

Analysis of Association Between the Serotonin Transporter and Antidepressant Response in a Large Clinical Sample

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Background: SLC6A4 encodes the serotonin transporter, the protein primarily responsible for the termination of serotonin neurotransmission. Because many antidepressants inhibit the transporter, it has been the focus of intense pharmacogenetic analysis. We sought to replicate our previous findings that SLC6A4 is associated with response to a selective serotonin reuptake inhibitor (SSRI) in a large case-control study.

Methods: Genotypes at the SLC6A4 locus were obtained for 1,914 subjects in the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study and then tested for association to treatment response of the SSRI citalopram.

Results: Nine tagging single nucleotide polymorphisms and two variants previously associated with antidepressant response, including a promoter repeat polymorphism, were genotyped. Single marker and haplotypic analyses failed to detect association with antidepressant response in the largest clinical sample studied to date.

Conclusions: The lack of association between response to an SSRI and variation at the SLC6A4 locus in this large sample, carefully characterized for response to citalopram, strongly suggests that SSRI response in major depression is not determined by DNA variation at this locus. These findings do not replicate findings of a number of studies with considerably smaller sample sizes. Other genetic determinants of SSRI response in depression should be sought.

Key Words: Association, case-control, citalopram, 5-HTTLPR, pharmacogenetics, SLC6A4, SNP, STAR*D

Pharmacologic treatment with selective serotonin reuptake inhibitors (SSRIs) is a mainstay in the treatment of major depressive disorder (MDD). There is prominent heterogeneity in response to antidepressants (Esposito and Goodnick 2003), and as of yet, there are no explanations for this observed variability. One possibility that has generated great interest, however, is that this variability results from genetic variation at the SLC6A4 locus (Lesch and Gutknecht 2005). A number of studies have tested the association between DNA variations in this gene and response to various SSRIs (Serretti and Artioli 2004). The results of these studies are inconclusive, however, particularly when different populations are investigated. In studies of the promoter variant (5-HTTLPR), some work finds no association with response (Joyce et al 2003; Peters et al 2004), whereas other reports show an association with treatment response (Serretti et al 2005), although the associated allele varies with ethnicity (Table 1). A limitation of the previous studies is that most did not systematically study variation across this gene, instead focusing almost exclusively on the promoter variant or, to a lesser extent, on an intronic tandem repeat polymorphism (Smits et al 2004). Although our group failed to find association between the 5-HTTLPR and SSRI response, we did detect asso-

ciation between two single nucleotide polymorphisms (SNPs), rs25531 and rs25533, near the 5-HTTLPR in the 5' region of this gene in studies of 96 subjects treated with fluoxetine (Kraft et al 2005; Peters et al 2004). We also tested 18 other exonic and intronic variants but found no association with these variants. The discrepancy among these previous studies (including ours) may be explained by small sample size (suggesting limited power), by differing analytical approaches, and by the use of different medications across studies. Although it is presumed that all SSRIs share the same response mechanism, this is not known. Table 1 summarizes the findings of these previous studies, which were chosen for inclusion if the study analyzed variants in the serotonin transporter in depressed samples being treated with an SSRI. In several of these studies, analyses were carried out by modeling recessive or dominant transmission of the short allele, but this was not done in others.

In this study, we sought to conduct a more definitive study by genotyping variants in the SLC6A4 gene in a large sample of subjects with major depression whose response to treatment had been carefully characterized. All of the subjects were treated with the SSRI citalopram. This sample was collected in the course of Sequenced Treatment Alternatives to Relieve Depression (STAR*D), a prospective, multicenter, randomized clinical trial involving 4,041 outpatients in both primary and specialty care settings (Rush et al 2004; Trivedi et al 2006).

Methods and Materials

Sample

Of the 4,041 subjects, DNA was obtained from 1,953 subjects as part of the National Institute of Mental Health (NIMH) Human Genetics Initiative. The design of STAR*D was to enroll adults experiencing a major depressive episode who exhibited neither an inadequate response nor intolerance to an adequate trial of any of the STAR*D protocol treatments during the current episode. The overall aim of STAR*D (principal investigator, A.

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Table 1. Summary of Studies Examining SLC6A4 Genotypes for Association with STAR*D Response in Unipolar Major Depression.

