Association Between a Functional Serotonin Transporter Promoter Polymorphism and Citalopram Treatment in Adult Outpatients With Major Depression

Xian-Zhang Hu, MD, PhD; A. John Rush, MD; Dennis Charney, MD; Alexander F. Wilson, PhD; Alexa J. M. Sorant, MA; George J. Papanicolaou, PhD; Maurizio Fava, MD; Madhukar H. Trivedi, MD; Stephen R. Wisniewski, PhD; Gonzalo Laje, MD; Silvia Paddock, PhD; Francis J. McMahon, MD; Husseini Manji, MD; Robert H. Lipsky, PhD

Context: The HTTLPR, a functional polymorphism of the serotonin transporter gene solute carrier family 6 (neurotransmitter transporter, serotonin), member 4 (SLC6A4), promoter, affects transcription and may be involved in antidepressant drug treatment outcome, although response rates with antidepressants can be lower in patients who experience adverse effects.

Objective: To test the hypothesis that HTTLPR is associated with treatment outcome to citalopram.

Design: A clinical effectiveness trial, Sequenced Treatment Alternatives to Relieve Depression, collected DNA samples from outpatients with nonpsychotic major depressive disorder who received citalopram in the first treatment step. The triallelic HTTLPR locus was genotyped in 1775 samples to discriminate between long (L) and short (S) alleles, followed by the A/H11022 substitution. The low-expression S and L_G alleles were grouped together compared with the high-expression L_A allele.

Setting: Eighteen primary care and 23 psychiatric care sites across the United States.

Participants: Ages 18 to 75 years, meeting criteria for single or recurrent nonpsychotic major depression.

Main Outcome Measures: Categorical response, remission, tolerance, and adverse effect burden.

Results: Expression-based grouping produced a significant finding of association between the L_A allele and adverse effect burden in the entire sample (P = .004 [genotype frequency]; P < .001 [allele frequency]). To control for bias from population stratification, a white American subsample was analyzed. A lesser adverse effect burden was associated with L_AL genotype frequency (P = .03) or L_A allele frequency (P = .007). These findings in white patients did not hold when the L allele was undifferentiated. No association was observed between treatment outcome phenotypes and HTTLPR. Development of diarrhea and the presence of the low-expression S or L_G alleles were the strongest risk factors associated with adverse effect burden.

Conclusions: The HTTLPR polymorphism is associated with citalopram adverse effects. Because the L_A allele confers increased SLC6A4 transcription, increased serotonin transporter levels in brain and other tissues may lead to fewer adverse effects for antidepressant medications that target the transporter.

Arch Gen Psychiatry. 2007;64(7):783-792

The serotonin transporter (HTT) in brain is the principal site of action for many antidepressant medications in treating major depressive disorder (MDD). Selective serotonin reuptake inhibitors (SSRIs), although widely prescribed, show variable response rates. Approximately 60% of patients improve, but fewer than 50% achieve complete remission of symptoms after the first treatment with a single antidepressant. In addition to genetic mechanisms affecting treatment response, environmental factors add to the variability.

The results of many studies have suggested that genetic variation at the serotonin transporter gene solute carrier family 6 (neurotransmitter transporter, serotonin), member 4 (SLC6A4), may be involved in vulnerability to affective disorders, including MDD, and in treatment response, although the overall conclusions are inconsistent. This issue may reflect differences in study design, selection of study populations, or phenotypic assessment. The effect of additional variation at SLC6A4 must also be considered.

A nucleotide sequence repeat polymorphism in the SLC6A4 promoter region

Author Affiliations are listed at the end of this article.

©2007 American Medical Association. All rights reserved.
(HTTLPR) has itself been the focus of many studies on the pharmacogenomics of antidepressant response in mood disorders.\(^7\)\(^8\)\(^9\)\(^10\) We recently described a common functional A > G variation in the long (L) allele of SLC6A4 HTTLPR. The L\(_c\) allele reduces SLC6A4 messenger RNA expression to levels nearly equivalent to those of the short (S) allele,\(^7\) whereas the L\(_s\) allele confers higher SLC6A4 expression, producing a gain-of-function phenotype. Prediction of SLC6A4 expression is significantly improved with knowledge of the L\(_c\) allele proportional to its frequency in the populations under study.\(^7\)

The Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial (http://www.star-d.org) collected DNA samples from 1953 participants in a clinical study who received the SSRI citalopram hydrobromide in primary and psychiatric care settings followed by regular assessment of outcome and adverse effects.\(^8\) To better understand the potential effect of variation in HTTLPR on outcome after antidepressant drug treatment, we performed a genetic association study of phenotypes measuring treatment outcomes and the adverse effect burden.

### STUDY DESIGN AND PARTICIPANTS

The rationale, methods, and design of the STAR*D study are described elsewhere.\(^9\)\(^10\) This multisite, prospective, randomized, multistep clinical trial of outpatients with nonpsychotic MDD was designed to compare treatment options for individuals who did not respond favorably to the SSRI citalopram. Only outcomes with citalopram are considered herein. Investigators at 14 regional centers implemented the protocol at 18 primary care and 23 psychiatric care sites across the United States. Media advertising was not used to recruit participants. All the participants provided written informed consent before study entry and separate consent for the DNA samples.

