# Monoamine Transporter Gene Polymorphisms and Antidepressant Response in Koreans With Late-Life Depression

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INITIAL DRUG TREATMENTS FAIL IN 30% to 40% of patients with major depression. Pharmacogenetic prediction of response is one possibility for improving the efficiency of antidepressant treatment. A number of studies have shown that *s/l* polymorphisms in the serotonin transporter gene (*5-HTT*) promoter region variation (*5-HTTLPR*) might predict treatment outcomes to selective serotonin reuptake inhibitors (SSRIs) such as antidepressant response[1-7] and adverse events.[8,9]

However, ethnic variation as well as choice of drug may influence genetic effects on antidepressant response. In white populations,[1-5,10] depressed patients with the long allele *5-HTTLPR* genotypes (*sl* and *ll*) generally show a greater response to SSRIs than those with a short allele genotype (*ss*). However, studies in Japanese and Korean populations[6,7] report an association in the opposite direction. The effect of this polymorphism on treatment outcome may also depend on the mechanism of antidepressant action.[2,7,11] A significant association between allelic variation of *5-HTTLPR* and antidepressant response was found with fluvoxam-

**Context** Polymorphisms in the serotonin transporter gene (*5-HTT*) may influence antidepressant response to selective serotonin reuptake inhibitors (SSRIs). The norepinephrine transporter (NET) is the analogous target for norepinephrine reuptake inhibitors (NRIs).

**Objective** To determine whether antidepressant responses to SSRIs or NRIs are associated with genetic polymorphisms of the corresponding monoamine transporters.

**Design, Setting, and Patients** A 6-week naturalistic treatment study with blinded outcome evaluation of 241 Korean inpatients and outpatients with major depression at an academic psychiatry service. Patients were recruited to the study from March 1998 through February 2003.

**Interventions** Treatment with an SSRI (fluoxetine or sertraline; n=136) or an NRI (nortriptyline; n=105) antidepressant. Adherence was assessed by measuring plasma concentration at 4 weeks. Patients were genotyped for *s/l* polymorphisms in *5-HTT* promoter region *(5-HTTLPR), 5-HTT* intron 2 *s/l* variation, and NET G1287A variation of exon 9.

**Main Outcome Measures** An SSRI and NRI response (defined as ≥50% decrease in Hamilton Rating Scale for Depression score at 6 weeks).

**Results** NRI response was associated with the NET G1287A polymorphism (odds ratio [OR], 7.54; 95% confidence interval [CI], 2.53-22.49; $P<.001$). An SSRI response was associated with the *5-HTT* intron 2 *s/l* variation (OR, 20.11; 95% CI, 4.27-94.74; $P<.001$). The *5-HTTLPR* was also associated with an SSRI response (OR, 3.34; 95% CI, 1.41-7.91; $P=.006$). In contrast to studies in white patients, the favorable allele for SSRI response was *S 5-HTTLPR*. The *S 5-HTTLPR* was associated also with NRI response (OR, 3.73; 95% CI, 1.32-10.53; $P=.01$). The NET polymorphism was not associated with an SSRI response. The NET G1287A *GG* genotype (56% of the population) was associated with better response to the NRI (83.3% [35/42]) than to SSRI (58.7% [44/75]) (OR, 3.52; 95% CI, 1.39-8.95; $P=.006$). Some genotype combinations were associated with high rates of antidepressant response and others with low rates of response.

**Conclusions** Monoamine transporter gene polymorphisms were associated with response to antidepressants with homologous monoamine transporter targets. Combinations of polymorphisms were informative for response and nonresponse. Confirmation of these preliminary findings would permit refined pharmacogenetic selection of antidepressant treatment.

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ine,[1,7] paroxetine[2] or sertraline,[10] but not with fluvoxamine plus pindolol,[1] or nortriptyline,[2] or milnacipran.[12]

In a previous study,[6] we found that allelic variation of the *5-HTT* gene was associated with variation in antidepressant response to SSRIs. Herein we extend that approach to allelic variation of the norepinephrine transporter (NET), which is the target of the norepinephrine reuptake inhibitor (NRI) class of antidepressant drugs. Due to reported interactions between the monoamine systems, we also included the dopamine transporter (DAT) polymorphism.[13] Based on previous studies, we chose *5-HTTLPR* and intron 2 variable number of tandem repeats (VNTR) of the *5-HTT* gene[1,5]; the G1287A polymorphism in exon 9, the C296T polymorphism (Thr99Ile) in exon 2, and the G1432A (Gly478Ser) in exon 10 of the *NET* gene[14,15]; and the 3′-untranslated region VNTR of the *DAT* gene[13] as candidate gene variants for the prediction of antidepressant responses. The C296T and G1432A polymorphisms of *NET* and 3′-untranslated region VNTR of the *DAT* gene were excluded from the analysis because minor alleles of these polymorphisms were relatively uncommon (<5% of the population).

As our primary hypothesis, we predicted significant associations between NRI efficacy and *NET* polymorphisms and between SSRI efficacy and *5-HTT* polymorphisms. If confirmed, these associations could provide a basis for the differential pharmacogenetic prediction of antidepressant response by the mode of the mechanism. In secondary analyses, we compared the NRI and SSRI response rates by genotype. In additional secondary analyses, we examined combinations of polymorphisms and NRI or SSRI response. We chose nortriptyline as the NRI and fluoxetine or sertraline as the SSRI because these are the agents most commonly used to treat late-life depression in Korea. In addition to treatment response, we also monitored adverse events to antidepressants by the UKU Side Effect Rating Scale.[16]

