

Genetic Markers of Suicidal Ideation Emerging During Citalopram Treatment of Major Depression

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Objective: Suicidal ideation is an uncommon symptom than can emerge during antidepressant treatment. The biological basis of treatment-emergent suicidal ideation is unknown. Genetic markers may shed light on the causes of treatment-emergent suicidal ideation and help identify individuals at high risk who may benefit from closer monitoring, alternative treatments, or specialty care.

Method: A clinically representative cohort of outpatients with major depressive disorder who enrolled in the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial were treated with citalopram under a standard protocol for up to 14 weeks. DNA samples from 1,915 participants were genotyped for 768 single-nucleotide polymorphisms in 68 candidate genes. Allele and genotype frequencies were compared between the 120 participants who developed treat-

ment-emergent suicidal ideation and those who did not.

Results: Two markers were significantly associated with treatment-emergent suicidal ideation in this sample (marker rs4825476, $p=0.0000784$, odds ratio=1.94; permutation $p=0.01$; marker rs2518224, $p=0.0000243$, odds ratio=8.23; permutation $p=0.003$). These markers reside within the genes GRIA3 and GRIK2, respectively, both of which encode ionotropic glutamate receptors.

Conclusions: Markers within GRIK2 and GRIA3 were associated with treatment-emergent suicidal ideation during citalopram therapy. If replicated, these findings may shed light on the biological basis of this potentially dangerous adverse event and help identify patients at increased risk.

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Since the introduction of selective serotonin reuptake inhibitor (SSRI) antidepressants in the 1980s, there has been controversy over whether they can trigger suicidal thoughts or behavior (1, 2). In 1991, the Psychopharmacologic Drugs Advisory Committee of the U.S. Food and Drug Administration (FDA) found no clear evidence of increased suicide risk associated with fluoxetine (3). In 2003, however, the U.K. Medicines and Healthcare Products Regulatory Agency concluded that paroxetine, citalopram, and other SSRIs were contraindicated in youths because of an increased risk of treatment-emergent suicidal ideation (4). Soon after that, a scrupulous analysis by the FDA and an independent group at Columbia University ultimately resulted in an FDA-mandated black box warning highlighting the potential for suicidal ideation in youths treated with SSRIs. The warning was later extended to all antidepressants (5). Since the black box warning was issued, the use of SSRIs in children has decreased (6) and concern about treatment-emergent suicidal ideation has extended to adults.

Treatment-emergent suicidal ideation and behavior are infrequent; the average incidence is 4% for antide-

pressants and 2% for placebo (7). The highest risk seems to be within a few weeks after initiation of treatment or dose adjustment (8, 9). It is not clear whether treatment-emergent suicidal ideation heralds actual suicidal behavior in children or adults. Of the 4,400 pediatric subjects who participated in the clinical trials of SSRIs analyzed by the FDA, no completed suicides were reported (7).

The Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial, the largest prospective treatment trial for major depressive disorder, provides a unique opportunity to ascertain treatment-emergent suicidal ideation prospectively in a large cohort of patients treated with the SSRI citalopram and to test whether specific genetic markers can identify patients who have an increased risk of developing this uncommon adverse event. Here we report an initial screen with 768 markers in 68 candidate genes. The results suggest that genetic markers may be able to identify some people at increased risk of treatment-emergent suicidal ideation. If replicated, these findings could have implications for the clinical management of major depression with SSRIs.

This article is featured in this month's AJP Audio and is discussed in an editorial by Dr. Gershon on p. 1460.

Method

Sample

Ascertainment and evaluation for the STAR*D study have been detailed elsewhere (10, 11). Briefly, investigators implemented a standard study protocol at 18 primary care and 23 psychiatric care settings across the United States. Participants provided written informed consent for both the treatment study and for DNA collection, although participation in DNA collection was optional. The genetic protocol was initiated approximately 12 months after treatment study initiation. Outpatients 18–75 years of age who had an initial score ≥ 14 on the 17-item Hamilton Depression Rating Scale (HAM-D; 12, 13) and who met DSM-IV (14) criteria for nonpsychotic major depressive disorder were eligible. Patients with bipolar or psychotic disorders were excluded, as were those with a primary diagnosis of obsessive-compulsive disorder or an eating disorder, a general medical condition in which study medications were contraindicated, substance dependence requiring inpatient detoxification, or clear nonresponse or intolerance to any protocol antidepressant during the current episode. Patients who were pregnant or breastfeeding were also excluded.

