospital TSGHC103-133, TSGH-C104-129, TSGH-C105-124 (SYH); and Medical Affairs Bureau, Ministry of National Defense, Taiwan, MAB-104-073 (SYH), DOD102-114 (SYH), 103-M077(CHY). These funding agencies played no role in the study design, collection, analysis or interpretation of data, the writing of the report, or the decision to submit the paper for publication. We would like to thank Miss Yun-Hsin Lin for her assistance in the preparing this manuscript.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Holmans P, Zubenko GS, Crowe RR, DePaulo JR Jr., Scheftner WA, Weissman MM, *et al.* Genomewide significant linkage to recurrent, early-onset major depressive disorder on chromosome 15q. Am J Hum Genet 2004;74:1154-67.
2. Sullivan GM, Oquendo MA, Huang YY, Mann JJ. Elevated cerebrospinal fluid 5-hydroxyindoleacetic acid levels in women with comorbid depression and panic disorder. Int J Neuropsychopharmacol 2006;9:547-56.
3. Stuerenburg HJ, Ganzer S, Müller-Thomsen T. 5-Hydroxyindoleacetic acid and homovanillic acid concentrations in cerebrospinal fluid in patients with Alzheimer's disease, depression and mild cognitive impairment. Neuro Endocrinol Lett 2004;25:435-7.
4. Salomon RM, Miller HL, Delgado PL, Charney D. The use of tryptophan depletion to evaluate central serotonin function in depression and other neuropsychiatric disorders. Int Clin Psychopharmacol 1993;8 Suppl 2:41-6.
5. Newberg AB, Amsterdam JD, Wintering N, Ploessl K, Swanson RL, Shults J, *et al.* 123I-ADAM binding to serotonin transporters in patients with major depression and healthy controls: A preliminary study. J Nucl Med 2005;46:973-7.
6. Reimold M, Batra A, Knobel A, Smolka MN, Zimmer A, Mann K, *et al.* Anxiety is associated with reduced central serotonin transporter availability in unmedicated patients with unipolar major depression: A [11C] DASB PET study. Mol Psychiatry 2008;13:606-13, 557.
7. Herold N, Uebelhack K, Franke L, Amthauer H,

Luedemann L, Bruhn H, *et al.* Imaging of serotonin transporters and its blockade by citalopram in patients with major depression using a novel SPECT ligand [123I]-ADAM. J Neural Transm (Vienna) 2006;113:659-70.
8. Bech P, Cialdella P, Haugh MC, Birkett MA, Hours A, Boissel JP, *et al.* Meta-analysis of randomised controlled trials of fluoxetine v. placebo and tricyclic antidepressants in the short-term treatment of major depression. Br J Psychiatry 2000;176:421-8.
9. Ramamoorthy S, Bauman AL, Moore KR, Han H, Yang-Feng T, Chang AS, *et al.* Antidepressant- and cocaine-sensitive human serotonin transporter: Molecular cloning, expression, and chromosomal localization. Proc Natl Acad Sci U S A 1993;90:2542-6.
10. Lesch KP, Wolozin BL, Murphy DL, Reiderer P. Primary structure of the human platelet serotonin uptake site: Identity with the brain serotonin transporter. J Neurochem 1993;60:2319-22.
11. Heils A, Teufel A, Petri S, Stöber G, Riederer P, Bengel D, *et al.* Allelic variation of human serotonin transporter gene expression. J Neurochem 1996;66:2621-4.
12. Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, *et al.* Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 1996;274:1527-31.
13. Greenberg BD, Tolliver TJ, Huang SJ, Li Q, Bengel D, Murphy DL. Genetic variation in the serotonin transporter promoter region affects serotonin uptake in human blood platelets. Am J Med Genet 1999;88:83-7.
14. Little KY, McLaughlin DP, Zhang L, Livermore CS, Dalack GW, McFinton PR, *et al.* Cocaine, ethanol, and genotype effects on human midbrain serotonin transporter binding sites and mRNA levels. Am J Psychiatry 1998;155:207-13.
15. Willeit M, Praschak-Rieder N, Neumeister A, Zill P, Leisch F, Stastny J, *et al.* A polymorphism (*5-HTTLPR*) in the serotonin transporter promoter gene is associated with DSM-IV depression subtypes in seasonal affective disorder. Mol Psychiatry 2003;8:942-6.
16. Collier DA, Stöber G, Li T, Heils A, Catalano M, Di Bella D, *et al.* A novel functional polymorphism within the promoter of the serotonin transporter gene: Possible role in susceptibility to affective disorders. Mol Psychiatry 1996;1:453-60.
17. Hoefgen B, Schulze TG, Ohlraun S, von Widdern O, Höfels S, Gross M, *et al.* The power of sample size and homogenous sampling: Association between the *5-HTTLPR* serotonin transporter polymorphism and major depressive disorder. Biol Psychiatry 2005;57:247-51.
18. Yu YW, Tsai SJ, Chen TJ, Lin CH, Hong CJ. Association study of the serotonin transporter promoter polymorphism and symptomatology and antidepressant response in major depressive disorders. Mol Psychiatry 2002;7:1115-9.
19. Willis-Owen SA, Turri MG, Munafò MR, Surtees PG, Wainwright NW, Brixey RD, *et al*. The serotonin transporter length polymorphism, neuroticism, and depression: A comprehensive assessment of association. Biol Psychiatry 2005;58:451-6.
20. Frisch A, Postilnick D, Rockah R, Michaelovsky E, Postilnick S, Birman E, *et al.* Association of unipolar major depressive disorder with genes of the serotonergic and dopaminergic pathways. Mol Psychiatry 1999;4:389-92.