## METHODS

All patients were of unrelated Korean ancestry. These patients were a completely separate sample from our previous report.[6] Patients were eligible if they were at least 18 years of age. Eligible patients were enrolled in the Clinical Trials Program of the Samsung Medical Center Geropsychiatry and Affective Disorder Clinics (Seoul, Korea). They received a semistructured diagnostic interview, the Samsung Psychiatric Evaluation Schedule.[6] The affective disorder section of the Samsung Psychiatric Evaluation Schedule uses the Korean version of the *Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*.[17] At least 1 family member who is living with the patient was interviewed to supplement the patient's report of symptoms, behaviors, level of functioning, duration of episode, and recent treatments. Patients fulfilled the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition,* criteria for major depressive episode. Diagnoses were confirmed by a board certified psychiatrist on the basis of the Samsung Psychiatric Evaluation Schedule, case review notes, and other relevant data. A minimum baseline 17-item Hamilton Rating Scale for Depression (HAM-D) score of 15 was required. No patient had received psychotropic medication within 2 weeks of the study or fluoxetine within 4 weeks. Potential study participants were excluded for pregnancy, significant medical conditions, abnormal laboratory baseline values, unstable psychiatric features (eg, suicidal), history of alcohol or drug dependence, seizures, head trauma with loss of consciousness, neurological illness, or concomitant Axis I psychiatric disorder. The protocol was approved by the ethics review board of the Samsung Medical Center. Signed informed consent was obtained from all participants.

A total of 241 patients were enrolled from March 1998 through February 2003. Patients were assigned by

a clinician to either an NRI (nortriptyline) or an SSRI (fluoxetine or sertraline). The clinician's drug choice was based on anticipated adverse effects of nortriptyline in at-risk individuals rather than by the symptomatic characteristics of the patients. Factors that determined use of fluoxetine or sertraline in preference to nortriptyline were frailty, osteoporosis, a history of falls, and known cardiovascular disease, including hypotensive episodes. A total of 105 patients received nortriptyline and 136 patients received an SSRI (fluoxetine [n=51] or sertraline [n=85]). Dose titration was completed within 2 weeks. Doses were titrated into the usual clinical range based on initial tolerability and adverse effects. The final daily median dosages were 55.0 mg/d of nortriptyline (interquartile range [IQR], 47.5-70.0 mg/d; range, 35.0-100.0 mg/d); 30.0 mg/d of fluoxetine (IQR, 20.0-40.0 mg/d; range, 20.0-50.0 mg/d); and 75.0 mg/d of sertraline (IQR, 75.0-100.0 mg/d; range, 50.0-100.0 mg/d). These are typical clinical doses for Asian populations and they result in comparable plasma drug levels as do higher drug dosages in white populations.[18] Trough serum samples to measure NRI and SSRI levels were drawn at the end of week 4. A 1- to 2-mg dose of lorazepam could be prescribed at bedtime for insomnia.

Patients were seen by a psychiatrist, who monitored their adverse events by the UKU Side Effect Rating Scale[16] at weeks 0, 0.5, 1, 2, 4, and 6. The 17-item HAM-D was administered by a single trained rater every 2 weeks. The rater and genotyper were blinded to the hypotheses of the study and to drug assignment. To maintain the blind, a trained research coordinator managed the data and schedules. The HAM-D and genotype data were not disclosed to the psychiatrist and the rater was blinded to the genotype data. Response was defined as a 50% or greater decrease in the HAM-D score at 6 weeks. Remission was defined as a HAM-D score of less than 8 at 6 weeks.[19]

**FIGURE 1** shows the flow of patients through the study. A total of 208 pa-

tients (86%) completed the 6-week treatment trial. Sixteen patients who received the NRI and 17 patients who received an SSRI dropped out of the study. Eight patients who received nortriptyline, 3 patients who received fluoxetine, and 5 patients who received sertraline discontinued treatment based on lack of efficacy or intolerable adverse effects. Six patients were excluded because their plasma drug concentrations were undetectable, consistent with nonadherence. Four patients were excluded because their plasma drug concentrations were consistent with extensive drug metabolism: <200 ng/mL for fluoxetine/norfluoxetine and <30 ng/mL for sertraline).[20] Seven patients failed to attend scheduled clinic visits. The clinical characteristics of the dropouts did not differ significantly from the completers who received either SSRI or the NRI (data not shown). These 33 dropouts were excluded from the data analyses.

### Genotyping

Genomic DNA was extracted from whole blood using a Wizard Genomic Purification kit (Promega, Madison, Wis). Patients were genotyped for *5-HTT* promoter region *s/l* variation *(5-HTTLPR)*, *5-HTT* intron 2 *s/l* variation, and NET G1287A variation of exon 9.

*NET* **Polymorphism.** The G1287A polymorphism in exon 9 was amplified by primers 8F (5′-TCCAGG-GAGACCCTAATTCC) and 8R (5′-TTGACTTTATTGAAATGCGGC) and digested by the restriction enzyme, *Sau*96I. Polymerase chain reaction was performed in a total volume of 25 μL that contained 40 ng of genomic DNA, 0.2 mM of dNTP, 10 pmol of primers, 10 mM of *tris* hydrochloric acid (pH, 8.3), 50 mM of potassium chloride, 3.5 mM of magnesium chloride, 0.1% of Triton-X100, and 0.5 U of Taq polymerase. The polymerase chain reaction condition was 1 cycle of predenaturation at 94°C for 5 minutes, 40 cycles at 94°C for 30 seconds, 57°C for 45 seconds, and 72°C for 45 seconds in series, and 1 cycle of postelongation at 72°C for 10 minutes. The expanded