Study participants aged 18 to 75 years with a baseline 17-item Hamilton Rating Scale for Depression\(^11\)\(^12\) score of at least 14 meeting DSM-IV criteria for single or recurrent nonpsychotic MDD were eligible. Patients with a history of bipolar or psychotic disorder or a primary diagnosis of obsessive-compulsive or eating disorder were excluded, as were patients with substance dependence that required inpatient detoxification, general medical conditions that precluded study medications, and a previous nonresponse or clear-cut intolerance to any trial medication used in the first 2 treatment steps and those who were pregnant or breastfeeding. We did not exclude patients with other Axis I conditions, although MDD had to be the primary diagnosis.

Patients completed the Psychiatric Diagnostic Screening Questionnaire,\(^13\)\(^14\)\(^15\) which estimated the presence of 11 different concurrent Axis I disorders using a threshold of 90% or greater specificity for each disorder.\(^16\) Clinical research coordinators administered the initial 17-item Hamilton Rating Scale for Depression\(^11\) at entry and exit from each treatment step and the 16-item Quick Inventory of Depressive Symptomatology–Clinician Rated (QIDS-C\(_{16}\))\(^17\) at baseline and at each clinical visit.\(^8\)\(^17\)\(^18\) The protocol recommended visits at 2, 4, 6, 9, and 12 weeks. The initial dosage of citalopram hydrobromide, 20 mg/d, could be raised to 40 mg/d by week 4 and then to 60 mg/d (final dosage) by day 42 (week 6). Dosage adjustments were based on duration, symptom changes, and adverse effect burden. The protocol ensured an adequate dose of citalopram of sufficient duration to maximize the likelihood of remission (ie, to ensure that those without improvement were not simply underdosed). The mean (SD) prescribed citalopram hydrobromide dosage at level 1 exit was 41.8 (16.8) mg/d, which was comparable for patients who did vs did not achieve remission.\(^8\) The present group of patients with MDD who were genotyped had a mean (SD) prescribed citalopram hydrobromide dosage at study exit of 45.1 (15.9) mg/d, which was not statistically significantly different from that of the entire patient group. Patients could discontinue citalopram use before the recommended 12 weeks of treatment if intolerable adverse effects required a change in medication, an optimal dosage was not possible owing to adverse effects, significant MDD symptoms (QIDS-C\(_{16}\) score \(\geq 9\)) were present after 9 weeks at the maximally tolerated dose, or the participant chose to discontinue use. It is also possible that many participants who reached remission did so as a result of the nonspecific or placebo effects of treatment (or the simple passage of time). On the other hand, this sample was characterized by more general medical and psychiatric comorbidities and chronicity than samples in efficacy trials that typically exhibit substantial placebo response rates. Possible effects due to race or ethnicity on SSRI treatment response\(^19\) (population stratification) were also considered (see the following subsection).

### RACE AND ETHNICITY

Study participants were enrolled without regard to race or ethnicity. Detailed data on ethnicity were not collected. Instead, participants self-reported their race as white, black, or “other” and ethnicity as Hispanic (yes/no). Classification into a population group was performed using marker allele frequencies using population structure in the self-identified white and black groups and was described elsewhere.\(^20\) Because 114 individuals self-identified as “other,” these samples were excluded from this analysis. The white and black groups behaved as 2 different populations. We excluded self-identified Hispanic individuals from the white group in association analyses to avoid potential unknown sources of population stratification. The control samples were composed of white (white non-Hispanic) and black individuals.

### OUTCOME MEASURES

All outcome definitions and measures were agreed on in advance and assigned before genotyping. Treatment outcomes were scored in 3 ways: responder status, remitter status, and tolerance status. Responders had to achieve at least a 30% reduction in baselineisd QIDS-C\(_{16}\) score by the last treatment visit while taking citalopram. Nonresponders did not achieve at least a 40% reduction from the baseline QIDS-C\(_{16}\) score at the last treatment visit. Nonresponders also had a QIDS-C\(_{16}\) score of 10 or greater at their last treatment visit. To avoid misclassification of patients with MDD as displaying symptomatic “remission” or “nonremission,” a QIDS-C\(_{16}\) score at study exit of 6 to 9 defined this group of patients as “undetermined,” and they were excluded from the remitter and nonresponder groups. Individuals with a baseline QIDS-C\(_{16}\) score less than 10, fewer than 6 weeks of treatment, nonadherence to the medication regimen (patients completed a global rating of treatment adherence), or intermediate results were also excluded from the analysis. Participants scored as “intolerant” or intermediate...
ADVERSE EFFECTS
AND TOLERABILITY

Tolerance to a medication is determined by the severity of the adverse effects associated with the drug. If adverse effects are serious enough, medication discontinuation may be necessary. Adverse effects were evaluated using the patient-rated Global Rating of Side Effect Burden (GRSEB) score, which incorporated 7-point subscales of self-reported adverse effects during treatment. A GRSEB score less than 4 classified a patient as having reduced adverse effects, whereas patients with a GRSEB score of 4 or greater had increased adverse effects and, thereby, a greater adverse effect burden. A few participants with missing GRSEB scores in the total analyzed sample (19 of 1659) were excluded from the analysis.