Authors	Year	Population†	UPD	Drug	Variant	Result
Smeraldi et al	1998	Italian?	69	Fluvoxamine	5-HTTLPR	s/s = poor response
Pollock et al	2000	?	51	Paroxetine	5-HTTLPR	s/s + s/l = slower response
Zanardi et al	2000	Italian	58	Paroxetine	5-HTTLPR	s/s = poor/slow response
Kim et al	2000	Korean	120	Fluoxetine, Paroxetine	5-HTTLPR, Int2 VNTR	l/l = poor response; Int2 VNTR 12 allele = good response
Zanardi et al	2001	Italian?	108	Fluvoxamine	5-HTTLPR	s/s>s/l>l/l for poor response
Rausch et al	2002	?	51	Fluoxetine	5-HTTLPR	s = poor response
Yu et al	2002	Chinese	121	Fluoxetine	5-HTTLPR	s/s/ + s/l = poor response
Yoshida et al	2002	Japanese?	66	Fluvoxamine	5-HTTLPR, Int2 VNTR	l = poor response, Int2 VNTR not associated
Ito et al	2002					
Arias et al	2003	Spanish	131	Citalopram	5-HTTLPR	s/s = non-remission
Joyce et al	2003	New Zealand?	86	Fluoxetine	5-HTTLPR	No association
Yoshida et al	2004	Japanese	96	Milnacipran	5-HTTLPR, Int2 VNTR	No association
Durham et al	2004	U.S. White‡	106	Sertraline	5-HTTLPR	l/l = early response
Murphy et al	2004	U.S. White‡	121	Paroxetine	5-HTTLPR	No association with efficacy
Peters et al	2004	U.S. White‡	96	Fluoxetine	5-HTTLPR, Int2 VNTR, Int7 STR, 18 SNPs	rs25531 and rs25533 minor alleles = poor response
Kraft et al	2005					
Kato et al	2005	Japanese	81	Paroxetine, Fluvoxamine	5-HTTLPR	No association in combined sample
Ng et al	2006	White, Chinese	35	Sertraline	5-HTTLPR	No association

UPD, number of subjects with unipolar major depression. Result, s = short allele of 5-HTTLPR; l = long allele of 5-HTTLPR; Int2 VNTR, intron 2 variable number tandem repeat; Int7 STR, intron 7 short tandem repeat. †, ethnicity of populations in publications that are without “?” are specifically described in the publications. ‡, populations for Durham et al, 95% White, 3% African American, <1% Asian, 1% other; Murphy et al, 89% White, 5% African American, 2% Asian, 4% other; and Peters et al and Kraft et al, 78% White, 6% African American, 7% Hispanic, 5% Asian, 3% other. Some studies grouped genotypes by the presence of the S allele, while others did not. Studies not included (Lee et al 2004; Minov et al 2001) were excluded because multiple classes of drugs were used and it was not possible to separate SSRIs from other drugs and antidepressant treatments for association to SLC6A4.

John Rush, NIMH Contract N01-MH-90003) was to determine prospectively which of a number of treatments are beneficial for subjects experiencing an unsatisfactory clinical outcome following treatment with citalopram. Because the STAR*D trial design has been described extensively (Fava et al 2003; Rush et al 2004; Trivedi et al 2006), it is summarized only briefly here. To make the findings as generalizable as possible, STAR*D used broad inclusion criteria (Fava et al 2003; Trivedi et al 2006) and enrolled a diverse population, including good minority representation. Diagnoses were made using the Psychiatric Diagnostic Screening Questionnaire (Zimmerman and Mattia 1999), and depressive symptoms were assessed with the 16-item Quick Inventory of Depressive Symptomatology Self-Report version (QIDS-SR) collected at clinic visits. The QIDS-SR is highly correlated with the 17-item Hamilton Rating Scale for Depression (HRSD₁₇), and scores can be converted readily between the two instruments (Rush et al 2003). Subjects meeting criteria and providing consent were administered citalopram as the initial treatment. The protocol encouraged 12 weeks of treatment with vigorous dosing of open-label citalopram (20–60 mg/day).

The subsample of 1,953 participants who consented to provide DNA samples was 61.8% female and 38.2% male, with ethnic proportions of 78.1% White, 16.1% African American, 3.5% multiracial, 1.1% Asian, 1.2% Pacific Islander/Native American, and 0.1% unspecified; 14.0% of the sample reported being Hispanic, and 43.5% of the sample came from primary care clinics, with the remaining 56.5% coming from specialty clinics. At the time of this report, we have received DNA from 1,914 participants (98%). Baseline demographic and clinical data on these 1,914 subjects are presented in Table 2. Access to the DNA samples and clinical data was approved by the STAR*D Ancillary Studies Committee, and clinical data were obtained from the Data Coordinating Center of STAR*D.

Approval to carry out the work described here was obtained by the Committee on Human Research at the University of California, San Francisco.

Marker Selection

To provide adequate coverage of the entire SLC6A4 locus, tagging SNPs were chosen based on our previous genotyping and variant discovery efforts (Kraft et al 2005; Peters et al 2004). Tagging SNPs were selected from a data set of SNP variants that were common (> 5% minor allele frequency) in a subset of White patients ($n = 75$) from our previous studies. Thirteen SNPs met this criteria, and seven were selected as tagging SNPs using a linkage disequilibrium (LD) threshold of $r^2 > .8$, as implemented in the program “ldselect.pl” (Carlson et al 2004). Because our initial variant characterization efforts were performed using a largely White population, we compared our tagging SNP set to HapMap data for this genomic region, which contained 10 common SNPs in a sample of 30 Yoruban trios (HapMap build 16c.1, June 2005). Two additional tagging SNPs from HapMap data were selected for this study (rs16965628 and rs2020933) because they had large (> .25) minor allele frequency differences between Yoruban samples and White samples. In addition, two markers that were previously reported to be associated with antidepressant response (5-HTTLPR, rs25531; Kraft et al 2005; Peters et al 2004; Serretti and Artioli 2004) were also included in this study, for a total of 11 markers (Table 3). The distribution of markers at the SLC6A4 is schematized in Figure 1.