**Figure 1.** Flow of Patients Through the Study
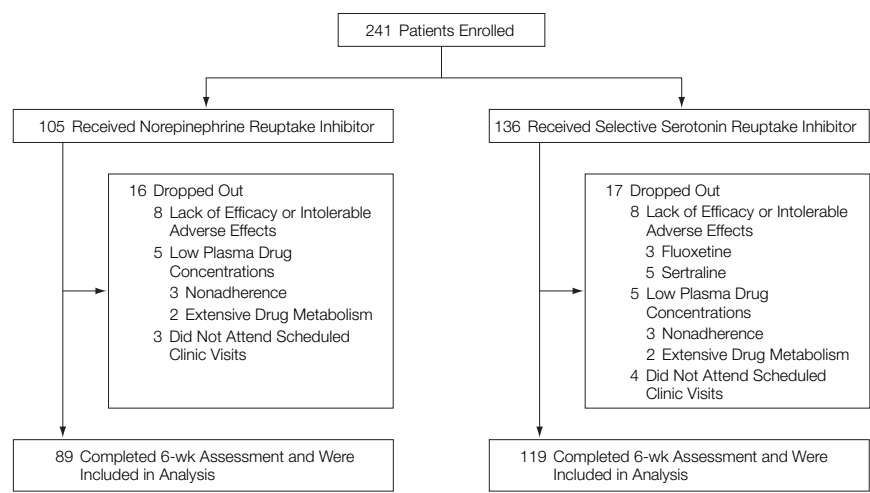


products were digested at 37°C for 1 hour with a restriction enzyme and detected in 12% polyacrylamide gel. Depending on the polymorphic *Sau*96I site, either a 1287A (113 + 97 + 31 bp) or 1287G (113 + 76 + 31 + 21 bp) fragment was produced.

*5-HTT* **Polymorphism.** The VNTR polymorphism in the intron 2 region and the *5-HTTLPR* (*5-HTT*–linked polymorphic region) element in the promoter region were also detected through polymerase chain reaction amplification as described previously.[6] The 9 and 10 VNTRs were designated as short alleles and the 12 VNTR was designated as the long allele of the *5-HTT* intron 2; the 14 copy of *5-HTTLPR* was designated as the short allele and copies 16, 18, 20, and 22 were designated as long alleles.

### Plasma Drug Levels

Plasma levels of nortriptyline, fluoxetine/norfluoxetine, and sertraline were quantified by previously described methods with liquid chromatography tandem mass spectrometry.[20-22]

### Statistical Analysis

Means and SDs, ranges of continuous variables, and proportions of categorical variables are presented as descriptive statistics. The Mann-Whitney *U* test was used for continuous variables be-

cause they were not normally distributed and the $\chi^2$ test was used for categorical variables. Hardy-Weinberg equilibrium was tested by the $\chi^2$ test. Power analyses were performed to examine if the number of patients was sufficient to produce a statistically significant result, given a true difference. The Fisher exact test was used for comparisons of the genotype and allele frequencies between the antidepressant responders and nonresponders. All 3 genes were entered in the multiple logistic regression model to evaluate the influence of each gene on the response to the medication, adjusting for other genes. The Bonferroni correction was applied for multiple testing. Results were considered significant at $P<.05$ after this correction. The *P* values from the Bonferroni correction are stated with the corrected values. The Fisher exact test was used to conduct limited exploratory, post hoc analyses by using a permutation method for multiple testing to examine response rates in relation to genotype combinations. The same method was used to compare differential response to NRI or SSRI by genotype. Measures of linkage disequilibrium were calculated using the Gold program.[23] All statistical analyses were performed using SAS software version 9.13 (SAS Institute Inc, Cary, NC).

## RESULTS

There were no major differences by sex, number of episodes, age at onset, or HAM-D scores before or after treatment between the NRI-treated and SSRI-treated groups (**TABLE 1**). Patients treated with SSRIs were older (mean [SD] age, 59.9 [12.6] years) than those treated with nortriptyline (mean [SD] age, 55.8 [12.4] years). The mean age at onset of major depressive disorder was in the early to mid-50s (Table 1). Rate of response to antidepressants was 124 (60%) of 208 patients who completed the 6-week treatment trial; 55 (62%) of 89 patients who received the NRI and 69 (58%) of 119 patients who received an SSRI (*P*=.58). Rate of remission did not differ by drug class (26 [29%] of 89 in NRI group vs 34 [29%] of 119 in SSRI group). The overall distribution of response, remission, and nonresponse did not differ by drug class (*P*=.84).

Genotype distribution for each monoamine transporter polymorphism was not significantly different between the NRI group and SSRI group (**TABLE 2**), and was not significantly different within the SSRI group (fluoxetine vs sertraline). The choice of drug in the SSRI group (fluoxetine vs sertraline) had no effect on drug responsiveness (*P*=.56). Genotypes were unrelated to dropout status or summed total score of the UKU Side Effect Rating Scale in either treatment group. There were no statistical differences in genotype distributions between early onset depression (age ≤59 years) and late-onset depression (age ≥60 years) (*P*=.25 for the *5-HTTLPR*; *P*=.82 for *5-HTT* intron 2; and *P*=.97 for NET G1287A). Comparisons between younger (age ≤59 years) and older (age ≥60 years) patients were similarly nonsignificant (*P*=.30, *P*=.35, and *P*=.44, respectively).

The mean (SD) plasma levels for responders and nonresponders were 116 (21) ng/mL and 123 (23) ng/mL for nortriptyline; 789 (236) ng/mL and 802 (206) ng/mL for fluoxetine/norfluoxetine; and 75 (29) ng/mL and 72 (28) ng/mL for sertraline and were not significantly different between responders and nonresponders. The responders did not differ significantly by lorazepam dosages from the nonresponders who received either an SSRI (*P*=.71) or the NRI (*P*=.46).

