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ORIGINAL RESEARCH ARTICLE

Association study of the serotonin transporter promoter polymorphism and symptomatology and antidepressant response in major depressive disorders

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The serotonin transporter (5-HTT) is the site of primary action for the selective serotonin reuptake inhibitors (SSRIs). Previous Western reports have demonstrated that the *l* allele of the 5-HTT gene-linked polymorphic-region (5-HTTLPR) polymorphism is associated with better SSRI antidepressive effects than the *s* allele, however, another study of a Korean population has produced a contrasting finding. The present study tested the hypothesis that the 5-HTTLPR genetic polymorphism is associated with SSRI antidepressant response by evaluating total and cluster depressive symptoms for 121 Chinese patients diagnosed with major depression. Analysis of the results reveals that patients with the *l/l* genotype had a significantly better response to SSRI (fluoxetine) when compared with *s* allele carriers, as evaluated on the basis of total ($P = 0.013$), core ($P = 0.011$), and psychic-anxiety ($P = 0.005$) and somatic-anxiety ($P = 0.002$) Hamilton Depression Rating Scale-score percentage change. Our findings confirm reports that the *l* allele is associated with better SSRI response.
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Introduction

The introduction of fluoxetine in 1988, followed by other selective serotonin reuptake inhibitors (SSRIs; citalopram, fluvoxamine, paroxetine and sertraline), has revolutionized the treatment of major depressive disorders (MDD) because of the favourable side-effect profiles. Not all MDD patients benefit from SSRI treatment, however, with partial or zero response demonstrated for 29–46% of MDD patients.[1] These inter-individual variations in response to SSRI treatment have prompted several studies aimed at finding biological markers to predict therapeutic response, facilitating determination of optimal drug selection (eg cortisol responses to *d*-fenfluramine,[2] plasma total tryptophan/large neutral amino acids ratio,[3] lymphocyte glucocorticoid receptor density,[4] and plasma free 3-methoxy-4-hydroxyphenylglycol[5]).

The primary mode of action for SSRIs is binding to the serotonin transporter (5-HTT), inhibiting its capacity to transport serotonin and thus modulating serotonergic activity. It has been determined that, in terms of transcriptional activity, the long (*l*) variant in the 5-HTT gene-linked polymorphic region (5-HTTLPR) is more than twice as active as the short (*s*) variant, with differences in 5-HTT mRNA synthesis and 5-HTT expression.[6] Lesch *et al* have demonstrated that carriers of the *s* variant of the polymorphism had higher anxiety-related traits than homozygotes of the *l* variant in healthy subjects.[7] Variations in the 5-HTTLPR polymorphism have been associated with major depression.[8] However, study of the 5-HTTLPR polymorphism in Japanese patients with major depressions could not find any differences between patients and the control group.[9] An association between fluvoxamine response and 5-HTTLPR polymorphism was first reported by Smeraldi *et al*, in 1998,[10] with better response to fluvoxamine demonstrated for the *l* allele carriers (*l*/*l* and *l*/*s*) in comparison to homozygotes for the *s* variant (*s*/*s*). Two subsequent studies produced similar findings.[11,12] For late-life MDD, Pollock *et al*, demonstrated significantly more rapid improvement for depressive symptoms for paroxetine-treated patients bearing the *l*/*l* genotype than for analogs carrying the *s* allele.[11] In another paroxetine study, *l*/*l* genotype patients had significantly better response than *s*/*s* genotype analogs, with those bearing heterozygote (*l*/*s*) falling between the two.[12] A contrasting finding was reported in the study of a Korean population, however, with the frequency

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of the *s* variant homozygote significantly higher for responders than for non-responders.[13] The hypothesis that the discrepancy between these studies is likely to be the result of ethnic differences prompted this investigation of the association between the 5-HTTLPR promoter polymorphism and SSRI treatment response in a Chinese population. In addition, the relationship between 5-HTTLPR genotypes and improvement for specific cluster symptoms was explored.

