Association study of the serotonin transporter promoter polymorphism and mirtazapine antidepressant response in major depressive disorder

Rhee-Hun Kang\textsuperscript{a,c,d,e}, Ma-Li Wong\textsuperscript{b}, Myoung-Jin Choi\textsuperscript{a,c,d}, Jong-Woo Paik\textsuperscript{a,c,d,e}, Min-Soo Lee\textsuperscript{a,c,d,e,}\textsuperscript{*}

\textsuperscript{a} Depression Center, Korea University, Seoul, Republic of Korea
\textsuperscript{b} Center for Pharmacogenomics and Department of Psychiatry and Behavioral Sciences, University of Miami Miller School of Medicine, Miami, Florida, USA
\textsuperscript{c} Clinical Research Center for Depression, Korea University, Seoul, Republic of Korea
\textsuperscript{d} Institute of Human Behavior and Gene, Korea University, Seoul, Republic of Korea
\textsuperscript{e} Department of Psychiatry, Korea University of College of Medicine, Seoul, Republic of Korea

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Abstract

Modulations of serotonergic and noradrenergic systems are thought to be critical to the therapeutic effect of most antidepressants, and their efficacies have been shown to depend on a functional polymorphism within the promoter region of the serotonin transporter gene (5-HTTLPR). Mirtazapine has a dual-action profile, combining the enhancement of the noradrenergic neurotransmitter system with specific actions on particular serotonergic receptor subtypes. The goal of this study was to elucidate whether the 5-HTTLPR polymorphism is associated with the mirtazapine antidepressant response in subjects with major depressive disorder (MDD). One hundred and one MDD patients were evaluated during 4 weeks of mirtazapine treatment. 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 results showed that responses at the 2nd and 4th weeks were significantly better for the s/s genotype of the 5-HTTLPR polymorphism than for l-allele carriers. These results support our hypothesis that the response to noradrenergic and specific serotonergic antidepressants is significantly associated with the 5-HTTLPR polymorphism.

Keywords: 5-HTTLPR; MDD; Mirtazapine

1. Introduction

Most of the newer classes of antidepressant drugs exploit the putative involvement of both the serotonergic and noradrenergic systems in depression (Blier and de Montigny, 1994; Delgado et al., 1990, 1993). By modulating both of these monoaminergic systems, newer dual-action agents may provide greater benefits to more severely depressed patients and may reduce the time to an antidepressant response, mimicking the effects of giving SSRI and NE-selective tricyclic antidepressants (Nelson et al., 1991; Seth et al., 1992).

Polymorphisms of the serotonin transporter (5-HTT) gene appear to be associated not only with the susceptibility of affective disorders (Ogilvie et al., 1996; Bellivier et al., 1998; Furlong et al., 1998), but also with the treatment response to SSRI (Kim et al., 2000; Zanardi et al., 2000; Serretti et al., 2001; Rausch et al., 2002). A functional polymorphism (5-HTTLPR) within the promoter of the serotonin gene has been identified, and the in vitro basal 5-HTT activity was found to be twofold higher in the 5-HTTLPR long (l)-allele carriers than in short (s)-allele carriers, suggesting that 5-HTT gene transcription is modulated by such variants (Ramamoorthy et al., 1993; Heils et al., 1996; Lesch et al., 1996). Although the association between the 5-HTTLPR polymorphism and antidepressant responses has been investigated in many studies, the results have been inconsistent (Kim et al., 2000; Yoshida et al., 2002; Smits et al., 2004). However, a meta-analysis of previous researches by Serretti et al. (2007) reported that l-allele carriers...
respond better to SSRI. There is also other increasing evidence of an association between SSRI and 5-HTTLPR.

We previously reported that the long-term antidepressant response was better in major depressive disorder (MDD) patients with the 5-HTTLPR l-allele (Lee et al., 2004). It has also been reported that the effect of polymorphisms on the outcome of treatment may depend on the mechanism of antidepressant action (Pollock et al., 2000; Minov et al., 2001; Yoshida et al., 2002). Among several types of antidepressants, mirtazapine works differently from SSRIs, is associated with an increase in serotonin and norepinephrine in the CNS (Kasper et al., 1997), and is commonly used clinically, but its association with the 5-HTTLPR polymorphism has not investigated in detail.