### NRI Response and Monoamine Transporter Gene Polymorphisms

The genotype frequencies in the NRI-treated group for the *5-HTTLPR* (*P*=.23), *5-HTT* intron 2 (*P*>.99), and NET G1287A polymorphisms (*P*=.34) were in Hardy-Weinberg equilibrium. For the power analysis in the NRI-treated group, the response rate of patients with the GG genotype was predicted to be 80% and

**Table 1.** Characteristics of Study Patients

| Characteristic | Overall | Responder | Nonresponder | P Value |
|---|---|---|---|---|
| Norepinephrine Reuptake Inhibitor | | | | |
| Response rate, No./total (%)* | 55/89 (61.8) | | | |
| Sex, No.* | | | | .89 |
| Male | 19 | 12 | 7 | |
| Female | 70 | 43 | 27 | |
| Age, mean (SD) [range], y†‡ | 55.80 (12.40) [22-76] | 54.78 (11.85) [26-76] | 57.44 (13.25) [22-74] | .33 |
| Episodes, mean (SD) [range]† | 1.72 (1.87) [1-15] | 1.58 (1.40) [1-10] | 1.94 (2.46) [1-15] | .38 |
| Age at onset, mean (SD) [range], y†‡ | 51.01 (13.69) [22-76] | 50.80 (13.37) [23-76] | 51.35 (14.39) [22-73] | .85 |
| Hamilton Rating Scale for Depression score, mean (SD) [range]†<br>Baseline | 24.88 (5.82) [15-42] | 24.67 (6.07) [15-42] | 25.21 (5.45) [17-41] | >.99§ |
| After treatment | 11.78 (6.34) [2-35] | 8.20 (3.37) [2-18] | 17.56 (5.73) [9-35] | .01§ |
| Selective Serotonin Reuptake Inhibitor | | | | |
| Response rate, No./total (%)* | 69/119 (58.0) | | | |
| Sex, No.* | | | | .38 |
| Male | 33 | 17 | 16 | |
| Female | 86 | 52 | 34 | |
| Age, mean (SD) [range], y†‡ | 59.91 (12.61) [22-85] | 58.12 (13.86) [22-81] | 62.38 (10.28) [38-85] | .07 |
| Episodes, mean (SD) [range]† | 1.90 (2.41) [1-25] | 1.68 (0.99) [1-6] | 2.20 (3.53) [1-25] | .25 |
| Age at onset, mean (SD) [range], y†‡ | 54.87 (14.08) [19-81] | 52.62 (15.67) [19-81] | 57.96 (10.94) [35-80] | .03 |
| Hamilton Rating Scale for Depression score, mean (SD) [range]†<br>Baseline | 23.52 (5.11) [16-40] | 23.55 (5.63) [16-40] | 23.48 (4.33) [16-36] | >.99§ |
| After treatment | 11.66 (5.94) [1-31] | 7.75 (3.23) [1-15] | 17.04 (4.40) [10-31] | .01§ |

*The χ² test was used.
†The *t* test was used.
‡*P*<.05 for comparison between selective serotonin reuptake inhibitor group and norepinephrine reuptake inhibitor group.
§Corrected by the Bonferroni correction for multiple testing.

the response rate of patients with the GA plus AA genotypes was predicted to be 50%. Based on 42 patients with GG genotype and 47 patients with GA plus AA genotypes, the power to detect this difference of 30% is 80% under the significance level of .05.

Response to NRI was strongly associated with the NET G1287A polymorphism (odds ratio [OR], 7.54; 95% confidence interval [CI], 2.53-22.49; $P$<.001 by multiple logistic regression). A response rate of 83.3% (35/42) was associated with the GG genotype, which was significantly greater than the response rate of 42.6% (20/47) in the GA plus AA genotypes ($P$=.01; **TABLE 3**). The GA and AA genotypes were combined for this analysis because the 1287AA genotype was found in only 6 patients. The reduction in HAM-D score after 6 weeks of NRI treatment was greater in the GG genotype than in the GA plus AA genotypes ($P$=.006; **FIGURE 2**).

The NRI response was also associated with the *5-HTTLPR* polymorphism (OR, 3.73; 95% CI, 1.32-10.53; $P$=.01 by multiple logistic regression). The favorable allele for the NRI response was *s* allele of *5-HTTLPR* polymorphism ($P$=.003; Table 3). A response rate of 76% (38/50) to the NRI was associated with the *ss* genotype of the *5-HTTLPR* polymorphism. In comparison, the response rates to the NRI were 48% (14/29) in those with the *sl* genotype and 30% (3/10) in patients who carried the *ll* genotype. The frequency of the *5-HTTLPR ss* genotype was significantly higher in the NRI responders than in the nonresponders ($P$=.006). The *l/s* polymorphism of *5-HTT* intron 2 region had no relationship with the NRI response (OR, 3.34; 95% CI, 0.70-15.91; $P$=.13 by multiple logistic regression).

## SSRI Response and Monoamine Transporter Gene Polymorphisms

Within the SSRI-treated group, the genotype frequencies for the polymorphisms of the *5-HTTLPR* ($P$=.83), *5-HTT* intron 2 ($P$>.99), and NET G1287A ($P$=.40) were in Hardy-Weinberg equi-

**Table 2.** Genotype Distributions of Monoamine Transporter Gene Polymorphisms

| | No. (%) of Participants | | | |
|---|---|---|---|---|
| | Overall (N = 208) | NRI (n = 89) | SSRI (n = 119) | *P* Value* |
| NET G1287A in exon 9 | | | | |
| GG | 117 (56.3) | 42 (47.2) | 75 (63.0) | |
| GA | 78 (37.5) | 41 (46.1) | 37 (31.1) | .21 |
| AA | 13 (6.3) | 6 (6.7) | 7 (5.9) | |
| *5-HTT* VNTR in promoter | | | | |
| *ss* | 120 (57.7) | 50 (56.2) | 70 (58.8) | |
| *sl* | 71 (34.1) | 29 (32.6) | 42 (35.3) | >.99 |
| *ll* | 17 (8.2) | 10 (11.2) | 7 (5.9) | |
| *5-HTT* VNTR in intron 2 | | | | |
| *ll* | 174 (83.7) | 77 (86.5) | 97 (81.5) | |
| *ls* | 33 (15.9) | 12 (13.5) | 21 (17.6) | >.99 |
| *ss* | 1 (0.5) | 0 | 1 (0.8) | |