Materials and methods

For this investigation, patients with moderate-to-severe depression were recruited from a psychiatric clinic. Inclusion criteria were: (1) diagnosis of MDD according to DSM-IV guidelines; (2) minimum baseline score of 18 on the 21-item Hamilton Depression Rating Scale (HAM-D);[14] and (3) presence of depressive symptoms for at least 2 weeks before entry into the study, without antidepressant treatment (patients were fresh cases or had quit antidepressants for more than 2 weeks). Exclusion criteria were additional diagnoses on Axis 1 (including substance abuse, generalized anxiety disorders, panic disorders or obsessive compulsive disorders) of the DSM-IV, personality disorders, pregnancy, attempted suicide, and major medical and/or neurological disorders. A total of 121 MDD patients (male/female: 70/51; mean age: 44.7 (SD: 16.7) years) were enrolled in this study. The sample consisted entirely of ethnic Chinese, with informed consents obtained from all participants. Treatment efficacy was evaluated by one investigator (YWY), blind to patient's genotype, administering the HAM-D before and after the 4-week antidepressant treatment. 'Responders' were defined as at least 50% decrease in the HAM-D total score after 4 weeks of mediation and 'remitters' were defined as subjects having a HAM-D total score of 7 or less points after 4 weeks of mediation. All the 121 patients took fluoxetine (range: 20–60 mg day^{-1}; mean 29.4 ± 10.4 mg day^{-1}).

Genomic DNA was extracted from EDTA-containing venous blood samples using Lahiri and Nurnberger's protocol.[15] For genotyping, fragments of 5-HTTLPR were amplified by polymerase chain reaction (PCR) using the primers as described with 5-HTTLPR-3: ATGCCAGCACCTAACCCCTAATG plus 5-HTTLPR-2: GAGGGACTGAGCTGGACAACCAC.[16] Polymorphisms of 5-HTTLPR were determined according to the size which was determined from agarose-gel electrophoresis. The sizes of the *s* and *l* 5-HTTLPR alleles were 469–470 bp and 511–513 bp, respectively.

To evaluate specific cluster depressive symptoms, the HAM-D items were grouped according to the following factors: core (Items 1, 2, 7, 8, 10, 13), sleep (Items 4, 5, 6), activity (Items 7, 8), psychic anxiety (Items 9, 10), somatic anxiety (Items 11, 12, 13), and delusion (Items 2, 15, 20), as described by Serretti *et al*.[17] Both the total and subcategory HAM-D scores were subjected to statistical analysis. Therapeutic response was evaluated by the percentage score reduction in total and subcategory HAM-D scores ((baseline score – 4-week score) × 100/baseline score).

The categorical data were analyzed using the chi-square test or the Fisher exact test if necessary. Gender differences for continuous variables were evaluated by student *t*-test. Genotype differences for continuous variables were evaluated using one-way analysis of variance followed by the LSD multiple range tests for comparison among groups. A linear-regression analysis was performed with total HAMD score percentage reduction as the dependent variable, and with 5-HTTLPR genotype as the predictor variables. The criterion for significance was set at $P < 0.05$ for all of the tests. Data are presented as mean (SD).

Results

The genotype distribution for the 5-HTTLPR polymorphism, which was in Hardy–Weinberg equilibrium, and the HAM-D scores for 121 MDD patients are presented in Table 1. No significant differences were demonstrated for age and sex comparing the three 5-HTTLPR genotype groups. There were also no significant differences comparing the three genotype groups for the baseline total HAM-D scores. Marginal significant differences were found in the core ($P = 0.030$) and sleep ($P = 0.037$) sub-HAM-D scores among the three 5-HTTLPR genotype groups.