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

2. Subjects and methods

2.1. Subjects

In-and-outpatients were recruited by the Pharmacogenomic Research Center for Psychotropic Drugs at the Department of Psychiatry, Korea University College of Medicine, during 2005 and 2006. Trained psychiatrists examined all the potential subjects using the Structured Clinical Interview for the fourth revision of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (Han and Hong, 2000).

The severity of depression was assessed using the 21-item Hamilton Depression Rating (HAMD-21) scale (Hamilton, 1967) and the CGI-S (Clinical Global Impression of Severity) scale (2 and 4 weeks). Only subjects with a minimum score of 18 on the HAMD-21 scale entered the study. Responders were defined as subjects exhibiting a decrease of at least 50% in the HAMD total score after 2 and 4 weeks of medication, and remitters were defined as subjects having a HAMD total score of 7 or less after 2 and 4 weeks of medication and a reduction in the HAMD scores from baseline of at least 50%.

Subjects with primary or comorbid diagnoses of schizophrenia, schizoaffective disorder, rapid cycling bipolar disorder, dementia, alcohol or substance dependence based on DSM-IV criteria within the previous 6 months were excluded from the study.

We also excluded subjects showing a personal or family history of substance abuse/dependence or major psychiatric disorders prior to study entry, and patients who were receiving medications were subjected to a 2-week washout period. Also, no patient had received psychotropic medication or other medication which can affect mirtazapine metabolism within 2 or 4 weeks of the commencement of the study, respectively. Demographic data, medical history, and laboratory data were documented, and patients with serious or unstable medical illness were also excluded from the study.

All subjects were at least 18 years of age. During the treatment period of the study, all subjects took mirtazapine (Remeron, Organon) daily at a dose of 15–60 mg. After obtaining written informed consent, venous blood was drawn from each subject using a protocol approved by the Ethics Committee of the Korea University Medical Center.

2.2. DNA analysis

DNA was extracted from the peripheral blood and a polymerase chain reaction was performed according to a previously described protocol (Heils et al., 1996) with the sense primer 5′-ACT CTT GAG AGC GTG AAT-3′ and the antisense primer 5′-ATA CTG TCA CAC ACG CTC-3′.

2.3. Statistical analysis

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 (ORs) with 95% confidence intervals (CIs) were calculated to estimate the effects of high-risk genotypes and alleles. The cutoff probability value was set at 0.05. A correction for multiple testing was not performed because this study applied an explorative approach to a genetically complex trait in which the relationship between genotype and phenotype has not been established, and thus such a correction might have inappropriately increased the likelihood of real effects being missed (i.e., increased type-II-error rates) (Rothman, 1990). All statistical analyses were performed using SPSS (version 10.0 for Microsoft Windows).

3. Results

Of the 136 patients who were chosen to participate in this study, 35 withdrew because of a failure to draw blood (n = 1), lack of efficacy (n = 1), personal conflict or other personal decision (n = 12), loss to follow-up (n = 12), or adverse events (n = 9). The intent-to-treat group thus comprised 101 subjects, and the 5-HTTLPR genotype of each of these subjects was ascertained. Chi-square tests applied to the three genotype frequencies (l/l, l/s, and s/s) revealed that the subjects were in Hardy-Weinberg equilibrium. Table 1 indicates that onset age, frequency of suicide attempts, family history of MDD, and gender distribution did not differ between the three 5-HTTLPR genotypes. The frequency of the 5-HTTLPR s/s genotype (78%) was very similar to that found (79%) in other Asian

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n (101)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>l/l</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>l/s</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>s/s</td>
<td>29</td>
<td></td>
</tr>
</tbody>
</table>

| Age       | 44.75±9.39 | 43.00±14.78 | 52.45±15.65 | 0.042* |
| Onset age | 45.00±10.53 | 39.65±15.14 | 47.85±16.19 | 0.125* |
| Suicide attempts | 0% (0) | 1% (25%) | 3% (75%) | 0.895* |
| Family history | 0% (0) | 4% (36.4%) | 7% (63.6%) | 0.293* |
| Males     | 32% (50%) | 32% (50%) | 36% (42.5%) | 0.373** |
| HAMD at baseline | 22.20±4.24 | 21.75±3.35 | 21.35±4.46 | 0.902* |

Table 1

Demographic characteristics of MDD subjects (intention-to-treat group, n = 101)

LOCF (last-observation-carried-forward) analysis was performed for missing data in HAMD scores.