Abbreviations: *5-HTT*, serotonin transporter; NET, norepinephrine transporter; NRI, norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; VNTR, variable number of tandem repeats.
*Comparisons of the genotype frequencies between the NRI and SSRI groups were performed using $\chi^2$ test followed by the Bonferroni correction for multiple testing.

librium. For the power analysis, the response rate of the patients with *ll* genotype in *5-HTT* intron 2 was assumed to be 70% compared with 30% in the patients with *ls* plus *ss* genotypes. Based on 97 patients with *ll* and 22 patients with *ls* plus *ss*, the power of detecting this difference of 40% is 90% under the significance level of .05.

The genotype distribution and allele frequencies in the SSRI responders and nonresponders appear in Table 3. Response to SSRI was significantly associated with the polymorphisms of the *5-HTT* intron 2 and the *5-HTTLPR*. Patients with the *ll* genotype of *5-HTT* intron 2 had a 69% (67/97) rate of response to SSRIs compared with only 9% (2/22) for the other 2 genotypes combined ($P$=.01). The *5-HTT* intron 2 polymorphism showed the strongest association with SSRI response among the monoamine transporter gene polymorphisms (OR, 20.11; 95% CI, 4.27-94.74; $P$<.001 by multiple logistic regression). **FIGURE 3** shows the difference in HAM-D score reduction after 6 weeks of SSRI medication between the two *5-HTT* intron 2 genotype groups (*ll* genotype vs *ls*+*ss* genotype, $P$<.001). Response to SSRI was also significantly associated with polymorphism of the *5-HTTLPR* (OR, 3.34; 95% CI, 1.41-7.91; $P$=.006). Patients with the *ss* genotype at this site

had a 71% (50/70) rate of response to SSRI compared with rates of 40% (17/42) with the *ls* genotype and 29% (2/7) with the *ll* genotype (*ss* genotype vs *sl* + *ll* genotypes, $P$=.003; Table 3). Polymorphisms in the *5-HTTLPR* and *5-HTT* intron 2 regions in our study population are in partial linkage disequilibrium ($r^2$=0.04; $D'$=.40), which indicates that *5-HTTLPR* and *5-HTT* intron 2 may play independent roles in determining drug response. The NET G1287A polymorphism showed no association with response to SSRI drugs (OR, 0.84; 95% CI, 0.34-2.09; $P$=.71 by multiple logistic regression).

## Transporter Gene Polymorphisms and Differential Drug Response

Although it was not a primary focus of this study, we compared the response rates to NRI and SSRI by genotype (Table 3). Only 1 strong association was detected: patients carrying the GG polymorphism of NET G1287A had a higher response rate of 83.3% to NRI treatment (35/42) than the rate of 58.7% to SSRI treatment (44/75), which is a statistically significant difference (OR, 3.52; 95% CI, 1.39-8.95; $P$=.006 by $\chi^2$).

## Combinations of Transporter Polymorphisms and Response

We examined combinations of 2 significant polymorphisms for their associa-

tion with response to each class of drug. Patients carrying unfavorable genotypes of both polymorphisms (NET G1287A polymorphism A + *5-HTTLPR l*) showed the lowest response rate among 4 genotype combination groups on NRI response (**TABLE 4**). The response rate of this group (21.7%) was sig-

nificantly lower than those of the other 3 genotype groups (NET A + *5-HTTLPR ss*, 62.5%, *P*=.02; NET GG + *5-HTTLPR l*, 75.0%, *P*=.008; and NET GG + *5-HTTLPR ss*, 88.5%, *P*<.001). In the SSRI group, patients carrying favorable genotypes of both *5-HTT* intron 2 *ll* and *5-HTTLPR ss* had the highest re-

sponse rate to SSRIs. The response rate of this genotype group to SSRIs (77.4%) was significantly higher than those of the other 3 genotype groups (*5-HTT* intron 2 *ll* + *5-HTTLPR l*, 54.3%, *P*=.06; *5-HTT* intron 2 *s* + *5-HTTLPR ss*, 25.0%, *P*=.01; and *5-HTT* intron 2 *s* + *5-HTTLPR l*, 0%, *P*<.001).

**Table 3.** Genotype and Allele Distributions of Monoamine Transporter Gene Polymorphisms in Responders and Nonresponders to Antidepressants