DNA Analysis and Genotyping

DNA from subjects was quantified and then used as a template to amplify specific regions of the gene via polymerase chain reaction (PCR). Variants were assayed by either fluorescence polarization detection of template-directed dye-terminator

Table 2. Baseline Clinical and Demographic Data from STAR*D Participants.

Baseline Variable	Total subjects in Analysis (N = 1,914)	Subjects classified as Responders (N = 991)	Subjects classified as Nonresponders (N = 669)	p value	Subjects classified as Specific Responders (N = 679)	Subjects classified as Non-Specific Responders (N = 187)	Subjects Classified as Remitters (N = 826)
Race/Ethnicity n (%)							
White	1,501 (78.4)	799 (80.6)	509 (76.1)	.06	559 (82.3)	146 (78.1)	679 (82.2)
African American	299 (15.6)	130 (13.1)	121 (18.1)		77 (11.3)	28 (15.0)	100 (12.1)
Other (Multiracial)	68 (3.6)	36 (3.6)	23 (3.4)		23 (3.4)	9 (4.8)	25 (3.0)
Asian	21 (1.1)	15 (1.5)	5 (0.7)		12 (1.8)	2 (1.1)	13 (1.6)
Pacific Islander/Native American	24 (1.2)	11 (1.1)	11 (1.6)		8 (1.2)	2 (1.1)	9 (1.1)
Unspecified	1 (.1)	0	0				
Number of Hispanic, n (%)	269 (14.1)	122 (12.3)	102 (15.2)	.09	82 (12.1)	25 (13.4)	96 (11.6)
Number of Females, n (%)	1,179 (61.6)	621 (62.7)	404 (60.4)	.35	424 (62.4)	126 (67.4)	518 (62.7)
Mean Age, Years (SD)	42.6 (13.4)	42.2 (13.4)	43.0 (13.2)	.11	41.7 (13.3)	43.4 (14.0)	42.0 (13.7)
Mean Years, Schooling (SD)	13.6 (3.3)	14.1 (3.3)	13.2 (3.1)	<.001	14.2 (3.3)	13.9 (3.3)	14.2 (3.3)
Marital Status							
Married	819 (42.8)	435 (43.9)	290 (43.3)	.27	301 (44.3)	76 (40.6)	377 (45.6)
Never Married	536 (28.0)	278 (28.1)	185 (27.7)		190 (28.0)	53 (28.3)	239 (28.9)
Divorced	483 (25.2)	249 (25.1)	162 (24.2)		169 (24.9)	52 (27.8)	189 (22.9)
Widowed	76 (4.0)	29 (2.9)	32 (4.8)		19 (2.8)	6 (3.2)	21 (2.5)
Clinical Characteristics							
Age at first MDE (SD)	26.1 (14.9)	26.2 (14.4)	25.6 (14.9)	.19	26.4 (14.6)	24.8 (13.7)	26.4 (14.5)
Months in current MDE (SD)	25.0 (53.9)	21.6 (45.8)	31.7 (65.8)	<.001	20.4 (45.5)	24.6 (51.5)	21.3 (45.9)
Index Length 24+ Months (%)	487 (25.4)	230 (23.2)	203 (30.3)	.001	142 (20.9)	53 (28.3)	187 (22.6)
Presence of Recurrent Depression (%)							
Depression (%)	1,347 (70.4)	701 (70.7)	469 (70.1)	.71	481 (70.8)	130 (70.0)	574 (69.5)
Presence of Family History of Depression (%)							
Depression (%)	1,037 (54.2)	557 (56.2)	346 (51.7)	.09	382 (56.3)	103 (55.1)	466 (56.4)
Baseline QIDS (SD)	16.4 (3.4)	16.2 (3.3)	16.6 (3.4)	.008	16.2 (3.3)	16.1 (3.1)	15.8 (3.3)
Years Since first MDE (SD)	16.6 (13.9)	16.0 (13.8)	17.5 (14.0)	.018	15.4 (13.8)	18.6 (14.6)	15.7 (13.8)

The p value corresponds to the test for differences in clinical or demographic variables in the responder vs. nonresponder groups, using the χ^2 for categorical data and t-test for continuous data.

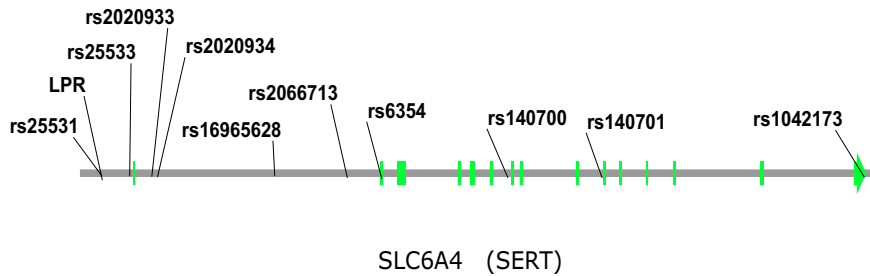


Figure 1. Schematic of the SLC6A4 region. The relative location of each genotyped variant is shown with respect to the 2 noncoding and 13 coding exons of SLC6A4. The direction of transcription is indicated by the arrow. Note that the orientation is reversed from the actual genomic orientation.

incorporation (FP-TDI) or by the use of restriction fragment length polymorphism analysis (RFLP). Additional details can be found in supplemental materials (Supplement 1).

