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Lack of Association between Serotonin Transporter Promoter Gene Polymorphism and Citalopram Response in Major Depressive Disorder

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Abstract

The 5-HTT gene is a candidate gene for influencing the clinical response to antidepressant treatment. The purpose of this gene study was to determine the relationship between serotonin transporter gene polymorphism at the SLC6A4 and the response to citalopram in a Korean population with major depressive disorder (MDD). Citalopram was administered for 8 weeks to the 80 patients who completed this study. The severity of depression was assessed with the 21-item Hamilton Depression Rating scale, and the 5-HTTLPR genotypes in the patients were determined using the polymerase chain reaction. Our result did not showed significant differences in, allele, and carrier distribution between the normal group and MDD patients. This study suggest that polymorphism of the 5HTT gene was not associated with citalopram response to MDD in the Korean population.

Keywords: MDD, 5-HTTLPR, Polymorphism, Citalopram

The serotonergic neurotransmitter system is involved in the regulation of a wide range of psychological, behavioral, and biological functions[1]. The 5-HTTLPR polymorphism in the serotonin transporter (5-HTT) gene (SLC6A4) promoter region has been studied as a marker for selective serotonin reuptake inhibitors (SSRIs) treatment response[2-4]. The mechanism of action of most clinically used antidepressants is inhibition of serotonergic neurotransmitter systems, among which SSRIs are the most popular agents for treating depression in clinical settings since they show the highest selectiveness to the molecular target[5], selectively affecting the neurotransmitter system and producing the same effects as TCAs but at lower dosages. SSRIs have been developed as drugs with high selectivity for their molecular target, and constitute the greatest advance in the pharmacological treatment of depression in the general-practice setting in recent years[6].

The serotonin transporter (5-HTT) gene is located on chromosome 17q11.1-17q12, and two common polymorphisms have been described: (1) a variable-number tandem repeat (VNTR) located in intron 2 (5-HTT-VNTR), and (2) a deletion-insertion in the transcriptional control region approximately 1 kilobase upstream of the transcription initiation site (5-HTTLPR)[7]. The short form of the variant, designated as "s", is associated with a lower basal and induced transcriptional efficiency of the 5-HTT gene promoter, resulting in lower serotonin uptake activity, when compared with the long form, designated "l"[7,8]: the l/l genotype yields higher expression levels of functional 5-HTT gene than the s/l and s/s genotypes, for which the expression levels do not differ significantly. Therefore, both *in vitro* and *in vivo* studies have shown that the s allele leads to reduced transcription and expression.

Based on this biological background, there is considerable evidence that alterations in serotonergic neuronal function are involved in the pathophysiology of depression[9]. The polymorphism of the 5-HTT gene appears to be associated not only with susceptibility to affective disorders but also with the treatment response to SSRIs, because they are thought to exert

Table 1. Frequencies of genotypes, alleles and allele carriers of the 5-HTTLPR in MDD patients after 8 weeks of medication.

	N	Genotype frequencies			Allele frequencies		Allele-carrier frequencies	
		ll	Ls	ss	l	s	ll+ls	ss
Rp	60	4 (6.7%)	14 (23.3%)	42 (70.0%)	22 (18.3%)	98 (81.7%)	18 (30.0%)	42 (70.0%)
Non-Rp	20	1 (5.0%)	5 (25.0%)	14 (70.0%)	6 (15.0%)	34 (85.0%)	6 (30.0%)	14 (70.0%)
		χ^2=0.084, d.f.=2, P=0.959			χ^2=0.014, d.f.=1, P=0.906		χ^2=0.000, d.f.=1, P=1.0	
Rm	42	3 (7.1%)	9 (21.4%)	30 (71.4%)	15 (17.9%)	69 (82.1%)	12 (28.6%)	30 (71.4%)
Non-Rm	38	2 (5.3%)	10 (26.3%)	26 (68.4%)	14 (18.4%)	62 (81.6%)	12 (31.6%)	26 (68.4%)
		χ^2=0.339, d.f.=2, P=0.844			χ^2=0.009, d.f.=1, P=0.926		χ^2=0.086, d.f.=1, P=0.769	