| | Response Rate, No./Total (%) | No. (%) | | P Value* | OR (95% CI)† | P Value† |
|---|---|---|---|---|---|---|
| | | Responders | Nonresponders | | | |
| **Norepinephrine Reuptake Inhibitor** | | | | | | |
| NET G1287A in exon 9 | | | | | | |
| GG | 35/42 (83.3) | 35 (63.6) | 7 (20.6) | | | |
| GA | 16/41 (39.0) | 16 (29.1) | 25 (73.5) | .01‡ | 7.54 (2.53-22.49) | <.001 |
| AA | 4/6 (66.7) | 4 (7.3) | 2 (5.9) | | | |
| G | | 0.78 | 0.57 | .01 | 3.48 (1.67-7.30) | .001 |
| A | | 0.22 | 0.43 | | | |
| *5-HTT* VNTR in promoter | | | | | | |
| ss | 38/50 (76.0) | 38 (69.1) | 12 (35.3) | | | |
| sl | 14/29 (48.3) | 14 (25.5) | 15 (44.1) | .006§ | 3.73 (1.32-10.53) | .01 |
| ll | 3/10 (30.0) | 3 (5.4) | 7 (20.6) | | | |
| S | | 0.82 | 0.57 | .003 | 4.85 (2.29-10.27) | <.001 |
| L | | 0.18 | 0.43 | | | |
| *5-HTT* VNTR in intron 2 | | | | | | |
| ll | 51/77 (66.2) | 51 (92.7) | 26 (76.5) | | | |
| ls | 4/12 (33.3) | 4 (7.3) | 8 (23.5) | .15‖ | 3.34 (0.70-15.91) | .13 |
| ss | 0 | 0 | 0 | | | |
| L | | 0.96 | 0.88 | .18 | 3.49 (0.92-13.24) | .07 |
| S | | 0.04 | 0.12 | | | |
| **Selective Serotonin Reuptake Inhibitor** | | | | | | |
| NET G1287A in exon 9 | | | | | | |
| GG | 44/75 (58.7) | 44 (63.8) | 31 (62.0) | | | |
| GA | 21/37 (56.8) | 21 (30.4) | 16 (32.0) | >.99‡ | 0.84 (0.34-2.09) | .71 |
| AA | 4/7 (57.1) | 4 (5.8) | 3 (6.0) | | | |
| G | | 0.79 | 0.78 | >.99 | 1.54 (0.74-3.20) | .25 |
| A | | 0.21 | 0.22 | | | |
| *5-HTT* VNTR in promoter | | | | | | |
| ss | 50/70 (71.4) | 50 (72.5) | 20 (40.0) | | | |
| sl | 17/42 (40.5) | 17 (24.6) | 25 (50.0) | .003§ | 3.34 (1.41-7.91) | .006 |
| ll | 2/7 (28.6) | 2 (2.9) | 5 (10.0) | | | |
| S | | 0.85 | 0.65 | .003 | 2.28 (1.17-4.47) | .02 |
| L | | 0.15 | 0.35 | | | |
| *5-HTT* VNTR in intron 2 | | | | | | |
| ll | 67/97 (69.1) | 67 (97.1) | 30 (60.0) | | | |
| ls | 2/21 (9.5) | 2 (2.9) | 19 (38.0) | .01‖ | 20.11 (4.27-94.74) | <.001 |
| ss | 0/1 | 0 | 1 (2.0) | | | |
| L | | 0.99 | 0.79 | .01 | 15.87 (3.47-71.43) | <.001 |
| S | | 0.01 | 0.21 | | | |

Abbreviations: *5-HTT*, serotonin transporter; CI, confidence interval; NET, norepinephrine transporter; NRI, norepinephrine reuptake inhibitor; OR, odds ratio; SSRI, selective serotonin reuptake inhibitor; VNTR, variable number of tandem repeats.
*Fisher exact test with Bonferroni correction for multiple testing.
†Multiple logistic regression.
‡Statistical analysis was performed between GG and GA plus AA.
§Statistical analysis was performed between *ss* and *sl* plus *ll*.
‖Statistical analysis was performed between *ll* and *ls* plus *ss*.

## COMMENT

Our results indicate that (1) antidepressant response to NRI is principally associated with the NET G1287A polymorphism; (2) response to NRI is also secondarily related to the *5-HTTLPR* polymorphism; (3) response to SSRI is associated with the *5-HTT* intron 2 and *5-HTTLPR* polymorphism; and (4) response to SSRI is not related to the NET G1287A polymorphisms. Thus, our primary hypotheses were confirmed and extended.

We observed significant associations of NRI efficacy with NET G1287A polymorphism and of SSRI efficacy with *5-HTT* polymorphisms. These findings support the concept that pharmacologically selective antidepressants act primarily through homologous neurotransmitter mechanisms. Thus, our findings are consistent with observations that selective depletion of monoamines leads to relapse in patients who have responded to the homologous monoamine transporter inhibitors.[24,25] Our results support our primary hypothesis that responses to NRI and SSRI are significantly associated with their respective transporter polymorphisms.

Our data revealed that the NET G1287A polymorphism plays a major role in the NRI antidepressant response. This polymorphism was reported by Stober et al[26] to have no functional consequences and to lack significant associations with major depression, bipolar disorder, schizophrenia, alcohol dependence, or panic disorder.[27-30] However, this polymorphism is associated with the cerebrospinal fluid concentration of 3-methoxy-4-hydrophenylglycol, a major norepinephrine metabolite,[13] and with the response to methylphenidate, a drug with noradrenergic action, in attention-deficit/hyperactivity disorder.[31] The association between the NET polymorphisms and the antidepressant response was previously examined in Japanese patients by Yoshida et al.[12] They reported that the NET T182C polymorphism was associated with a superior response to milnacipran, a serotonin

and norepinephrine reuptake inhibitor, and that the NET G1287A polymorphism was associated with the onset of response but not the final clinical improvement. Thus, our data add to the basis for considering that the NET G1287A polymorphism does have functional consequences.

The *5-HTTLPR* polymorphism had significant associations with both the SSRI (*P* = .003) and nortriptyline responses (*P* = .006). Few studies have examined the relationship between the *5-HTTLPR* polymorphism and NRI response. Pollock et al[2] examined this polymorphism and response to nortriptyline in 23 patients and found no differences. Tsapakis et al[32] reported a trend association between the *5-HTTLPR* and response to tricyclic antidepressant treatment. Our study is the largest to date to show that the *5-HTTLPR* is associated with a response to nortriptyline as well as to an SSRI. We see this finding as consistent with preclinical studies that indicate facilitation of multiple neurotransmitter systems by antidepressant agents.[33,34] "Cross-talk" between the noradrenergic and serotonergic systems may explain why drugs acting selectively on either one or other of these systems are both active at relieving symptoms of depression.[35]