Data are mean±SD values or n (%) as appropriate.

Genotypes were compared using ANOVA*, chi-square test**, or Fisher’s exact test*. Please cite this article as: Kang R-H et al. Association study of the serotonin transporter promoter polymorphism and mirtazapine antidepressant response in major depressive disorder. Prog Neuro-Psychopharmacol Biol Psychiatry (2007), doi:10.1016/j.pnpbp.2007.05.018
Table 2

Distributions of genotypes, alleles, and allele carriers of 5-HTTLPR polymorphism among responders and nonresponders after 2 and 4 weeks of mirtazapine treatment

<table>
<thead>
<tr>
<th>Genotype</th>
<th>2 weeks</th>
<th>4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>l/l</td>
<td>l/s</td>
</tr>
<tr>
<td><strong>Responders (n=42)</strong></td>
<td>1 (2.4%)</td>
<td>4 (9.5%)</td>
</tr>
<tr>
<td><strong>Nonresponders (n=59)</strong></td>
<td>3 (5.1%)</td>
<td>16 (27.1%)</td>
</tr>
<tr>
<td><strong>Responders (n=62)</strong></td>
<td>2 (3.2%)</td>
<td>7 (11.3%)</td>
</tr>
<tr>
<td><strong>Nonresponders (n=39)</strong></td>
<td>2 (5.1%)</td>
<td>13 (33.3%)</td>
</tr>
</tbody>
</table>

Comparison of genotype frequencies among responders and nonresponders after 2 weeks of mirtazapine treatment:
- \( \chi^2 = 5.615, df = 2, p = 0.06 \)
- \( \chi^2 = 5.580, df = 1, p = 0.018, OR = 3.515, CI = 1.192 - 10.369 \)

Comparison of genotype frequencies among responders and nonresponders after 4 weeks of mirtazapine treatment:
- \( \chi^2 = 7.894, df = 2, p = 0.019 \)
- \( \chi^2 = 7.577, df = 1, p = 0.006, OR = 3.681, CI = 1.414 - 9.582 \)

Studies (Kunugi et al., 1997; Kim et al., 2000; Yoshida et al., 2002). Although there was difference in the mean age between the 5-HTTLPR genotypes, statistical significance was marginal (\( p = 0.042 \)). Furthermore ANCOVA was used to establish the potential confounding effect of genotype and responder on mean age. Confounding effects could not be detected (data not shown).

Table 2 lists the frequencies of 5-HTTLPR polymorphism genotypes, alleles, and allele carriers in the responders and nonresponders. The therapeutic response to antidepressant mirtazapine was significantly better in patients with the s/s genotype than in l-allele carriers, both at the 2nd week (\( \chi^2 = 5.580, df = 1, p = 0.018, OR = 3.515, CI = 1.192 - 10.369 \)) and the 4th week (\( \chi^2 = 7.577, df = 1, p = 0.006, OR = 3.681, CI = 1.414 - 9.582 \)). However, the genotype and carrier distributions for the 5-HTTLPR polymorphism did not differ significantly between remitters and nonremitters.

4. Discussion

The present study showed that the antidepressant response to mirtazapine is significantly associated with the 5-HTTLPR polymorphism, which represents evidence that this polymorphism plays a major role in the mirtazapine antidepressant response in Koreans.

Previously, the association between 5-HTTLPR and antidepressant response has been studied mostly using SSRIs due to these drugs acting directly on the serotonin system, and the difference in locus may produce a different treatment result (Lesch, 2001). 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 (Kim et al., 2000; Yoshida et al., 2002). The allele frequency of l-variant 5-HTTLPR is 25% in Koreans and Japanese (Kim et al., 2000; Yoshida et al., 2002) compared with around 55% in whites (Pollock et al., 2000). Thus, other genetic explanation for this ethnic difference must be sought.

Our results obtained with mirtazapine – an antidepressant that acts via a different mechanism compared with SSRIs – were similar to Kim et al. (2000) and Yoshida et al. (2002) showing that Koreans and Japanese with the s/s genotype respond better to antidepressants in a protocolized-dosing 6-week study.