To determine tolerability to treatment, an algorithm was used that considered study exit data and the Frequency, Intensity, and Burden of Side Effects Rating (FIBSER) Scale score21 to place the patient into 1 of 4 categories: “tolerant,” “probably tolerant,” “probably intolerant,” or “intolerant.” In addition, study participants who elected to continue treatment with citalopram after the first stage of the trial were considered tolerant. In the group of tolerant participants, individuals with a high FIBSER Scale score (intolerable adverse effects) who were willing to continue treatment (presumably because they were receiving benefit) and who continued to experience similar adverse effects were considered intolerant. This included the reason for study exit given by the participant as “intolerant” to citalopram. If the reason for exit was not given, an individual with a GRSEB score of 4 or greater and a treatment duration of 4 weeks or less was considered intolerant.

Because GRSEB and FIBSER Scale scores cannot identify specific adverse effects and because SSRIs are also associated with peripheral serotonergic adverse effects, including treatment-emergent diarrhea, we performed another test based on whether the patient reported having diarrhea at the first treatment visit (diarrhea-1) or the last treatment visit (diarrhea-2) in which they met 1 of 2 criteria: the patient received the same dose of citalopram on subsequent treatment visits or a higher dose of citalopram before the last visit. The lack of a baseline score was one reason we determined whether the patient developed diarrhea twice during the initial phase of the trial, at the first visit and at the last visit after commencing medication treatment. Another reason for evaluating patients in this way was to determine whether the diarrhea was due to a long-term effect of taking citalopram.

DNA SAMPLES

Blood samples were collected in citrate-treated vacuum tubes from each of the 1953 STAR*D study participants. Genomic DNA was extracted from whole blood and cryopreserved at the Rutgers University Cell and DNA Repository (Piscataway, New Jersey). In this study, 1775 DNA samples from the STAR*D study and 751 control DNA samples were genotyped. Control DNA samples were obtained through the National Institute of Mental Health Center for Collaborative Genetic Studies on Mental Disorders (http://zork.wustl.edu/nimh/NIMH_initiative/NIMH_initiative_link.html). A CONSORT (Consolidated Standards of Reporting Trials) chart for genotyping and analysis is shown in the Figure. Samples were arrayed using a liquid handler (Genesis; Tecan US, Durham, North Carolina) (R.H.L., Laboratory of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health). The samples were sex verified with a set of 3 X-linked and 2 Y-linked markers (F.J.M., Mood and Anxiety Program, National Institute of Mental Health, National Institutes of Health). Five samples revealed sex discrepancies and were excluded from further analysis. In addition, 2 samples were genetically identical on all 768 tested markers and were excluded because of uncertainty about patient identity.

A summary of the major demographic and clinical characteristics of the 1775 patients with MDD and the 751 controls is given in Table 1. Participants in this study were similar to the 1953 participants who consented to have blood samples collected for DNA isolation. No statistically significant differences were observed between the samples from the STAR*D study cohort for the sociodemographic and clinical characteristics listed. There were differences in several variables between patients with MDD and controls. The control group was older but comprised a near equal distribution of men and women.
Reduced: stage 1 standards were introduced. Briefly, stage 1 of the method differentiated between samples as 1 of 6 genotypes: AL, AG, LL, LG, LL, and LG. Stage 1 and stage 2 genotypes were combined to assign genotypes. Genotypes were generated using sequence detection primers (100 nM each), dimethyl sulfoxide (4% by volume), 25 to 50 ng of DNA, 120 nM allele-discriminating probe, and fluorogenic probes were labeled at the 5' end with either FAM (6-carboxy-fluorescein) or VIC (Applied Biosystems Inc, Foster City, California). Sequences of the allele-specific detection probes and control probes are as described by Hu et al.7 A polymerase chain reaction was conducted in a 25-μL volume: 25 to 50 ng of DNA, 120 nM allele-discriminating probe, 80 nM internal control probe, polymerase chain reaction primers (100 nM each), dimethyl sulfoxide (4% by volume), and 1X Master Mix (Applied Biosystems Inc). Amplification conditions were 2 minutes at 50°C, 10 minutes at 95°C, and then 40 cycles at 96°C for 15 seconds and 62.5°C for 90 seconds. Genotypes were generated using sequence detection system software (ABI PRISM 7700; Applied Biosystems Inc). Stage 1 and stage 2 genotypes were combined to assign samples as 1 of 6 genotypes: SS, SL, SL, LL, LL, and LL. Each plate, previously sequenced standards were introduced: stage 1 standards were SS, SL, and SS and stage 2 standards were LL, LL, and LL. To evaluate genotyping accuracy, 10% of randomly selected samples were genotyped in duplicate. The overall error rate was 1.2%. The completion rate was greater than 99%.