[a]Intention-to-treat group. LOCF analysis was performed for missing data in HAMD scores

*Responsive group is defined by patients who shows more than or equal to 50% relative decreases of HAMD score at week 8 comparing to that of baseline

**Remission group is defined by patients whose HAMD score at week 8 is 7 or lower

their effects through binding to the 5-HTT gene and inhibiting serotonin reuptake. However, this remains controversial due to results differing between studies.

Among antidepressants, citalopram is distinguished by it being the most selective inhibitor of the serotonin transporter molecule. Its capability of inhibiting serotonin reuptake without appreciably inhibiting the uptake of noradrenaline or dopamine makes it a perfect target in pharmacogenetic studies aimed at minimizing interfering factors.

The goal of the present study was to elucidate whether the 5-HTTLPR polymorphism is associated with the citalopram antidepressant response in MDD patients.

Genotype Frequencies of the 5-HTTLPR in MDD Patients

Genotype and allele frequencies of 5-HTTLPR in this case-control study are listed in Table 1 lists the percentages of 5-HTTLPR polymorphism genotypes and carriers in the patient subgroups. In one subgroup, 60 patients were Rp and 20 patients were nonresponders (Non-Rp), while 42 patients were Rm and 38 patients were nonremitters (Non-Rm) in the other subgroup. Genotype and carrier distributions for the 5-HTTLPR polymorphism did not differ significantly between Rp/Rm and Non-Rp/Non-Rm.

Discussion

A functional polymorphism (5-HTTLPR) within the promoter of the serotonin gene has been identified, and the *in vitro* basal 5-HTT activity in the 5-HTTLPR long (l) allele carriers was found to be twice stronger than that in the short (s) allele, suggesting that serotonin transporter gene transcription is modulated by such variants[7,12,13].

With this background, the association between the polymorphism of 5-HTTLPR and antidepressant response has been investigated repeatedly in many researches, but the results have been inconsistent[4,14,15].

Studies with Caucasians have found that the response to SSRI treatment is better in patients with the 5-HTTLPR l/l genotype than in s-allele carriers[2,4]. This result appears to contradict studies involving Japanese and Korean populations that have found an association in the opposite direction[14,15].

However, our data revealed no correlation of the different 5-HTTLPR genotypes with Rp, Rm, Non-Rm, Non-Rp after either 4 or 8 weeks of citalopram treatment, Which does not confirm the previous studies of Arias *et al*. (s/s genotype of the 5-HTTLPR polymorphism was associated with Non-Rm at week 12)[16].

Thus, we cautiously consider the following two conclusions based on the results of the present study:

1. 5-HTTLPR is linked with unknown functional variants. It may be a marker in linkage disequilibrium associated with a functional site, rather than a functional polymorphism itself[17].

2. 5-HTTLPR and its genotypes do not appear to directly influence drug responses, but rather act as modifying conditioning agents. It is highly possible that drug response is a multifactorial phenotype, whose expression can be determined by several fac-

tors linked to different neurotransmitter systems.

This study is subject to several limitations. First, the plasma levels of citalopram were not analyzed, although their effect is expected to be minimal because a recent study has demonstrated no significant correlation between the plasma level of citalopram and clinical responses in depressed patients[16]. Second, although a single gene may affect the response to an antidepressant, it would only play a relatively minor role in a complex mechanism; that is, we should consider interactions between many different genes. Thirds, only 80 patients completed 8 weeks of citalopram treatment, and this small sample limits the generalizability of our findings since it widens the CIs and diminishes the power to detect associations between genotypes and phenotypes. Finally, we cannot exclude the presence of population stratification bias in this case-control study. However, because the Korean population is characterized by a relatively high degree of genetic homogeneity[18], we consider that such bias is unlikely in our sample.