Phenotypic Definitions

We define five interrelated response phenotype definitions of response to citalopram. The first two are responders and nonresponders: responders are subjects who had at least 42 days of treatment and whose QIDS-SR on their final clinical visit shows $\geq 50\%$ reduction in score; the remaining subjects, who had at least 42 days of treatment, were then considered nonresponders. The $\geq 50\%$ reduction in symptom severity on the HRSD₁₇ is the conventional definition of response in clinical trials. We used the QIDS-SR score to estimate severity because all subjects had this rating, and it correlates highly with the HRSD₁₇ scores (Rush et al 2003). We required this 42-day (or 6-week) threshold to ensure an adequate exposure to citalopram and to enhance the power to find associations between genotype and response by reducing potential heterogeneity. Using this threshold, we found no statistical difference in the average total dosage of citalopram between those who were on the trial for at least 42 days (average total dosage = 29.88 mg) and those who were not (average total dosage = 30.43 mg). The 254 subjects with < 42 days of treatment were excluded from analysis.

The third phenotype definition is remission. Remission was defined as a QIDS-SR score ≤ 5 , which closely corresponds to the conventional definition of an HRSD score of ≤ 7 (Rush et al 2003).

The final two phenotypes are based on our attempt to reduce heterogeneity further by attempting to separate placebo response from true drug response in antidepressant trials (Ross et al 2002). Some response to antidepressant medication is a placebo response, which we posit may have either no genetic determinant or a different genetic substrate than “true” drug response. Thus, it is of interest to limit our definition of response to true pharmacologic response rather than placebo response. For these phenotypes, a “specific” pattern of response was defined by persistence, or the maintenance of response for the remainder of the study once it was attained. Previous studies considered “specific” patterns to be further characterized by delayed response, that is, after the first 2 weeks (Quitkin et al 1987; Stewart et al 1998). We were unable to employ this criterion because the STAR*D study design did not include ratings before week 2. We defined persistent responders as those subjects who had a sustained response at all consecutive visits following the first visit with a response, as measured by $\geq 50\%$ reduction in QIDS-SR scores. Those whose response occurred only at the last visit were removed from the analysis. In contrast, “nonspecific” responders were those subjects who did not maintain their response following the first visit with a response. Note that “specific” and “nonspecific” responders are a subset of responders (as defined by the response phenotype described

earlier). Moreover, because visits were at least 2 weeks apart, we assumed that intervening weeks were characterized by the response defined by the previous visit. We compared “specific” responders to nonresponders, allowing us to test the hypothesis that the “specific” response to citalopram represented a more genetically homogenous group of persons taking citalopram. We also compared “specific” responders to “nonspecific” responders to test whether there are genetic difference between “true” drug responders and “placebo” responders, as suggested in our previous work (Peters et al 2004).

Statistical Analysis

The frequency distributions of demographic and clinical variables were examined in the combined sample and by the five phenotypes. To control for any potential population stratification, all analyses were stratified by race categories: White and African American. Other racial categories were not considered because of the small numbers of those samples. We tested for Hardy–Weinberg equilibrium within each of the White and African American groups, and all subjects from a stratum were used in the analysis because all subjects had depression, and the evaluated polymorphisms were not suspected to influence risk of depression. Linkage disequilibrium was estimated using r^2 .

We used unconditional logistic regression analysis to examine associations of the 11 genetic polymorphisms and each of the four phenotypic comparisons. These comparisons are 1) Responder versus Nonresponder, 2) Remitter versus Nonresponders, 3) “Specific” Responders versus Nonresponders, and 4) “Specific” Responders versus “Nonspecific” Responders. Each polymorphism was modeled individually as gene–dosage effects in the regression models. This coding scheme was chosen because of its robustness to departure from the true additive genetic model (Freidlin et al 2002). Regression analyses were either unadjusted or adjusted for potential confounding effects, including gender, age, education (years of school), months in current major depressive episode (MDE), and years since first MDE. We estimated odds ratios (OR) and 95% confidence intervals (CIs) for the carriers of the minor allele versus noncarriers of the minor allele. Because of the large number of statistical tests, significance threshold was set at .01, and permutation tests were performed on any test that resulted in an asymptotic p value of .01 or less.