### Table 1. Demographic and Clinical Characteristics of the STAR*D Study Cohort and the Control Sample

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Depressive Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 1775)</td>
<td>(n = 751)</td>
</tr>
<tr>
<td>Sociodemographic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, mean ± SD, y</td>
<td>42.4 ± 13.4</td>
<td>51.9 ± 16.8</td>
</tr>
<tr>
<td>Sex, No.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>688</td>
<td>376</td>
</tr>
<tr>
<td>Female</td>
<td>1087</td>
<td>375</td>
</tr>
<tr>
<td>Race, No.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>1418</td>
<td>642</td>
</tr>
<tr>
<td>Black</td>
<td>250</td>
<td>109</td>
</tr>
<tr>
<td>Other (mixed)</td>
<td>107</td>
<td>0</td>
</tr>
<tr>
<td>Education, mean ± SD, y</td>
<td>13.6 ± 3.2</td>
<td>NA</td>
</tr>
<tr>
<td>Employment, No.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>995</td>
<td>NA</td>
</tr>
<tr>
<td>Unemployed</td>
<td>648</td>
<td>NA</td>
</tr>
<tr>
<td>Retired</td>
<td>132</td>
<td>NA</td>
</tr>
<tr>
<td>Clinical, mean ± SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at first MDE, y</td>
<td>25.8 ± 14.8</td>
<td>NA</td>
</tr>
<tr>
<td>Time since first MDE, y</td>
<td>16.7 ± 13.9</td>
<td>NA</td>
</tr>
<tr>
<td>Duration of current episode, mo</td>
<td>24.6 ± 53.5</td>
<td>NA</td>
</tr>
<tr>
<td>HRSD-17 score</td>
<td>19.8 ± 6.5</td>
<td>NA</td>
</tr>
<tr>
<td>QIDS-C16 score</td>
<td>16.3 ± 3.4</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: HRSD-17, 17-item Hamilton Rating Scale for Depression; MDE, major depressive episode; NA, not applicable; QIDS-C16, 16-item Quick Inventory of Depressive Symptomatology–Clinician Rated; STAR*D, Sequenced Treatment Alternatives to Relieve Depression.

### HHTTLPR Genotyping

**INCLUDING S, L, AND L ALLELES**

A 2-stage 5'-exonuclease method, detailed elsewhere,7 was used. Briefly, stage 1 of the method differentiated between S and L alleles. Stage 2 differentiated between L and L alleles. A total of 4 allele-specific fluorogenic detection probes were used in the assay, 2 for each stage. For stage 1, the allele-specific probe was capable of hybridizing only once to the 43-base pair L repeat. We also used an internal control probe that hybridized to a sequence in the same amplicon but specific to a divergent repeat found only once in the amplicon and not involved in the repeat polymorphism. For stage 2, probes were designed that were specific for the L and L alleles. The fluorogenic probes were labeled at the 5' end with either FAM (6-carboxy-fluorescein) or VIC (Applied Biosystems Inc, Foster City, California). Sequences of the allele-specific detection probes and control probes are as described by Hu et al.7 A polymerase chain reaction was conducted in a 25-μL volume: 25 to 50 ng of DNA, 120 nM allele-discriminating probe, 80 nM internal control probe, polymerase chain reaction primers (100 nM each), dimethyl sulfoxide (4% by volume), and 1X Master Mix (Applied Biosystems Inc). Amplification conditions were 2 minutes at 50°C, 10 minutes at 95°C, and then 40 cycles at 96°C for 15 seconds and 62.5°C for 90 seconds. Genotypes were generated using sequence detection system software (ABI PRISM 7700; Applied Biosystems Inc). Stage 1 and stage 2 genotypes were combined to assign samples as 1 of 6 genotypes: SS, SL, SL, LL, LL, and LL. Each plate, previously sequenced standards were introduced: stage 1 standards were SS, SL, and SS and stage 2 standards were LL, LL, and LL. To evaluate genotyping accuracy, 10% of randomly selected samples were genotyped in duplicate. The overall error rate was 1.2%. The completion rate was greater than 99%.

### RESULTS

**GENOTYPE- AND ALLELE-BASED ASSOCIATION OF HHTTLPR WITH TREATMENT RESPONSE**

We initially tested for association in the total sample of 1659 patients between HHTTLPR genotype and allele frequencies and treatment tolerance, response, and remission. A few participants (n = 98) were excluded from the analysis for nonadherence to the medication regimen or for other reasons. In addition, based on the a priori definition, patients with MDD with a QIDS-C16 score at study exit of 6 to 9, defined as “undetermined,” were excluded from the remitter and nonremitter groups (312 from the entire sample). For testing association in the nonresponder groups, patients scored as intolerant or probably intolerant were excluded from the analysis. In testing for association with tolerance, patients in the intermediate groups “probably intolerant” (45 from the entire sample) and “probably tolerant” (225 from the entire sample) were excluded.

Based on knowledge of HHTTLPR function, we grouped the low-expression S and L alleles together to compare with the high-expression L allele.7 We also tested for association without distinguishing the L and L alleles. The functional grouping (L vs S and L alleles) produced no significant finding of association with tolerance, response, or remission (Table 2). Association was also tested between the undifferentiated L allele and the 3 outcome measures. No association was found between the L allele and treatment outcome.