The observed association of the *5-HTTLPR* polymorphism with the response to both NRIs and SSRIs may signal a more general association of this polymorphism with response to multiple interventions for depression, including drugs of several classes, placebo, sleep deprivation, and light therapy. It was reported in a white population that patients with the long allele (especially *ll* genotype) of *5-HTTLPR* are more responsive to placebo, sleep deprivation, and light therapy as well as more responsive to drug than those with the short allele.[4,36]

In this independent sample, we replicated our finding[6] that a variable repeat sequence of *5-HTTLPR* is associated with response to SSRI drugs in depressed patients (*P* = .003; Table 3), although the favorable allelic variant for

**Figure 2.** Hamilton Rating Scale for Depression Score Changes During 6 Weeks of Treatment With Norepinephrine Reuptake Inhibitor
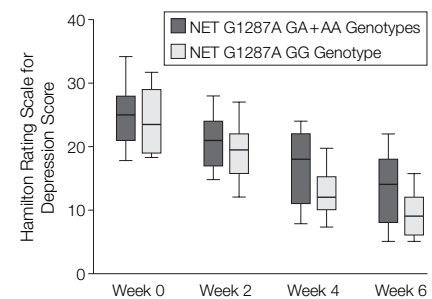


A significant difference was found in Hamilton Rating Scale for Depression score changes between the GG group and GA + AA group at week 6. The score change was −14 (95% confidence interval, −19 to −11) for the GG genotype and was −11 (95% confidence interval, −15 to −6) for the GA + AA genotypes (*P* = .006 by Mann-Whitney *U* test). Each box displays the median, 75th percentile, and 25th percentile values; horizontal bars indicate the highest and lowest observed values. NET indicates norepinephrine transporter.

**Figure 3.** Hamilton Rating Scale for Depression Score Changes During 6 Weeks of Treatment With Selective Serotonin Reuptake Inhibitor
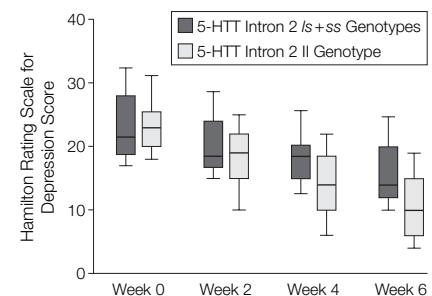


A significant difference was found in Hamilton Rating Scale for Depression score changes between the *ll* group and *ls* + *ss* group at week 6. The score change was −12 (95% confidence interval, −16.5 to −9) for the *ll* genotype and was −6.5 (95% confidence interval, −8.75 to −5) for *ls* + *ss* genotypes (*P* < .001 by Mann-Whitney *U* test). Each box displays the median, 75th percentile, and 25th percentile values; horizontal bars indicate the highest and lowest observed value. *5-HTT* indicates serotonin transporter gene.

response again is at odds with studies in white patients.[1-5] The reason for this ethnic difference remains unclear. However, the data suggest several speculations. First, allelic frequencies for *5-HTTLPR* in Korean and Japanese populations differ greatly from white popu-

**Table 4.** Response Rates With Combinations of Monoamine Transporter Polymorphisms

| NET G1287A | 5-HTT Promoter | 5-HTT Intron 2 | Response Rate, No./Total (%) | P Value* |
|---|---|---|---|---|
| | | Norepinephrine Reuptake Inhibitor | | |
| GG | ss | Any genotype | 23/26 (88.5) | <.001 |
| GG | l Carrier | Any genotype | 12/16 (75.0) | .008 |
| A carrier | ss | Any genotype | 15/24 (62.5) | .02 |
| A carrier | l Carrier | Any genotype | 5/23 (21.7) | Comparator |
| | | Selective Serotonin Reuptake Inhibitor | | |
| Any genotype | ss | ll | 48/62 (77.4) | Comparator |
| Any genotype | l Carrier | ll | 19/35 (54.3) | .06 |
| Any genotype | ss | s Allele carriers | 2/8 (25.0) | .01 |
| Any genotype | l Carrier | s Allele carriers | 0/14 | <.001 |

Abbreviations: 5-HTT, serotonin transporter; NET, norepinephrine transporter.
*Fisher exact test.

lations. The allele frequency of *l* variant *5-HTTLPR* in Korean and Japanese populations is about 25%[6,7,37,38] compared with about 55% in white populations.[1-3,39] If we assumed from studies of white patients that the *l* variant *5-HTTLPR* is the favorable allele for response to SSRIs, we might expect a low rate of response of Korean and Japanese patients to those agents. However, a consistent finding is that 60% to 70% of depressed patients respond to SSRI drugs in multicenter clinical trials regardless of ethnic group.[40-43] Thus, other genetic explanations for this ethnic difference must be sought.

The second speculation is that *5-HTTLPR* is linked with unknown functional variants. It may be an associated marker in linkage disequilibrium 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. Similarly, there may be a functional site closely linked with the second intronic VNTR in the Korean population (but not in the white population) that is associated with SSRI response. Different ethnic populations may have other polymorphisms in linkage disequilibrium with *5-HTT* polymorphisms.

Moreover, it may not be possible to explain the ethnic difference by analyzing only these 2 polymorphisms. Thus, although studies to date have fo-

cused on these 2 polymorphisms of the *5-HTT* gene, the ethnic variation observed between this study and others suggests that these alleles are only indirectly responsible for the observed interactions with treatment response. New approaches that include whole gene sequencing or entire single nucleotide polymorphism (SNP) analyses are needed to identify the responsible loci. In this regard, we note that the current SNP database from the National Center for Biotechnology Information Web site shows 111 SNPs in the *5-HTT* gene, and all of these should be considered in the search for functional polymorphisms. Our finding illustrates the importance of comparing ethnic groups to confirm candidate pharmacogenetic markers.