Therapeutically, patients bearing the *l/l* genotype had a significantly better response to antidepressants when compared with *s* allele carriers, as evaluated on the basis of total ($P = 0.013$; power is 73% with effect size = 0.0708), core ($P = 0.011$), psychic anxiety ($P = 0.005$) and somatic anxiety ($P = 0.002$) HAM-D score percentage reduction (Table 1). A linear-regression analysis was performed with 5-HTTLPR genotype as the predictor variables, and it was demonstrated that the 5-HTTLPR *l/l* genotype was a significant predictor of therapeutic response ($P = 0.007$; $r^2 = 0.051$). 'Responders' were also more commonly found in patients of the 5-HTTLPR *l/l* genotype than in patients of other genotypes ($P = 0.019$; power = 75%), while the percentage of the 'remitters' was similar among the three genotype patients ($P = 0.150$; power = 47%) (Table 1). No difference in total HAM-D score percentage reduction was found between either gender ($P = 0.862$).

Discussion

In this study of a Chinese sample population, it was demonstrated that patients bearing the 5-HTTLPR *l/l* genotype had a better response to SSRI treatment than *s* allele carriers. Although this finding confirms three previous Western reports,[10–12] it contrasts with a Korean study.[13] There are several possible explanations for this discrepancy. Firstly, the 5-HTTLPR polymorphism may be in linkage disequilibrium with a functional variant that affects SSRI response, and the extent

Table 1 Demographic data and fluoxetine therapeutic response among three 5-HTTLPR genotype groups

	5-HTTLPR genotypes			*P*
	l/l (n = 13)	*l/s (n = 36)*	*ss (n = 72)*	
Sex (M/F)	4/9	16/20	31/41	0.673
Age	53.4 (16.2)	46.1 (14.4)	42.2 (18.1)	0.305
Baseline HAM-D score				
Core	10.2 (2.3)	11.6 (2.7)	11.9 (1.8)	0.030
Sleep	4.6 (1.5)	4.6 (1.8)	5.3 (1.2)	0.037
Activity	2.2 (0.8)	2.9 (1.4)	2.9 (1.1)	0.132
Psychic anxiety	3.6 (1.0)	4.1 (1.0)	4.2 (1.0)	0.122
Somatic anxiety	5.5 (1.6)	5.5 (1.4)	5.6 (1.6)	0.842
Delusion	3.2 (1.2)	3.1 (1.6)	3.4 (1.3)	0.769
HAMD total	28.2 (5.8)	29.3 (5.6)	31.0 (3.8)	0.053
Responders/non-responders	9/4	10/26	21/51	0.019
Remitters/non-remitters	2/11	1/35	2/70	0.150
HAM-D score change (%)				
Core	51.3 (21.8)	35.8 (21.4)	30.7 (23.2)	0.011
Sleep	65.4 (30.8)	52.8 (31.4)	48.1 (31.3)	0.181
Activity	47.4 (35.1)	36.3 (32.8)	29.3 (30.3)	0.140
Psychic anxiety	56.5 (13.3)	32.6 (23.9)	30.5 (28.9)	0.005
Somatic anxiety	44.8 (23.3)	21.2 (22.2)	20.8 (22.8)	0.002
Delusion	32.3 (29.5)	27.4 (30.3)	21.8 (34.0)	0.468
HAMD total	52.4 (17.6)	36.4 (20.3)	32.7 (23.1)	0.013

Data are mean (SD).
Responders were defined as at least 50% decrease in the HAM-D total score after 4 weeks of mediation.
Remitters were defined as subjects having a HAM-D total score of 7 or less points after 4 weeks of mediation.
HAM-D score change (%) = (baseline score − 4-week score) × 100/baseline score.

of this linkage disequilibrium is not similar for all ethnic populations. Thus, the association between 5-HTTLPR genetic variants and SSRI treatment response may be ethnicity-dependent. This is less likely, however, since it seems reasonable to assume that the Chinese and Koreans are more likely to be similar genetically, given their geographical proximity. Secondly, this discrepancy may have resulted from differences in MDD severity or subtype for MDD populations enrolled in the various studies. Thirdly, compared with the European American population, the *l* allele frequency in Chinese or Korean populations was much lower.[10–13] The *s*/*s* population is five times bigger than the *l*/*l* and two times bigger than the heterozygous in our or Korean studies. Only 13 of our 121 patients and five of the 120 Korean patients were *l*/*l* homozygotes. Thus, the results will be likely influenced by a chance finding and further study with a larger sample is needed. Finally, differences in therapeutic-response assessment, duration of treatment and SSRI type may have produced the different results. For example, a study stratifying the patients into responders and non-responders may lead to the reclassification of some non-responders as responders in a longer follow-up. In this study, patients were assessed after 4-week medication, while in the Korean study patients were assessed after a 6-week trial.