However, when the entire sample was tested for association between genotype frequencies and allele frequencies and adverse effect burden, as measured by GRSEB scores, a significant finding of association was observed (P = .004 based on genotype frequency and P = .001 based on allele frequency). Association was also observed between the undifferentiated L allele and adverse effect burden (P = .006 for genotype-based association and P = .001 for allele-based association). We recently showed that HHTTLPR S, L, and L alleles frequencies differ between white and black groups. In white patients, the

### Statistical Methods

Associations with clinical outcomes were evaluated using χ² and logistic regression analyses. Association was tested on the basis of genotype or allele frequency, where alleles were grouped by functionality (L vs S and L), because L is the high-expressing allele; S and L were grouped together because they have nearly equivalent expression. We also compared S and L. Univariate and multivariate logistic regression analyses were performed to identify risk factors for adverse effects considering HTTLPR genotype, sex, citalopram dosage, diarrhea-1, and diarrhea-2 as possible factors. All variables with a P < .20 in univariate analyses were used in the multivariate model. A backward elimination procedure was used to define the final risk factors. Variables with a P > .05 were eliminated from the model. Similar univariate and multivariate logistic regression analyses were performed considering the HTTLPR genotype, sex, and citalopram dosage as possible risk factors for diarrhea-1 and diarrhea-2. Analyses were performed using SPSS statistical software (Version 12.0; SPSS Inc, Chicago, Illinois).
We determined the contribution of HTTLPR genotype on adverse effect burden to citalopram treatment. For the entire sample, 7% of $L_AL_A$ homozygotes displayed a greater adverse effect burden from the drug compared with 13% of those without the $L_A$ allele. Therefore, the $L_A L_A$ genotype conferred a 6% reduction in absolute risk of adverse effects in the total sample. Nearly identical results were obtained when only the white non-Hispanic subgroup was considered.

### CASE-CONTROL ASSOCIATION

The HTTLPR genotype and allele frequencies were compared in 1131 patients with MDD and 641 nondepressed control subjects. These patient and control groups were entirely composed of white non-Hispanic individuals. No evidence of association was detected using genotype- or allele-based association approaches, grouped either by predicted SLC6A4 expression ($L$ vs $S$ and $L_G$) or using $S$ and $L$ HTTLPR alleles, by combining $L_A$ and $L_G$ (Table 4).

### ADVERSE EFFECTS AND INTOLERANCE

The SSRI medications, such as citalopram, act to block serotonin transporter function, leading to elevated serotonin levels in the central nervous system and throughout the body. This action seems particularly important in the gastrointestinal tract. Subgroups of patients with irritable bowel syndrome have reduced SLC6A4 messenger RNA and HTT immunoreactivity in their mucosa,22 suggesting that decreased HTT function may play a role

### STRENGTH OF ASSOCIATION

We determined the contribution of HTTLPR genotype on adverse effect burden to citalopram treatment. For the entire sample, 7% of $L_A L_A$ homozygotes displayed a greater adverse effect burden from the drug compared with 13% of those without the $L_A$ allele. Therefore, the $L_A L_A$ genotype conferred a 6% reduction in absolute risk of adverse effects in the total sample. Nearly identical results were obtained when only the white non-Hispanic subgroup was considered.

### CASE-CONTROL ASSOCIATION

The HTTLPR genotype and allele frequencies were compared in 1131 patients with MDD and 641 nondepressed control subjects. These patient and control groups were entirely composed of white non-Hispanic individuals. No evidence of association was detected using genotype- or allele-based association approaches, grouped either by predicted SLC6A4 expression ($L$ vs $S$ and $L_G$) or using $S$ and $L$ HTTLPR alleles, by combining $L_A$ and $L_G$ (Table 4).

### ADVERSE EFFECTS AND INTOLERANCE

The SSRI medications, such as citalopram, act to block serotonin transporter function, leading to elevated serotonin levels in the central nervous system and throughout the body. This action seems particularly important in the gastrointestinal tract. Subgroups of patients with irritable bowel syndrome have reduced SLC6A4 messenger RNA and HTT immunoreactivity in their mucosa,22 suggesting that decreased HTT function may play a role

### STRENGTH OF ASSOCIATION

We determined the contribution of HTTLPR genotype on adverse effect burden to citalopram treatment. For the entire sample, 7% of $L_A L_A$ homozygotes displayed a greater adverse effect burden from the drug compared with 13% of those without the $L_A$ allele. Therefore, the $L_A L_A$ genotype conferred a 6% reduction in absolute risk of adverse effects in the total sample. Nearly identical results were obtained when only the white non-Hispanic subgroup was considered.

### CASE-CONTROL ASSOCIATION

The HTTLPR genotype and allele frequencies were compared in 1131 patients with MDD and 641 nondepressed control subjects. These patient and control groups were entirely composed of white non-Hispanic individuals. No evidence of association was detected using genotype- or allele-based association approaches, grouped either by predicted SLC6A4 expression ($L$ vs $S$ and $L_G$) or using $S$ and $L$ HTTLPR alleles, by combining $L_A$ and $L_G$ (Table 4).