As to the prediction of differential drug response, our preliminary data analysis suggests that patients carrying the GG polymorphism of NET G1287A will have a statistically significantly superior rate of response to NRI treatment than to SSRI treatment (83.3% vs 58.7% [*P*=.006]; OR, 3.52 [95% CI, 1.39-8.95]). As this genetic subgroup comprised 56% of the population (117/208 cases), this result may prove to have salience for clinical practice. This preliminary finding needs to be tested in studies specifically designed to examine differential response to drug class by genotype.

At least some of the individual variation in antidepressant treat-

ment outcome has a genetic basis.[44] Although the functional influence of these transporter polymorphisms is not fully understood, it is related to the transcription of individual genes. The *l* and *s* variants of the promoter polymorphism have functional differences in modulating transcription of the *5-HTT* gene as well as subsequent *5-HTT* availability.[45] These allele-specific functional differences have been confirmed in human tissues including the brain.[46,47] Thus, the *5-HTT* polymorphisms might influence response to treatment by modulating transcription of *5-HTT*, a direct target of SSRIs.

Our exploratory post hoc analyses point to the predictive potential of combinations of polymorphisms. Two significant polymorphisms, NET G1287A and *5-HTTLPR,* contributed to the prediction of NRI response. For instance, in patients carrying the A allele of the NET G1287A polymorphism, the rate of response to nortriptyline was 43% (20/47) (Table 4). After stratifying by the *5-HTTLPR* polymorphism, however, the rates of response to nortriptyline were 63% (15/24) in those with the combination of NET A plus *5-HTTLPR ss* and only 22% (5/23) in those with the combination of NET A plus *5-HTTLPR l* (*P*=.02). For SSRIs, two *5-HTT* polymorphisms contributed to the prediction of response, with no contribution from NET polymorphism. As the number of favorable genotypes (*ss* in *5-HTTLPR* and *ll* in *5-HTT* intron 2)

increased, the response rate to SSRIs increased (77.4% for 2 favorable genotypes, 54.3% and 25.0% for 1 favorable genotype, and 0% for no favorable genotype). Notwithstanding the small size of the subgroup samples for these secondary analyses, these differential response rates by polymorphism combination are statistically significant or nearly so and they are potentially clinically meaningful.

It is also interesting that the effect of the *5-HTT* intron 2 polymorphism varies by drug. That is, even within a single gene, the association between this polymorphism and outcome is drug-class specific. Likewise, the effect of the NET G1287A polymorphism is drug-class specific.

The primary limitation of this study is its seminaturalistic design, in which the antidepressant was selected by clinician's choice, driven by safety considerations, and titrated to a final dosage based on therapeutic responses or intolerable adverse effects. However, the data confirm that choice of drug was not related to the underlying genotypes. The 2 treatment groups did not differ meaningfully in their demographic or clinical features, and the primary hypotheses did not involve a comparison between the drugs. Moreover, compliance with treatment was checked by drug plasma levels at 4 weeks, and outcome was evaluated by a trained rater who was blinded to both drug status and genotype.

The second limitation of this study is the absence of a placebo-treated group. Because we do not know the rate of nonspecific or nondrug attributable response, we cannot determine from the present data whether subgroups with high rates of response (Table 3 and Table 4) reflect specific drug effects or combinations of drug effect with high nonspecific response rates. Stronger inference is possible with respect to the subgroups in which low rates of response were seen. These groups must have a low placebo-response rate as well as a low drug-response rate. In future studies it will be important to resolve this issue of nonspecific drug response in relation to geno-

type. Nevertheless, the naturalistic design of our study reflects the de facto conditions of managing depression in primary care, in which drugs are a first-line treatment and placebo is not used.[48] Whether these monoamine transporter genotypes additionally predict response to placebo or to psychotherapy of depression remains to be studied.

Our patients were mostly elderly (77% age >50 years), and most (60%) had late-onset illnesses with few previous depressive episodes. Eighty-eight percent of all cases were in their first or second lifetime episode of depression. We adopted strict criteria for a previous major depressive episode, excluding minor depression or dysthymia. It is unclear whether late-life depression has distinctive genetic contributions. It is generally accepted that familial risk for an affective disorder is reduced after age 50 years and that patients with late-onset depression are less likely to have psychiatric comorbidity and more likely to have medical comorbidity. However, studies have demonstrated that depression symptoms in older adults might be more heritable than previously thought,[49] and that early onset and late-onset groups do not differ from each other in genotype frequency distribution of the two *5-HTT* gene polymorphisms.[50,51] Likewise, it is not known whether antidepressant response and pharmacogenetic effects are affected by age in general or by the age at onset.[52,53] We found no differences in genotype distributions between early onset (126 patients aged ≤59 years) and late-onset (82 patients aged ≥60 years) by $\chi^2$ tests for *5-HTTLPR* ($P = .25$), *5-HTT* intron 2 ($P = .82$), and NET G1287A ($P = .97$). The result was similar when we compared genotype distributions between mid-life (89 patients aged ≤59 years) and later in life (119 patients aged ≥60 years) by $\chi^2$ tests for *5-HTTLPR* ($P = .30$), *5-HTT* intron 2 ($P = .35$), and NET G1287A ($P = .44$). As for treatment response, some previous pharmacogenetic studies reported similar results with elderly and younger patients when controlling for ethnicity and drug.[4,8,54]

Despite these limitations, this study demonstrates that the responses to antidepressants with different targets have significant associations with homologous monoamine transporter gene polymorphisms. Our data confirm a relationship between SSRI response and *5-HTT* polymorphisms, and establish an association between NRI response and the NET G1287A polymorphism. We also found that the *5-HTTLPR s/l* variation plays a role in the treatment of depression with both NRI and SSRI agents. The results of this study need to be confirmed in other populations, using selective NRIs other than nortriptyline. Additional studies in younger populations with depression are also needed.

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