At the first treatment step (Level 1), all participants received citalopram, typically starting at 20 mg/day, with dose increases following recommended procedures (up to 40 mg/day by week 4 and 60 mg/day by week 6) (15). The protocol required an adequate dose of citalopram for a sufficient time to ensure that those whose symptoms did not improve were most likely unresponsive to the medication (15). No concomitant psychotropic medications were allowed aside from benzodiazepines, hypnotics, or trazodone up to 200 mg/day for sleep, if needed. The sample characteristics have been presented elsewhere (15, 16).

DNA samples were collected from 1,953 participants. A sample of 20 ml of whole blood was collected and shipped to the Rutgers Cell and DNA Repository, where lymphocytes were extracted and cryopreserved by standard methods. DNA was extracted by standard methods (16). Samples were arrayed robotically, then gender-verified with a set of three X-linked and two Y-linked markers. A CONSORT (Consolidated Standards of Reporting Trials) diagram of the study sample is shown in Figure 1.

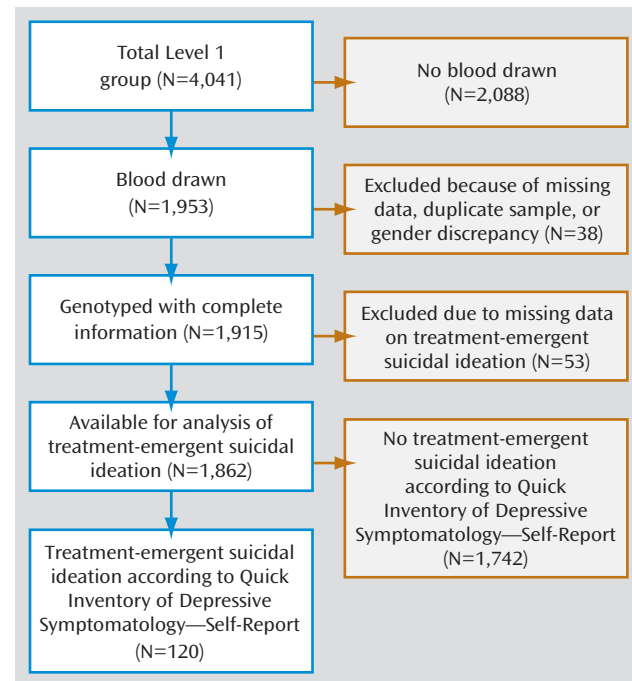
Participants who consented to DNA collection were similar to those in the full sample but differed in some variables (see reference 16 for details). These differences cannot affect the genetic association results, which derive from comparisons among the genotyped subjects, but they do limit generalizability. Those who provided DNA did not differ in the frequency of treatment-emergent suicidal ideation in this sample (Table 1).

Phenotype Definition

Consistent with previous definitions of treatment-emergent suicidal ideation, we used the “thoughts of death or suicide” question (item 12) from the 16-item Quick Inventory of Depressive Symptomatology—Self-Report (QIDS-SR), a reliable and well-validated measure of symptom severity that has been shown to correlate well with the HAM-D (17–21). The QIDS-SR was chosen over the clinician-rated version (the QIDS-C) because suicidal ideation is a subjective phenomenon, and we wished to avoid any clinician bias. The QIDS-SR has been shown to successfully substitute for the QIDS-C and the HAM-D (21). Secondary testing with the QIDS-C was carried out for markers significantly associated with treatment-emergent suicidal ideation by the QIDS-SR, solely to test the robustness of the findings. The primary outcome phenotype was decided prior to any data analysis.

The QIDS-SR was administered at baseline and at each of the protocol-recommended clinic visits around weeks 2, 4, 6, 9, and 12. Possible responses to item 12 include: “I do not think of suicide or death” (coded 0), “I feel that life is empty or wonder if it is worth living” (coded 1), “I think of suicide or death several times

FIGURE 1. CONSORT Chart of Genotyping and Analysis of STAR*D Sample



a week for several minutes” (coded 2), and “I think of suicide or death several times a day in some detail, or I have made specific plans for suicide, or have actually tried to take my life” (coded 3). Participants who scored 0 on this item before citalopram treatment and 1, 2, or 3 at least once during treatment were defined as having treatment-emergent suicidal ideation (N=120 cases).