### ADVERSE EFFECTS AND INTOLERANCE

The SSRI medications, such as citalopram, act to block serotonin transporter function, leading to elevated serotonin levels in the central nervous system and throughout the body. This action seems particularly important in the gastrointestinal tract. Subgroups of patients with irritable bowel syndrome have reduced SLC6A4 messenger RNA and HTT immunoreactivity in their mucosa,22 suggesting that decreased HTT function may play a role

### STRENGTH OF ASSOCIATION

We determined the contribution of HTTLPR genotype on adverse effect burden to citalopram treatment. For the entire sample, 7% of $L_A L_A$ homozygotes displayed a greater adverse effect burden from the drug compared with 13% of those without the $L_A$ allele. Therefore, the $L_A L_A$ genotype conferred a 6% reduction in absolute risk of adverse effects in the total sample. Nearly identical results were obtained when only the white non-Hispanic subgroup was considered.

### CASE-CONTROL ASSOCIATION

The HTTLPR genotype and allele frequencies were compared in 1131 patients with MDD and 641 nondepressed control subjects. These patient and control groups were entirely composed of white non-Hispanic individuals. No evidence of association was detected using genotype- or allele-based association approaches, grouped either by predicted SLC6A4 expression ($L$ vs $S$ and $L_G$) or using $S$ and $L$ HTTLPR alleles, by combining $L_A$ and $L_G$ (Table 4).
effects resulting from citalopram in the white non-Hispanic subgroup suggested that SLC6A4 genotype and treatment-emergent diarrhea may contribute to intolerance and was the basis for the subsequent analyses.

We thus performed univariate and multivariate logistic regression analyses using adverse effect burden to citalopram as the dependent variable. Based on univariate logistic regression analysis, risk factors for adverse effects to citalopram were HTTLPR genotype (using the functional grouping of S with Lc vs Ltet alleles), \(^7\) citalopram dosage, and treatment-emergent diarrhea at the final treatment visit (diarrhea-2) (**Table 5**). No effect on risk was predicted for sex or other variables, such as age,
weight, family history, disease history, and disease severity. In multivariate analyses, factors for increased risk of adverse effects to citalopram were diarrhea-2, genotypes including S and (L, allele, and citalopram dosage. In this model diarrhea-1 was not significantly associated with a greater adverse effect burden.

This study was designed to determine the possible effect of variation at the SLC6A4 promoter on treatment effectiveness to a single SSRl, citalopram, in a large clinical study of representative outpatients with nonpsychotic MDD treated in a primary or psychiatric care setting. We used 3 categorical measure outcomes to define response, remission, and tolerance. We found no association between SLC6A4 HTTLPR and symptomatic response or remission, which supports the previous treatment outcome findings based on 4 SLC6A4 markers in the same study population. The study had adequate statistical power to detect even a weak association with treatment outcome.

Several pharmacogenetic studies have been performed using SSRIs with the aim of using HTTLPR genotype as a predictor of treatment response (as reviewed in other studies). That evidence suggested a less favorable response to SSRl treatment in patients with the S/S genotype, although differences in study design or patient samples (eg, inclusion of patients with bipolar depression, differences in diagnosis, history of depression, choice of SSRI dosage, and population origin) make comparisons difficult. In addition, interpretation of some associations was based on expression data from cell lines, platelets, and brain using the assumption that the S allele acted dominantly, an assumption that will need to be reconsidered because it was recently shown that HTTLPR alleles act co-dominantly based on previously unrecognized L, alleles in SL and LL genotypes. With these limitations in mind, note that all of the previous studies were also conducted on small patient populations, which may have biased a particular result.

In contrast to the HTTLPR polymorphism, relatively few pharmacogenetic studies have investigated the effect of other SLC6A4 polymorphisms on antidepressant pharmacodynamics. One of these variants is functionally relevant and lies in the second intron of SLC6A4. It is a variable number tandem repeat (VNTR) polymorphism composed of 9, 10, or 12 repeat units. The 12-repeat variant increases transcription compared with the 10-repeat variant in a transfection-based reporter gene assay. Recently, Popp et al showed that patients homozygous for the higher-expression, 12-repeat allele had more adverse effects.

Serotonin receptors have also been logical candidates for identifying possible contributions of genetic variation to antidepressant medication efficacy. A 2006 finding was the first significant and reproducible association between variation at the serotonin 2A receptor gene (HTR2A) and treatment response. In that same study there was no significant association between adverse drug events and HTR2A. A recent study by Kato and colleagues showed that polymorphisms at the HTR2A and HTR3A genes were associated with response and that HTR2A was associated with adverse drug reactions, particularly nausea, while failing to show significant differences between genotype groups for HTTLPR in 81 depressed Japanese patients treated with different SSRIs.

We hypothesized that allelic variation at HTTLPR may have a role in citalopram-induced adverse effects leading to medication intolerance. The SSRIs are associated with adverse effects, where an initial study supported an association of the S allele with the development of treatment-emergent insomnia or agitation. Subsequently, Putzhammer et al observed that nocturnal motor activity was increased during SSRI treatment in individuals with MDD who were homozygous for the L allele. Although these previous studies focused on specific adverse effects associated with HTTLPR polymorphism, other investigators used measures related to a series of adverse events, culminating in discontinuation of treatment or a retrospective assessment of adverse drug events. In those studies, one involving a geriatric population and the other a population with a wider range of ages, patients with the S/S genotype who received SSRI therapy had more adverse effects.