The 5-HTTLPR allele frequency examined differed for this Chinese population in comparison to the European-American samples.[10–12] Thus, if carriage of the *l* allele is associated with better SSRI response, it is likely that Chinese MDD patients would have a less favourable response rate than their Western counterparts. In this study, however, it was determined that the 5-HTTLPR polymorphism accounts for 5.1% of the variance in SSRI response. This is very close to the value of 7% reported in previous studies.[18] Moreover, the same article reported that a tryptophan hydroxylase genetic polymorphism (A218C) explained about 5% of the variance in antidepressant efficacy and the effect was independent from that of the 5-HTTLPR polymorphism, suggesting an additive effect.[18] As therapeutic antidepressant effect may involve the interaction of many different genes, a single gene may, therefore, play only a relatively minor role in an intricate mechanism and, thus, not be strongly associated with antidepressant response. For example, a recent study of MDD patients demonstrated that angiotensin *I*-converting enzyme *D*-allele carriers had a better therapeutic outcome than analogs bearing the *I/I* genotype.[19] Analyses of the interactions of multiple genes, implicated in the pharmacokinetics or pharmacodynamics of SSRIs, may merit attention in future pharmacogenetic study of antidepressants.

We demonstrated that, for our patient population, there were no significant differences comparing the

three genotype groups for the baseline total HAM-D scores. However, marginal significant differences were found in the core and sleep sub-HAM-D scores among the three 5-HTTLPR genotype groups (without correction for multiple comparisons). A previous report using similar analytical techniques found no association of the 5-HTTLPR variants and MDD symptomatology.[17] This finding and our results suggest that this particular 5-HTTLPR polymorphism plays no major role in MDD symptomatology. In our analysis of the associations between specific symptoms and this 5-HTTLPR polymorphism, however, improvements were demonstrated for core, psychic and somatic anxiety item clusters, but not sleep, activity and delusion analogs. This finding is of interest since a relationship has been demonstrated between the 5-HTTLPR variants and anxiety-related traits.[7] In addition, SSRIs have recently been widely used for anxiety disorders such as panic and obsessive-compulsive disorder, social phobia and generalized anxiety disorder.[20] It would be of interest to test whether the 5-HTTLPR polymorphism may also be a predictor for efficacy of SSRI treatment for these dysfunctions.

Since the 5-HTTLPR polymorphism may affect 5-HTT gene transcription,[6] we suggest that using the 'knockout' or 'knockdown' strategy may provide further elaboration of 5-HTT's contribution for mediation of the effects of SSRIs. For example, a leading hypothesis for the therapeutic actions of SSRI is desensitization of somatodendritic serotonin 5-HT1A autoreceptors in the midbrain raphe.[21] Recent studies demonstrated that altered expression and function of serotonin 5-HT1A receptor in mice lacking the 5-HTT may partially explain the association of the 5-HTTLPR polymorphism and SSRI therapeutic effects.[22–24]

One limitation of this study is that plasma levels of fluoxetine were not analyzed. However, this effect is minimal since a previous study had demonstrated that there were no significant relationships between fluoxetine blood levels and clinical response in depressed patients.[25]

In summary, it was demonstrated that, for our Taiwanese Chinese population, patients bearing the 5-HTTLPR *l/l* genotype had a superior response to SSRI treatment than *s* allele-carrier analogs, supporting Western reports. Further, our results suggest an association between 5-HTTLPR genotypes and improvement for anxiety-cluster symptoms.

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