The control group (N=1,742) consisted of all participants who scored 0 on item 12 of the QIDS-SR during up to 12 weeks of citalopram treatment. This included participants who denied any suicidal ideation at the initial and subsequent visits (N=765) and participants who acknowledged suicidal ideation at the initial visit before the start of treatment (N=977). Participants for whom suicidal ideation data were missing at the initial visit or at all subsequent visits were excluded (N=53). We chose this set of control subjects in order to avoid detecting markers that might be associated with general suicidal thoughts unrelated to treatment.

Population Structure

We evaluated the possibility of population structure leading to inflated association results in three ways. First, we used self-reported race as a forced covariate in the logistic regression analysis. We have previously shown that self-reported race corresponds well in this sample to population assignment on the basis of multilocus allele frequencies (15). Second, we stratified the sample by self-reported race, then investigated the possibility of cryptic structure within the largest subset, those self-described as white (N=1,473). Within this subset, we used the Kolmogorov-Smirnov test to assess whether the p values of the association test were consistent with a uniform distribution under the assumption of one population, as recommended by Pritchard et al. (22). Finally, we used STRUCTURE and STRAT (23) to evaluate the worst-case scenario of two cryptic populations within the white subset by assessing the multilocus chi-square value in a set of 344 unlinked single-nucleotide polymorphisms selected from the total data set without regard to the association results. We ran STRUCTURE for 20,000 burn-in steps followed by 20,000 replications.

TABLE 1. Selected Demographic and Clinical Characteristics of the STAR*D Sample^a

Characteristic	Total (N=4,041)		Blood Drawn				Analysis ^b	
			Yes (N=1,954)		No (N=2,087)			
	N	%	N	%	N	%	χ^2	df
Sex							0.75	1
Male	1,509	37.3	743	38.0	766	36.7		
Female	2,532	62.7	1,211	62.0	1,321	63.3		
Race							16.0**	2
White	3,055	75.6	1,531	78.4	1,524	73.0		
Black	709	17.6	309	15.8	400	19.2		
Other	277	6.8	114	5.8	163	7.8		
Ever attempted suicide							5.51*	1
Yes	667	16.5	295	15.1	372	17.8		
No	3,370	83.5	1,658	84.9	1,712	82.2		
Treatment-emergent suicidal ideation ^c							2.53	1
Yes	234	5.8	125	6.4	109	5.3		
No	3,789	94.2	1,821	93.6	1,968	94.7		
Remission ^d							62.91***	1
Yes	1,396	34.6	795	40.7	601	28.8		
No	2,644	65.4	1,159	59.3	1,485	71.2		
After Level 1							562.28***	2
Went to next level	1,439	35.6	885	45.3	554	26.6		
Went to follow-up phase	1,475	36.5	861	44.1	614	29.4		
Exited study	1,127	27.9	208	10.6	919	44.0		
	Mean	SD	Mean	SD	Mean	SD	t	df
Age (years)	40.5	13.3	42.6	13.4	38.5	12.9	10.05***	4,037
Maximum citalopram dose (mg/day)	40.9	16.9	45.1	15.9	37.1	16.9	15.49***	4,026
Quick Inventory of Depressive Symptomatology—Clinician Rating score	16.4	3.4	16.3	3.4	16.5	3.5	1.03	4,026
Quick Inventory of Depressive Symptomatology—Self-Report score	15.4	4.3	15.3	4.3	15.6	4.3	2.64*	4,015

^a Variables presented are those used as covariates in the current analysis. Additional variables can be found in McMahon et al. (16).

^b t=absolute value of Student's t; χ^2 =chi-square or Kruskal-Wallis test for continuous variables.

^c Treatment-emergent suicidal ideation was defined as having scored 0 on item 12 of the Quick Inventory of Depressive Symptomatology—Self-Report before citalopram treatment and 1, 2, or 3 at least once during treatment.

^d Remission was defined as a score ≤ 5 on the Quick Inventory of Depressive Symptomatology—Clinician Rating on the last recorded visit.

* $p \leq 0.05$. ** $p \leq 0.001$. *** $p \leq 0.0001$.

Candidate Genes

Sixty-eight genes were chosen for study from among a larger list of plausible candidates, which has been detailed elsewhere (16). Genes were selected to sample five broad signaling pathways of potential importance in antidepressant effects: serotonin (20 genes), glutamate (16 genes), dopamine (three genes), norepinephrine (four genes), and neurotrophins (four genes), along with selected genes in other pathways (21 genes).