Association between haplotypes and the four phenotypic comparisons were calculated using a score test implemented in the computer program HAPLO.SCORE (Schaid et al 2002). This test uses the expectation–maximization algorithm to estimate the posterior probability of each person’s haplotype. These posterior probabilities are then used to calculate a person’s expected haplotype score in the logistic regression analyses. All haplotypes with frequencies $> .01$ were simultaneously tested in the analysis. Global p values and individual haplotype p values were

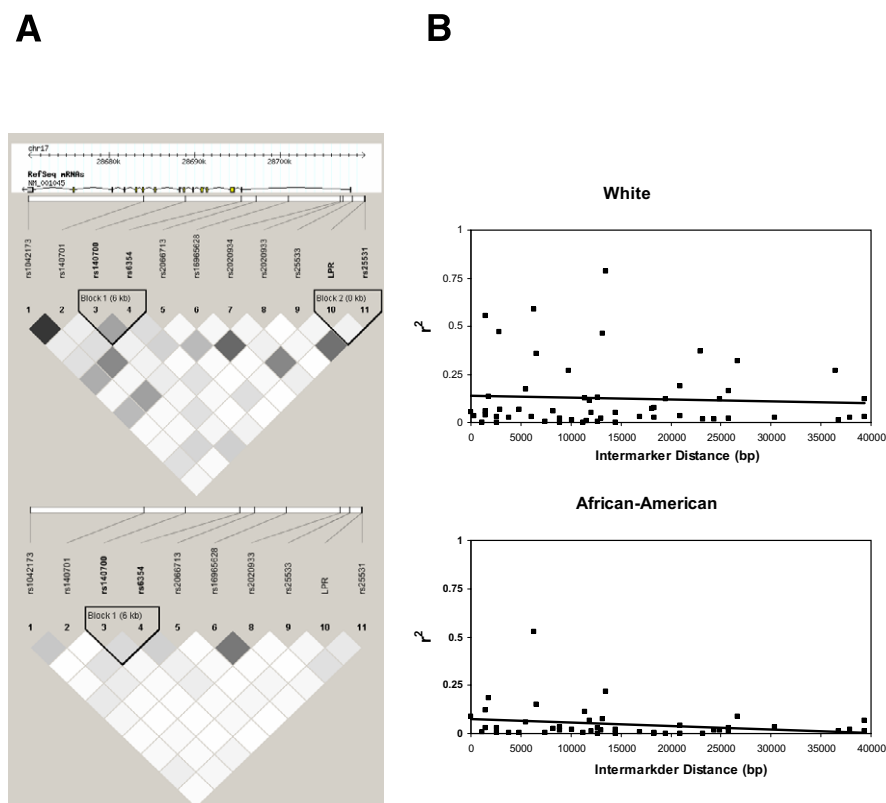


Figure 2. Linkage disequilibrium at the SLC6A4 locus. r^2 was estimated in White (top panels) and African American (bottom panels) samples. **(A)** r^2 is displayed using Haploview (Barrett et al 2005, version 3.2), with darker boxes representing larger values of r^2 . The orientation and gene structure of SLC6A4 (GenBank accession NM_001049) matches the genomic orientation. Haplotype “blocks” are represented using the criteria of Gabriel et al (2002). **(B)** Plot of r^2 versus intermarker distance for White and African American samples.

obtained. Statistical tests were performed in SAS version 8.2 or Spplus version 6.2.1 statistical packages.

Results

Of the 1,953 subjects who consented to give DNA, data for 1,914 subjects are described in this report. The 39 samples that were unavailable for genotyping did not differ from the remaining samples in demographic or clinical variables. Using our responder versus nonresponder phenotype (R/NR), 1,660 of the 1,914 samples could be categorized, with 991 responders and 669 nonresponders, for a response rate of 59.7%. We excluded 254 because they did not reach the 6-week treatment threshold. The clinical and demographic characteristics of the 1,914 samples is shown in Table 2. Significant demographic and clinical differences between responders and nonresponders within the current study included years of schooling ($p < .001$), months in current MDE ($p < .001$), length of current MDE >24 months ($p = .001$), baseline QIDS-SR ($p = .008$), and years since first MDE ($p = .02$). The presence of recurrent depression or a family history of depression did not differ between responders and nonresponders. Of the responders, 826 (83.3% of responders) were considered to be remitters. For the analysis of specificity of the 991 responders, 679 (68.5%) were categorized as “specific” responders, and 187 (18.9%) as “nonspecific” responders, with the remaining 125 (12.6%) responders unclassifiable for the specificity response phenotype. The ratio of “specific” to “nonspecific” responders is similar to that seen in our previous work (Peters et al 2004).

We used the phenotypes described to test two general hypotheses. First, we sought to determine whether SLC6A4 variants are associated with general indicators of response (responders vs. nonresponders, remitters vs. nonresponders) based on changes in the QIDS-SR. Second, we sought to examine

whether SLC6A4 variants influences response in a subgroup of responders likely to exhibit a “true” drug response (“specific” responders versus nonresponders, “specific” responders versus “nonspecific” responders). All analyses were stratified by ethnicity, which for this analysis are White and African American. None of the variants showed significant deviation from Hardy–Weinberg equilibrium within any of the ethnic groups (results not shown). Linkage disequilibrium (LD) was present among the polymorphisms. Figure 2A shows the extent of LD for each ethnic group, using the r^2 metric, and Figure 2B shows the correlation between intermarker distance in base pairs and r^2 . Given our SNP ascertainment strategy, we did not expect prominent association between markers.