21. Zaboli G, Jönsson EG, Gizatullin R, De Franciscis A, Asberg M, Leopardi R. Haplotype analysis confirms association of the serotonin transporter (5-HTT) gene with schizophrenia but not with major depression. Am J Med Genet B Neuropsychiatr Genet 2008;147:301-7.
22. Nakamura M, Ueno S, Sano A, Tanabe H. The human serotonin transporter gene linked polymorphism (*5-HTTLPR*) shows ten novel allelic variants. Mol Psychiatry 2000;5:32-8.
23. Hu XZ, Lipsky RH, Zhu G, Akhtar LA, Taubman J, Greenberg BD, *et al.* Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive disorder. Am J Hum Genet 2006;78:815-26.
24. Zalsman G, Huang YY, Oquendo MA, Burke AK, Hu XZ, Brent DA, *et al.* Association of a triallelic serotonin transporter gene promoter region (*5-HTTLPR*) polymorphism with stressful life events and severity of depression. Am J Psychiatry 2006;163:1588-93.
25. Nurnberger JI Jr., Foroud T, Flury L, Meyer ET, Wiegand R. Is there a genetic relationship between alcoholism and depression? Alcohol Res Health 2002;26:233-40.
26. Huang SY, Lu RB, Ma KH, Shy MJ, Lin WW. Norepinephrine transporter polymorphisms T-182C and G1287A are not associated with alcohol dependence and its clinical subgroups. Drug Alcohol Depend 2008;92:20-6.
27. Endicott J, Spitzer RL. A diagnostic interview: The schedule for affective disorders and schizophrenia. Arch Gen Psychiatry 1978;35:837-44.
28. Merikangas KR, Stevens DE, Fenton B, Stolar M, O'Malley S, Woods SW, *et al.* Co-morbidity and familial aggregation of alcoholism and anxiety disorders. Psychol Med 1998;28:773-88.
29. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. Fourth edition. Washington, DC: American Psychiatric Association; 1994.

30. Cusin C, Yang H, Yeung A, Fava M. Rating scales for depression. In: Baer L, Blais MA, editors. Handbook of Clinical Rating Scales and Assessment in Psychiatry and Mental Health. New York: Humana Press; 2010. p. 7-35.
31. Wendland JR, Martin BJ, Kruse MR, Lesch KP, Murphy DL. Simultaneous genotyping of four functional loci of human SLC6A4, with a reappraisal of 5-HTTLPR and rs25531. Mol Psychiatry 2006;11:224-6.
32. Buchner A, Faul F, Erdfelder E. G-power: A Priori, *Post Hoc*, and Compromise Power Analyses for the Macintosh. Ver. 2.1.1. Trier, Germany: University of Trier; 1996.
33. Ohara K, Nagai M, Tsukamoto T, Tani K, Suzuki Y, Ohara K. Functional polymorphism in the serotonin transporter promoter at the SLC6A4 locus and mood disorders. Biol Psychiatry 1998;44:550-4.
34. Huang SY, Lin WW, Ko HC, Lee JF, Wang TJ, Chou YH, *et al*. Possible interaction of alcohol dehydrogenase and aldehyde dehydrogenase genes with the dopamine D2 receptor gene in anxiety-depressive alcohol dependence. Alcohol Clin Exp Res 2004;28:374-84.
35. Serretti A, Lilli R, Smeraldi E. Pharmacogenetics in affective disorders. Eur J Pharmacol 2002;438:117-28.
36. Serretti A, Lilli R, Lorenzi C, Lattuada E, Cusin C, Smeraldi E. Serotonin transporter gene (*5-HTTLPR*) and major psychoses. Mol Psychiatry 2002;7:95-9.
37. Eley TC, Sugden K, Corsico A, Gregory AM, Sham P, McGuffin P, *et al.* Gene-environment interaction analysis of serotonin system markers with adolescent depression. Mol Psychiatry 2004;9:908-15.
38. Kidd KK. Associations of disease with genetic markers: Déjà vu all over again. Am J Med Genet 1993;48:71-3.
39. Lu RB, Lin WW, Lee JF, Ko HC, Shih JC. Neither antisocial personality disorder nor antisocial alcoholism is associated with the MAO-A gene in Han Chinese males. Alcohol Clin Exp Res 2003;27:889-93.
40. Hsu YP, Loh EW, Chen WJ, Chen CC, Yu JM, Cheng AT. Association of monoamine oxidase A alleles with alcoholism among male Chinese in Taiwan. Am J Psychiatry 1996;153:1209-11.
41. Chang CC, Lu RB, Chen CL, Chu CM, Chang HA, Huang CC, *et al.* Lack of association between the norepinephrine transporter gene and major depression in a Han Chinese population. J Psychiatry Neurosci 2007;32:121-8.
42. Kunugi H, Hattori M, Kato T, Tatsumi M, Sakai T, Sasaki T, *et al.* Serotonin transporter gene polymorphisms: Ethnic difference and possible association with bipolar affective disorder. Mol Psychiatry 1997;2:457-62.
43. Angst J, Cassano G. The mood spectrum: Improving the diagnosis of bipolar disorder. Bipolar Disord 2005;7 Suppl 4:4-12.
44. Rybakowski JK, Suwalska A, Lojko D, Rymaszewska J, Kiejna A. Types of depression more frequent in bipolar than in unipolar affective illness: Results of the Polish DEP-BI study. Psychopathology 2007;40:153-8.
45. Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, *et al.* Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. Science 2003;301:386-9.