Selection of Single-Nucleotide Polymorphism Markers

A total of 768 single-nucleotide polymorphisms were selected to sample common variation, as detailed elsewhere (16). Briefly, genotype data spanning the coding region and up to 2 kilobases of flanking sequence were downloaded from HapMap database (www.hapmap.org, accessed Nov. 2004). The program "LDSelect" (24) was used to choose an optimal set of available single-nucleotide polymorphisms to genotype, at an r^2 threshold of >0.8 , excluding single-nucleotide polymorphisms with a minor allele frequency $<7.5\%$. (The complete list of single-nucleotide polymorphisms genotyped is available from the first author upon request.)

Genotyping Methods

Samples were shipped to Illumina, Inc. (San Diego), where they were genotyped on their BeadArray platform, a highly accurate assay (25). The genotyping success rate was 99.9%, and 99.73% of samples were successfully genotyped, including 35,052 blind duplicate genotypes, all of which matched exactly.

Power analysis was performed with the Genetic Power Calculator (26; <http://pngu.mgh.harvard.edu/~purcell/gpc/>). The num-

ber of cases was set at the observed 120, with a control-to-case ratio at the observed value of 15. The high-risk allele frequency was set at 0.3. The trait prevalence was set at the observed value of 0.062. Marker-disease allele linkage disequilibrium (D') was set to 0.81, equal to the median D' value observed in the actual data (16). In an allele-wise test, power was greater than 80% to detect association at the $p=0.001$ level with a variant conferring a heterozygote relative risk of 1.8. Power dropped to 60% at $p=0.0001$ but exceeded 94% at $p=0.01$. In a genotypic association test, with a heterozygote relative risk of 1 and a homozygous relative risk of 4, power was 68% at $p=0.001$.

Statistical Methods

To improve the sensitivity of our initial analyses, we performed both allelic and genotypic association tests on all markers. Allelic tests are most powerful for alleles that confer risk in a codominant or dominant fashion, while genotypic tests are more powerful when a recessive model applies (27). Allelic comparisons were performed with Cocaphase in the UNPHASED package (28), which estimates a likelihood-based test of association under the null hypothesis of all odds ratios being equal to one. Genotypic comparisons were carried out using a Pearson chi-square test on a 2 \times 3 contingency table. To properly account for X-linked markers, these were analyzed separately. The Hardy-Weinberg equilibrium for the chosen markers was calculated using PEDSTATS (29). Odds ratios for the genotypic test are based on a comparison between the two groups of homozygotes.

Experimentwise p values that correct for the number of single-nucleotide polymorphisms tested were estimated by permuting case-control labels 10,000 times. Allelic p values were permutation-tested in an allelic test; genotypic p values were permuta-

TABLE 2. Demographic and Clinical Features of Case and Control Subjects (N=1,862)

Characteristic	Cases (N=120)		Controls (N=1,742)		Analysis
	N	%	N	%	χ^2
Female	71	59.2	1,072	61.5	0.2664
Race					1.0563
White	91	75.8	1,382	79.3	
Black	20	16.7	262	15.0	
Other	9	7.5	98	5.6	
Non-Hispanic	102	85.0	1,501	86.2	0.1273
Employment status					1.9630
Employed	64	53.3	981	56.3	
Retired	6	5.0	129	7.4	
Unemployed	50	41.7	632	36.3	
Marital status					0.6588
Married	52	43.3	745	42.8	
Divorced	33	27.5	435	25.0	
Never Married	31	25.8	492	28.2	
Widowed	4	3.3	70	4.0	
Remission ^a	30	25.0	748	42.9	15.529**
Psychomotor agitation ^b	16	13.3	234	13.4	0.0010
Initial Insomnia ^c	16	13.3	212	12.2	0.1414
Drug Abuse ^d	11	9.2	124	7.1	0.1404
Alcohol Abuse ^d	15	12.5	199	11.4	0.0254
History of suicide attempts	19	15.8	261	14.9	0.0713
Medication tolerability ^e					3.1386
Intolerant	12	10.1	198	11.4	
Probably intolerant	2	1.7	34	1.9	
Probably tolerant	26	21.9	273	15.7	
Tolerant	79	66.4	1,232	70.9	
Exit from study Level 1					14.834*
Follow-up	35	29.2	805	46.2	
Next level	75	62.5	782	44.9	
Study exit	10	8.3	155	8.9	
	Mean	SD	Mean	SD	t
Maximum citalopram dose (mg/day)	51.8	13.7	46.2	15.3	3.86**
Age at onset of first major depressive episode (years)	24.3	15.8	25.9	15.0	-1.12
Months in current episode	24.1	49.4	24.5	53.4	-0.09
Quality of Life Enjoyment and Satisfaction Questionnaire, general activities index	40.4	15.7	42.2	15.7	-1.21
Baseline score on Quick Inventory of Depressive Symptomatology—Clinician Rating, rated by clinical research coordinator	16.1	3.1	16.3	3.4	-0.83
Baseline score on Hamilton Depression Rating Scale (17-item), rated by research outcomes assessor	20.8	6.5	19.8	6.5	1.58
Age at enrollment (years)	42.3	13.3	42.5	13.4	-0.22