Table 4 shows the association results for the 11 polymorphisms for the response versus nonresponse comparison for the two ethnic groups. The minor allele frequencies among the nonresponders within the Whites ranged from 5% (rs2020933) to 46% (rs2020934). None of the variants were found to be associated with response at the significance level of .01. We found similar results for our other three phenotypic comparisons: “specific” responders versus nonresponders; “specific” responders versus “nonspecific” responders; and remitters versus nonresponders (data not shown). We also found no evidence of confounding adjusting for gender, age, education, months in current MDE, and years since first MDE in the regression analyses (results not shown).

We then constructed haplotypes and tested for association with the four phenotypic comparisons to account for possible interactions among the 11 variants within the serotonin transporter. Among the Whites, our global test of association with the responder versus nonresponder phenotypic comparison was found to be nonsignificant ($p = .55$). Likewise, the global test of association in the African

Table 3. Oligonucleotide Sequences for PCR Primers and FP-TDI Probes.

Polymorphism	Major/Minor Allele	Product Size	Forward Primer	Reverse Primer	Probe Sequence
rs25531	A/G	419/376 bp	atgccagcacctaaccctaagt	ggaccgcaaggtggcgcca	N/A
5-HTTLPR	16RPT/14RPT	419/376 bp	atgccagcacctaaccctaagt	ggaccgcaaggtggcgcca	N/A
rs25533	A/G	249 bp	aagtaaagagcaggaaagtcag	agaattttcgctcactttg	N/A
rs2020933	T/A	145 bp	tcctttccatttgggactct	gctagcaggctcataaataatcca	cagttttgtccagaaaagtgaacc
rs2020934	T/C	125 bp	tctgtgtgaagccactgagg	ttgctcaatttcacaaacc	gggtgagcagctgacctcaa
rs16965628	G/C	121 bp	gtctgtggcctcagtttc	tctagcacagggaagcatca	gctagggtatgaagttagaaaggca
rs2066713	C/T	104 bp	ctctctaccaggccaga	actgctcactgctgctgta	gatggaccgcatttccttc
rs6354	T/G	256 bp	ccagagctgagctgacttc	cactgctgctcaccattgt	gctaagccctgttattctgcaa
rs140700	C/T	110 bp	gaggtgggtgaatggatgc	atccgatccctgtgtgactc	tgaagacctgagaaaggagg
rs140701	C/T	139 bp	agtgtgaggacgcactgtgt	agaggaggaggtggtgaca	aaaactcagccacaacacagtta
rs1042173	A/C	130 bp	aaactcgttagagagaacagg	cttcttctctgatgccaca	aggttctagtagattccagcaataaaatt

dbSNP accession numbers for the SNPs and PCR fragment sizes are provided. Genotyping methods are described in Methods and Materials.

American sample was nonsignificant ($p = .28$). Similar results were observed for the other phenotypic comparisons (results not shown).

Discussion

We failed to detect association between any of the SNPs within the SLC6A4 and antidepressant response phenotype. Our failure to detect association in a large sample is strong evidence against a role for variation in this gene as a factor in response to SSRIs. In our primary phenotype, categorical response versus nonresponse, our results differed with regard to a number of other studies in which associations were found between response and SLC6A4. There may be a number of reasons for this including differences in outcome measures, drugs, ethnicities, and analytical approaches.

For our outcome measures, we used the QIDS-SR. This instrument has been shown to have high correlation (Pearson's correlation = .81) with the standard HRSD (Rush et al 2003), which has been used in many of the previous studies. Despite this high correlation, however, variability in these measurements may explain the difference in results among the studies, especially if the effect size of any SLC6A4 genotypic effect is modest (Ioannidis 2005).

All of the studies in Table 1 and this study tested for association between SLC6A4 and an SSRI. It would be ideal to compare all of the previous studies to our own. Unfortunately, each of the previous studies differs strikingly from the others,

with wide variation in treatment trial design, drug choice, marker choice, outcome measures, and statistical methods in each of the studies reviewed in Table 1. For example, the systematic and vigorous dosing strategy employed by STAR*D differs from the forced titrations or flexible dosing approaches used in the other studies, raising the possibility that those studies may be more sensitive to effects due to genotype \times dose interactions. Although it is presumed that the SSRIs are equivalent in terms of mechanism, this has not been proven definitively and thus remains a potential explanation for the difference between studies using different SSRIs. Another difference may involve the inclusion criteria applied to the various studies. The inclusion criteria for STAR*D was HRSD₁₇ ≥ 14 , which was somewhat lower than several of the previous studies that used the HRSD₁₇ for assessment, suggesting that the STAR*D sample may be less severely affected. The only previous study that is most directly comparable to ours based on drug is by Arias et al (2003), which investigated only the 5-HTTLPR variant. This study used citalopram and defined response as $\geq 50\%$ reduction in HRSD₂₁ at 4 weeks and remission as HRSD₂₁ ≤ 7 at 12 weeks. Because this definition is different from ours, we carried out analyses to emulate this approach ($\geq 50\%$ reduction in QIDS-SR at 28 days or QIDS-SR ≤ 5 at 84 days) and still found no association between SLC6A4 and treatment response (data not shown). In this context, if the association between this gene and antidepressant response is not consistently found in samples with adequate

Table 4. Association Results for SLC6A4 and Citalopram Response.