However, since mirtazapine has not been studied in Asian populations, the analogous hypothesis for that population cannot be formulated. The accumulation of the corresponding data as for SSRIs in the future may make it possible to reconsider this hypothesis. Also, we consider that 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. The authentic functional sequence variants may be in strong linkage disequilibrium with the l-allele in whites and also in linkage disequilibrium with the s-allele in Koreans (Kim et al., 2006).

Each type of antidepressant exhibits a different selectivity and affinity to biogenic amine transporters, and hence the therapeutic response to antidepressants may differ with their type.

The antidepressant activity of mirtazapine is associated with the enhancement of the serotonergic and noradrenergic systems in the CNS (Kasper et al., 1997). The noradrenergic effect is attributed to the blockade of inhibitory presynaptic α2-auto-receptors. This blockade enhances the release of norepinephrine to the synaptic cleft and the postsynaptic availability of this neurotransmitter. However, mirtazapine does not inhibit norepinephrine reuptake. In addition, mirtazapine antagonizes α2-heteroreceptors in the serotonergic nerve terminals, thereby increasing serotonin release (de Boer, 1995).

In addition, norepinephrine release at synapses of serotonergic neurons augments serotonin release. Although mirtazapine does not directly interact with 5-HTT, individual differences in the abundance of this protein could affect the response to mirtazapine-induced changes in serotonergic neuronal activity.

Tsapakis et al. (2005) reported an association between 5-HTTLPR and tricyclic antidepressant treatment, and Kim et al. (2006) reported that 5-HTTLPR is secondarily associated with the response to nortriptyline. Murphy et al. (2004) reported that s-allele-carrying geriatric patients treated with mirtazapine for 14 days showed a lower HAMD-17 score (\( p = 0.03 \)), indicating that they were less depressed. We found that the mirtazapine...
response at the 2 and 4th weeks was significantly better in patients with the s/s genotype of the 5-HTTLPR polymorphism than in l-allele carriers, which suggests that the s/s genotype is predictive of the response to treatment with mirtazapine. Furthermore, since the response time to treatments with an antidepressant has a significant effect on drug compliance, dose adjustment, and the patient’s well-being, when treating depression patients with mirtazapine the s/s genotype can function as an early predictor and bring several benefits to them.

This study was subject to several limitations. First, only 101 patients completed 4 weeks of mirtazapine 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. The powers to detect an association between the allele-carrier frequencies of the 5-HTTLPR polymorphism and mirtazapine response at 2 and 4 weeks were 0.64 and 0.77, respectively. This indicates that our sample provides a moderate power, considering that a power cutoff of 0.8 is commonly used for \( \alpha < 0.05 \). Second, the association of the 5-HTTLPR polymorphism with mirtazapine response should be studied in a placebo-controlled trial. However, previous studies (Smith et al., 1990; Kasper, 1997) have already found that the improvement in symptoms was significantly higher in mirtazapine groups than in placebo control groups. Third, though a single gene may affect the responses to antidepressants, it would play only a relatively minor role in a complex mechanism—we should therefore consider interactions between many different genes. Fourth, the plasma levels of mirtazapine were not analyzed, but we made efforts to exclude patients with low or no drug-compliance as much as possible through checking the tablet count and detailed interview by psychiatrists and research nurses. Fifth, 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 (Kim, 2003), we consider that such bias is unlikely in our sample. Finally, in previous meta-analyses of 5-HTTLPR and anxiety-related traits, the polymorphism was shown to be most strongly associated with neuroticism (Sen et al., 2004). It can be argued that the association seen in our sample is actually conferred by the underlying association with this personality trait rather than with the diagnostic entity MDD. Therefore, we excluded patients with serious personality disorders based on face-to-face interviews performed by two psychiatrists and the familial history of diseases, and minimized diagnostic errors by applying the Korean version of the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (First et al., 1997).

Notwithstanding these limitations, this study suggests that the response to noradrenergic and specific serotonergic antidepressants is significantly associated with the 5-HTTLPR polymorphism, with the presence of the s/s genotype resulting in a better response to mirtazapine treatment. The 5-HTTLPR s/s genotype also acts as a predictor of an early response to the treatment of depression with mirtazapine.

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