^a Remission was defined as a score ≤ 5 on the Quick Inventory of Depressive Symptomatology—Clinician Rating on the last recorded visit.

^b Item 16 on the Quick Inventory of Depressive Symptomatology—Self-Report.

^c Item 1 on the Quick Inventory of Depressive Symptomatology—Self-Report.

^d Based on self-report responses to the Psychiatric Diagnostic Screening Questionnaire, set at 90% specificity (31).

^e As defined by an algorithm detailed in McMahon et al. (16).

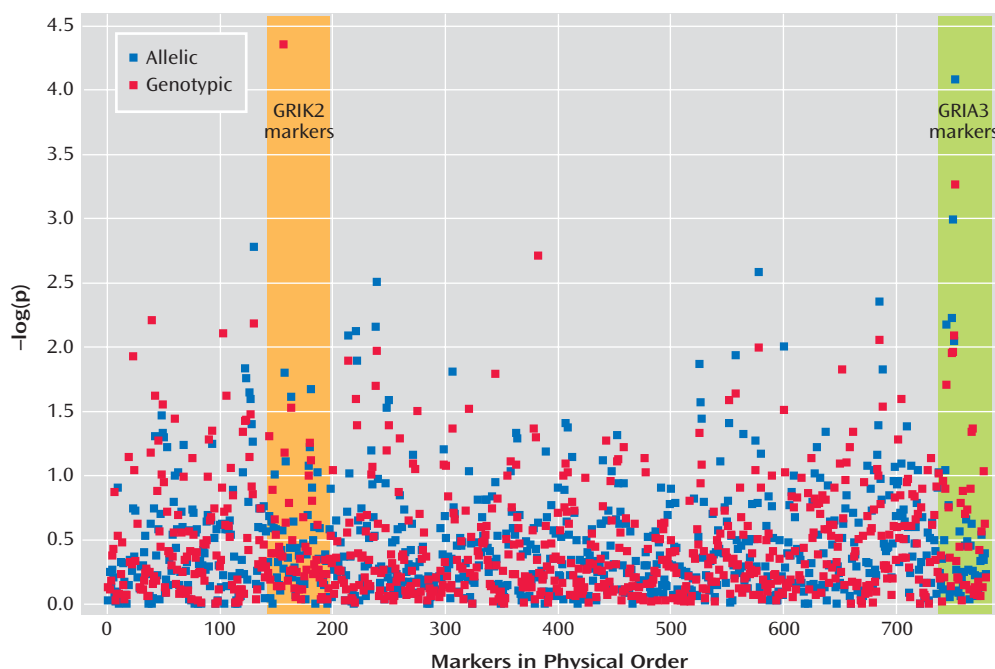
* $p \leq 0.001$. ** $p \leq 0.0001$.

tion-tested in a genotypic test. Since autosomal and X-linked markers require different analytic procedures, permutation tests were run separately for autosomal and X-linked markers. The total number of p values less than or equal to the lowest p value observed in the actual data were tallied across the autosomal and X-linked permutation results and then divided by 10,000 to yield the experimentwise p value.