In conclusion, our data showed that the polymorphism of the 5HTT gene at the SLC6A4 locus was not associated with citalopram response to MDD in the Korean population.

Methods

Subjects

Subjects were recruited by the Pharmacogenomic Research Center for Psychotropic Drugs at the Department of Psychiatry, Korea University College of Medicine, during 2003 and 2005. A total of 80 patients (females : males, 57 : 23; age, 52.66 ± 15.66 (mean ± SD) years) with MDD were included in the study. Trained psychiatrists examined all the subjects according to the Structured Clinical Interview for DSM-IV and the K-DIGS (Korean version of the Diagnostic Interview for Genetic Studies). The severity of depression was assessed using the HAMD. Only subjects with a minimum score of 17 on the HAMD scale were included in the study. The presence of any concomitant Axis-I diagnosis, single major depressive episode, together with somatic or neurological illnesses impairing the psychiatric evaluation represented exclusion criteria. The normal control group comprised 128 (females : males, 78 : 50; age, 51.13 ± 12.29 (mean ± SD) years) randomly selected and physically healthy individuals who visited the hospital for regular health screening and were ascertained to be free of major psychiatric problems following interviews with well-trained psychiatrists. We excluded subjects showing a personal or family history of substance abuse/dependence or major psychiatric disorders. The subjects in both the control and patient groups were all unrelated Korean individuals. Venous blood was drawn from each subject after obtaining written informed consent using a protocol approved by the Ethics Committee of the Korea University Medical Center.

Procedure

Prior to study entry, a 2-week wash-out period was applied to those MDD patients who were being treated with different drugs. During treatment, all 80 patients took citalopram daily (10-40 mg). Their clinical symptoms were evaluated with the HAMD at baseline and also after 1, 2, 4 and 8 weeks of treatment. Responders (Rp) were defined as a reduction in the HAMD scores of at least 50% relative to baseline, and remitters (Rm) were defined as an HAMD total score of 7 points or less after either 4 or 8 weeks of treatment.

DNA Analysis

The polymerase chain reaction (PCR) analysis employed 5′-GGC GTT GCC GCT CTG AAT GCC-3 and 5′-CAG GGG AGA TCC TGG GAG AGG T-3′ as the forward and reverse primers, respectively. The PCR was carried out in a total volume of 30 μL containing 10 × Taq buffer [500 mM KCl, 100 mM Tris-HCl (pH 8.3), and 15 mM $MgCl_2$], 2.4 μL of 2.5 mM dNTP, 1 μL of each of the primers (10 pmol/μL), and 0.5 μL of Taq polymerase (5 U/μL). Samples were amplified using a thermocycler (Perkin-Elmer, Boston, MA, USA) for an initial 5 min at 94°C, followed by 35 cycles of 30 sec at 94°C, 30 sec at 54°C, and 30 sec at 72°C. After a final 5 min at 72°C, the reaction was terminated at 4°C. PCR products were separated on 3% agarose gels supplemented with ethidium bromide to allow identification of the l (265 bp) and s (221 bp) variants.

Statistical Analysis

The presence of Hardy-Weinberg equilibrium for genotype frequencies in patients and normal control samples was assessed using chi-square tests. The categorical data were analyzed using the chi-square test or Fisher's exact test as appropriate. Genotype differences for continuous variables were evaluated using the *t*-test or one-way ANOVA, followed by the LSD multiple-range test for comparisons among groups. Odds ratios and 95% confidence intervals were calculated to estimate the effects of high-risk genotypes and alleles. The cutoff probability value for statistical significance was set at 0.05. A correction for multiple testing was not performed, because the study represented an exploration of a genetically complex trait in which the relationship between genotype and

phenotype has not been established. Consequently, such corrections might inappropriately increase the likelihood that real effects are missed (i.e., increased type II error rates)[10].

The power to detect associations in the sample size used was analyzed using G · Power[11]. All statistical analyses were performed using SPSS (version 10.0 for Microsoft Windows).

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