The present study detected a significant association between a lesser adverse effect burden resulting from citalopram treatment and the high-expression, gain-of-function L, allele in a large representative sample. To minimize the risk of spurious associations with the gain-of-

### Table 5. Univariate and Multivariate Logistic Regression Analyses for Predictors of Adverse Effects to Citalopram

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea-1</td>
<td>1.45 (0.98-2.15)</td>
<td>.06</td>
</tr>
<tr>
<td>Diarrhea-2</td>
<td>2.13 (1.37-3.34)</td>
<td>.001</td>
</tr>
</tbody>
</table>

**Abbreviations:** CI, confidence interval; diarrhea-1, patient-reported diarrhea at the first treatment visit; diarrhea-2, patient-reported diarrhea at the last treatment visit; L, long; OR, odds ratio; S, short.

a Expression-based grouping. Because S and L, alleles are nearly equivalent in serotonin transporter expression, these alleles were grouped and compared with the L, allele.

b Statistically significant.
function allele and genotypes due to differences in HTTLPR allele frequencies between populations we also performed the analysis in a subgroup of exclusively white non-Hispanic patients. The association between reduced adverse effects and \( L_\alpha \) allele and \( L_\alpha \)-containing genotypes remained significant with this subgroup, but when \( L_\alpha \) and \( L_\beta \) alleles were not distinguished, the association of HTTLPR with adverse effects was not significant (Table 3).

Based on the idea that colon motility is affected by antidepressant drug therapy, we sought to determine whether treatment-emergent diarrhea contributed to SSRIs intolerance. Treatment-emergent diarrhea was the strongest risk factor for adverse effects, where a 2.31-fold increase in risk was predicted at the last treatment visit. Presence of the low-expression \( S \) or \( L_\beta \) alleles was the second strongest risk factor. Taken together, these results and those supporting an association of the \( L_\beta \) allele with reduced adverse effect burden during citalopram treatment suggest that increased expression of HTT may moderate some citalopram-induced adverse effects. The lack of association of HTTLPR with tolerance is not as clear. Most participants who withdrew from the study owing to adverse effects had higher FIBSER Scale scores, indicating that they had a higher frequency, greater intensity, and increased burden of adverse effects than those who continued citalopram therapy. In addition, patients experiencing adverse effects had their medication dosage lowered in an effort to reduce adverse effects. Because the goal of treatment was to seek relief of depressive symptoms a patient may elect to continue taking the medication even in the face of severe adverse effects. In the present study design, these patients would be classified as tolerant. A likely molecular mechanism to partially explain intolerance is the effect of prolonged availability of serotonin as a result of HTT inhibition, which may lead to desensitization of their cognate receptors, in particular inhibitory autoreceptors.

What can explain the fact that the present study found no association between \( SLC6A4 \) HTTLPR and symptomatic response or remission? The findings are most likely not due to a lack of effectiveness of citalopram for the following reasons: (1) the number of responders in this group was similar to that seen in other studies and (2) in the STAR*D study citalopram dose was increased in nonresponsive patients in an effort to achieve responsiveness. We also did not detect in the same sample an association between treatment outcome and any of 4 \( SLC6A4 \) markers among a total of 768 markers covering a set of 68 candidate genes. This may be due to the study design: we excluded patients who were medication intolerant. Since medication-intolerant patients are less likely to respond to treatment, the present results suggest that the apparent association between HTTLPR and treatment response reported in some previous studies might actually reflect association with tolerance.

However, the lack of association of HTTLPR does not completely rule out a role of \( SLC6A4 \) in SSRI response and remission. In the present study, we analyzed a single polymorphic locus, albeit a functional one. We did not determine genotypes for the VNTR in the STAR*D study cohort, which will be the subject of future studies. Note that HTTLPR resides in a genomic region of low linkage disequilibrium based on HapMap Project-generated data on genomewide levels of variation in different human populations and our own data (X.-Z.H. and R.H.L., unpublished observation, 2006). Therefore, we cannot exclude the possibility that an association may be detected when additional markers are genotyped and the data are analyzed using approaches designed to capture additional information even in the absence of functional loci.

There are several limitations to this study. First, the findings may have been affected by how we defined response, remission, and intolerance. However, because these clinical classifications were determined a priori, without knowledge of the patient’s genotype, the results are independent. In addition, the STAR*D study cohort consisted of individuals with many comorbidities; although representative of patients with MDD, these comorbidities could have affected response, remission, or tolerance to citalopram treatment. Another issue was the lack of a standard measure for determining medication regimen adherence in this outpatient population, which could also have affected the results. Finally, note that there was no “placebo control” group in this study. Participants who remitted largely held the benefit in the longer-term naturalistic follow-up used herein (A.J.R., S.R.W., data available from the authors), which suggests that a “placebo response” could not explain most of the benefits because placebo effects often wear off with time.