Marker	White (n = 799 vs. 509)				African American (n = 130 vs. 121)			
	MAF		Additive model p value	Dominant OR (95% CI)	MAF		Additive model p value	Dominant OR (95% CI)
	NonResp	Resp			NonResp	Resp		
rs25531	.07	.08	.22	1.25 (.90,1.73)	.25	.27	.54	1.19 (.72,1.96)
5-HTTLPR	.44	.42	.27	.89 (.70,1.13)	.20	.22	.60	1.19 (.71,2.01)
rs25533	.06	.06	.48	1.15 (.80,1.64)	.07	.13	.05	1.81 (.92,3.56)
rs2020933	.05	.05	.83	1.06 (.71,1.58)	.32	.36	.38	1.27 (.74,2.19)
rs2020934	.46	.49	.10	1.31 (.99,1.72)	.23	.18	.28	.68 (.37,1.25)
rs16965628	.06	.07	.66	1.10 (.77,1.57)	.33	.35	.63	.96 (.56,1.65)
rs2066713	.38	.42	.09	1.29 (1.00,1.67)	.26	.29	.42	1.41 (.82,2.41)
rs6354	.20	.20	.95	1.03 (.81,1.32)	.34	.34	.90	1.07 (.62,1.82)
rs140700	.07	.10	.03	1.48 (1.04,2.10)	.04	.09	.02	2.57 (1.07,6.19)
rs140701	.44	.42	.42	1.02 (.80,1.31)	.27	.28	.95	.97 (.56,1.68)
rs1042173	.45	.43	.28	.90 (.70,1.16)	.23	.22	.82	.94 (.55,1.61)

Response versus nonresponse was assessed in the sample, and analyzed by stratifying by ethnicity. Minor allele frequencies (MAF) are shown for each variant by response category, with an associated p-value for an additive model and odds ratio (OR) for a dominant genetic model. The minor allele corresponds to that indicated in Table 3, except for rs2020934, for which the minor allele in African Americans is the major allele from Table 3.

power, the previous findings are likely spurious because of small sample sizes and low prior probability of the genetic variants affecting response (Wacholder et al 2004). Finally, using four markers and differing phenotypes, McMahon et al (2006) reported no association between SLC6A4 and antidepressant response in the STAR*D data set.

An important issue in case–control association studies of antidepressant response (or association studies in general) is population stratification, which in theory may lead to spurious associations. To correct for potential ethnic stratification, we analyzed each population subgroup separately to test for association. Although methods have been developed for quantitating and correcting for stratification (Bacanu et al 2000; Pritchard and Rosenberg 1999), previous studies have shown that self-reported ethnicity closely corresponds with clustering of marker allele frequencies (Tang et al 2005). We found dramatic differences in allele frequencies among the ethnic groups for many of the markers used here, with the average allele frequency difference between Whites and African Americans of .17 (Table 4). Because of these differences across the ethnic groups found in our study, ethnicity may explain the differences in results across the previous studies. In a number of the previous studies, ethnicity is not clearly delineated, however (Table 1).

A major strength of our study is that we attempted to reduce heterogeneity of the clinical phenotype. Accurate assessment of clinical phenotype is essential in pharmacogenetic studies. This is particularly true with antidepressant therapy, because placebo response rates can be as high as 60% for patients with MDD (Walsh et al 2002). Previous studies with serotonin pathway gene variants and SSRI antidepressant response have failed to address these concerns. Given the high placebo response rate for many antidepressants, it may prove necessary to control for nonspecific responses in pharmacogenetic studies of antidepressant response (Licinio and Wong 2005). In this regard, we examined two phenotypes that might better represent those subjects who are responding to the biochemical effects of the medication, “specific” responders and remitters. Our goal for these refined phenotypes was to decrease phenotypic heterogeneity among the responders, possibly introduced by any placebo response. We failed to observe any association to the serotonin transporter using these phenotypes, however.

Another strength of our study is that we more fully interrogated the SLC6A4 gene than previous studies. Our tag SNP approach, using HapMap information, and our own previous dense genotyping of the gene (Peters et al 2004) has sufficiently covered the gene. Furthermore, our in-depth sequencing survey of this gene (Kraft et al 2005) has provided few useful markers beyond publicly available common SNPs. In addition to single-locus analysis, we used haplotypic analyses to allow us to determine whether combinations of alleles were associated with treatment response. The use of both single-locus and haplotypic association has allowed us to take a more comprehensive look at the role of DNA variation in the serotonin transporter locus in antidepressant treatment response. Thus far, the vast majority of the previous studies on serotonin transporter variants in antidepressant response have focused on single polymorphism associations.

A possible limitation of our study may be our choice of clinical phenotype, that is, antidepressant response, as our primary phenotype. We restricted our analyses to subjects receiving 6 weeks of treatment and required a 50% reduction in symptomatology. In doing this, we excluded some 250 subjects, although we believe requiring sufficient medication exposure to

reduce placebo response should increase the probability of detecting an association to drug response. Note that the rates of response and remission in our analysis were higher when compared with that of a recent analysis of 2,876 STAR*D subjects, in which subjects with less than 6 weeks of treatment were included (Trivedi et al 2006), suggesting a strong correlation between response and length of treatment. Nevertheless, it might be argued that an intent-to-treat approach may be useful. We have done this for the White and for the African American subjects who had at least one treatment assessment and found that a single marker, rs140700, resulted in a p value of .009 in the White subsample. In a longitudinal analysis using generalized estimating equations and the raw QIDS-SR scores measured at each treatment assessment, we found results similar to that in Table 4, that is, none of the SNPs had p values $< .01$ for either race. Thus, the fact that these alternative post hoc approaches resulted in similar results to those reported further support our findings of no association between this gene and antidepressant response (see Supplemental Material for details). An additional potential limitation arises because this open study, without placebo control or measure of adherence (i.e., serum level monitoring), might have shown only placebo response and therefore may not provide a valid phenotype for gene finding, although this seems less plausible given the similarity in response and remission rate to controlled studies where compliance is measured.

Another limitation of this report involves its generalizability to the STAR*D sample as a whole. There are significant differences between the sample providing DNA and the 2,087 STAR*D subjects who did not provide DNA for the genetics study. For example, the subject who gave DNA was significantly more likely to 1) come from a primary care clinic (43.5% vs. 34.7%, $p < .0001$); 2) be a White subject and not be an African American subject (78.4% vs. 73.0%, 15.8% vs. 19.2%, $p = .0003$); 3) be Hispanic 14.6% vs. 11.2%, $p = .006$; 4) be married (42.6% vs. 39.9%, $p = .003$); 5) have recurrent depression (76.0% vs. 71.5%, $p = .002$); 6) be older (42.6 vs. 38.5, $p < .0001$); 7) be more educated 13.6 vs. 13.3 years, $p = .007$); 8) have more MDEs (6.4 vs. 5.4, $p = .003$); and 9) have a longer illness (16.6 vs. 13.6 years, $p < .0001$). It is difficult to formulate how these differences, typically of small magnitude, would affect the generalizability of our results. In any case, it must be stated that the results may not be generalizable to subjects who are not inclined to provide DNA samples, who in the STAR*D sample appear to have observable differences with the subjects who provided DNA samples. Furthermore, the clinical importance of some of these statistically significant differences, which are presumably driven by the large sample, is unknown. For example, the average number of years of education was 13.6 in subjects who gave DNA, which was significantly different from the average of 13.3 years in the subjects who did not provide blood samples. The effect size for this observation of a .3 year difference in schooling is minute, so that even if it is unlikely to arise by chance, it would be difficult to imagine a scenario or mechanism through which this would affect attempts to find a genetic association. The difference between primary care and specialty care subjects in STAR*D has been shown to be negligible (Gaynes et al 2005). We also compared baseline QIDS-SR in our sample between specialty and primary care clinics and found no significant difference for all subjects ($p = .70$) or for groups stratified by ethnicity (Whites $p = .30$, African Americans $p = .23$), comparable to the findings in an analysis of 2,876 STAR*D subjects (Trivedi et al 2006). The response rates between primary care and specialty clinics were

similar for our primary response phenotype (57.8% vs. 60.5%, $p = .32$) and for our remission phenotype (53.3% vs. 56.3%, $p = .38$). Although the age of onset of MDD in STAR*D subjects has been shown to be related to a number of clinical variables (Zisook et al 2004), we found that it was not correlated with treatment response. Finally, we cannot exclude the fact that our results may be false negatives. Given our sample size in the White sample, we have 80% power to detect a minimal odds ratio of 1.39, assuming 5% significance level, dominant model, and common allele frequency greater than .2. Our largest observed odds ratio in the White sample, given a common allele frequency of at least .2, was 1.31 (range 1.02–1.31). To detect effects of this magnitude or less, we would need to increase our sample size by at least 50%. To date, we have the largest sample collection of patients.

In summary, we have looked in depth at the molecular target of SSRIs, the serotonin transporter, in a very large clinical sample and tested the role of DNA variants within this gene in citalopram response and failed to find any associations using both single loci and haplotypic analyses. At this point, we cannot definitively answer the ultimate question: at what point can we say that the contribution of genotype in SLC6A4 to antidepressant response is negligible? We have not accounted for the possibility of gene–gene or gene–environment interaction. By itself, however, this gene does not affect response to drug in our representative population with citalopram using our outcome assessment. Because it appears serotonin plays an important role in depression, this study may simply suggest that variation within other genes in the serotonin pathway such as enzymes that affect serotonin levels (TPH1, TPH2, MAOA) or the serotonin receptors (5HT2A, 5HT2C) may contribute to SSRI response. Similarly, it is possible that serotonin itself may be part of a cascade of events and any genetically determined variability in antidepressant response may lie elsewhere in the cascade or in another neural system all together.

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