Despite these limitations, the present results show that the HTTLPR polymorphism is associated with citalopram adverse effects in a large patient sample with MDD. Because the \( L_\alpha \) allele predicts increased \( SLC6A4 \) transcription, increased HTT levels in tissues outside the central nervous system may be responsible for reducing adverse effects from SSRIs. A better understanding of the effect of genetic variation in developing adverse effects to medications may aid in improving response through increased treatment adherence and better treatment regimens. The future of patient care may rely on individualized therapies, in which pretreatment genotyping will be an essential part of the decision-making process to provide appropriate care and management of patients with MDD.
Financial Disclosures: Dr Rush has received research support from the Robert Wood Johnson Foundation, the National Institute of Mental Health, and the Stanley Medical Research Institute; has been on the advisory board of or has been a consultant to Advanced Neuromodulation Systems Inc, Best Practice Project Management Inc, Bristol-Myers Squibb Co, Cyberonics Inc, Eli Lilly & Co, Forest Pharmaceuticals Inc, Gerson Lehman Group, GlaxoSmithKline, Healthcare Technology Systems Inc, Jazz Pharmaceuticals Inc, Merck & Co Inc, Neuronetics, Ono Pharmaceutical, Organon USA Inc, Personality Disorder Research Corp, Pfizer Inc, the Urban Institute, and Wyeth-Ayerst Laboratories Inc; is on the speaker’s bureaus of Cyberonics Inc, Eli Lilly & Co, Forest Pharmaceuticals Inc, GlaxoSmithKline, and Merck & Co Inc; owns stock (excluding mutual funds/blended trusts) in Pfizer Inc; and has royalty (patent and other income) from Guilford Publications and Healthcare Technology Systems Inc. Dr Charney has been a consultant to Abbott Laboratories, AstraZeneca, Bristol-Myers Squibb Co, Cephalon, Eli Lilly & Co, Forest Laboratories Inc, GeneLogic Inc, the Institute of Medicine, Neurogen Corp, the Neuroscience Education Institute, Novartis Pharmaceuticals Corp, Orexigen Therapeutics Inc, Organon International Inc, Otsuka America Pharmaceutical Inc, Quintiles Inc, and Sepracor Inc. Dr Fava has received research support from Abbott Laboratories, Alkermes, Aspect Medical Systems, AstraZeneca, Bristol-Myers Squibb Co, Cephalon, Eli Lilly & Co, Forest Pharmaceuticals Inc, GlaxoSmithKline, J & J Pharmaceuticals, Lichtwer Pharma GmbH, Lorex Pharmaceuticals, Novartis Pharmaceuticals Inc, Organon Inc, PatLab LLC, Pfizer Inc, Pharmavite Roche, Sanofi-Synthelabo, Solvay Pharmaceuticals Inc, and Wyeth-Ayerst Laboratories Inc; has been an advisor or consultant to Aspet Medical Systems, AstraZeneca, Bayer AG, Biovail Pharmaceuticals Inc, Brain Cells Inc, Bristol-Myers Squibb Co, Cephalon, Compellis, Cypress Pharmaceuticals, Dov Pharmaceuticals, Eli Lilly & Co, EPIX Pharmaceuticals, Fabre-Kramer Pharmaceuticals Inc, Forest Pharmaceuticals Inc, GlaxoSmithKline, Grunenthal GmbH, J & J Pharmaceuticals, Janssen Pharmaceutical, Jazz Pharmaceuticals, Knoll Pharmaceutical Co, Lundbeck, MedAvante Inc, Neuronetics, Novartis Pharmaceuticals Corp, Nutrition 21, Organon Inc, PatLab LLC, Pfizer Inc, PharMacia, Pharmavite Roche, Sanofi-Synthelabo, Sepracor Inc, Solvay Pharmaceuticals Inc, Somaxon, Somerset Pharmaceuticals, and Wyeth-Ayerst Laboratories Inc; has been on the speaker’s bureaus for AstraZeneca, Boehringer-Ingelheim, Bristol-Myers Squibb Co, Cephalon, Eli Lilly & Co, Forest Pharmaceuticals Inc, GlaxoSmithKline, Novartis Pharmaceuticals Corp, Organon Inc, Pfizer Inc, PharMacia, and Wyeth-Ayerst Laboratories Inc; and owns stock in Compellis and MedAvante.

Funding/Support: This work was supported in part by National Institutes of Health intramural research programs of the National Institute on Alcohol Abuse and Alcohoholism (Dr Lipsky), the National Human Genome Research Institute (Drs Wilson and Papanicolaou and Ms Sorant), and the National Institute of Mental Health (Dr McMahon) and by extramural contract N01MH90003 from the National Institute of Mental Health (Dr Rush). The Rutgers Cell and DNA Repository provided DNA samples, and Forest Laboratories provided citalopram at no cost to the STAR*D study.

Additional Contributions: We thank Katrina Neyer and Tanglea R. Anderson, for technical assistance, and the study participants, without whom this work could not have been possible.

REFERENCES

17. Rush AJ, Trivedi MH, Ibrahim HM, Carmody TJ, Arnow B, Klein DN, Markowitz (REPRINTED) ARCH GEN PSYCHIATRY/Vol. 64 (No. 7), JULY 2007 WWW.ARCHGENPSYCHIATRY.COM ©2007 American Medical Association. All rights reserved.