Single-nucleotide polymorphisms that passed the initial tests with an experimentwise $p < 0.05$ were studied further. Tests of association included a logistic regression model calculated with SAS 9.1.3 Enterprise Guide 3.0 (SAS Institute, Cary, N.C.), with a nominal dependent variable for treatment-emergent suicidal ideation. X-linked markers were analyzed in males and females separately. Single-marker tests were carried out under codominant, dominant, and recessive models. Models were compared

with the likelihood ratio test. The best-fitting model was used for the multimarker analyses. The reference model was based on the single-nucleotide polymorphisms with the largest identified odds ratios in the single-marker models. The remaining covariates were added in a stepwise fashion in descending order of effect size, as recommended by Cordell and Clayton (30). The -2 log-likelihood was used to assess the improvement of fit as each variable was removed from the model. The Hosmer-Lemeshow test was used to test for final model fit. For the X-linked marker in GRIA3, these analyses were carried out separately in males and females because there is no method in SAS Enterprise Guide that corrects for X-linked markers in a regression analysis.

Case and control subjects were characterized clinically using univariate tests. Chi-square tests were used to assess differences

FIGURE 2. Genotypic and Allelic Comparisons for Each of the 768 Markers^a

^a Ordered by physical position along the autosomes (markers 1–719) and X-chromosome (markers 720–768) on Build 34 of the Draft Human Genome Sequence. The boxed areas encompass all the markers genotyped in GRIK2 and GRIA3.

between groups for nominal and ordinal variables, and *t* tests for continuous variables.

Results

Descriptive Variables

The demographic and clinical characteristics of the sample are presented in Table 2. There were no significant demographic differences between case and control subjects, and no differences in several clinical variables that are known predictors of suicide (32). Participants who developed suicidal ideation received a significantly higher maximum citalopram dose, were significantly less likely to go into remission (defined as a score ≤ 5 on the QIDS-C on the last recorded visit during citalopram treatment [Level 1]), and were more likely to move on to a secondary treatment phase (Level 2) after citalopram (15). Only 25% of those who had treatment-emergent suicidal ideation, compared with 42.9% of control subjects, achieved remission, although those with and without suicidal ideation did not differ significantly in initial symptom scores (Table 1). Consistent with this poorer outcome, 62.5% of those with suicidal ideation went on to Level 2 to receive a change in treatment, compared with 44.9% of control subjects. There was no difference in the rates of exit from citalopram treatment.

Consistent with previous reports (8), treatment-emergent suicidal ideation developed relatively early in treatment: 21% of those who developed suicidal ideation did so by visit 2 (median=14 days after starting treatment), 69% by visit 3 (median=21 days), and 92% by visit 5 (median=28

days). By the end of Level 1, 48% of those who developed suicidal ideation had returned to their baseline score of 0, while 37% persisted, and 15% had a fluctuating course. None of the participants with treatment-emergent suicidal ideation are known to have attempted suicide.

Association Analysis

Figure 2 presents results of the allelic and genotypic association tests. Two markers produced significant evidence of association at the experimentwise $p < 0.05$ level. Both markers were in Hardy-Weinberg equilibrium in this sample.

A marker in the first intron of GRIK2 on chromosome 6 (marker rs2518224), which encodes the kainate-sensitive ionotropic glutamate receptor GluR6, was associated with treatment-emergent suicidal ideation in the genotypic test (CC genotype, nominal $p = 2.43 \times 10^{-5}$, odds ratio=8.23; permutation $p < 0.003$). This marker was not significantly associated in the allelic test.

A marker in the third intron of GRIA3 on chromosome X (marker rs4825476), which encodes the α -amino-5-hydroxy-3-methyl-4-isoxazole propionic acid-sensitive ionotropic glutamate receptor AMPA3, was associated with treatment-emergent suicidal ideation in the allelic test (G allele, nominal $p = 7.84 \times 10^{-5}$, odds ratio=1.94; permutation $p < 0.01$).

Regression Analysis

Having established experimentwise significant associations between two markers and treatment-emergent sui-

TABLE 3. Logistic Regression Models With Stepwise Selection^a

Model and Step	Model Variables	–2 Log-Likelihood	Likelihood Ratio χ^2	df	p
Women^b					
1	rs2518224 (CC genotype) and race	519.6	6.3	3	0.0982
2	rs2518224 (CC genotype), remission, and race	512.2	13.7	4	0.0084
3	rs2518224 (CC genotype), rs4825476 (G allele), remission, and race	505.2	20.7	5	0.0009
Men^c					
1	rs2518224 (CC genotype) and race	346.5	11.3	3	0.01
2	rs2518224 (CC genotype), maximum citalopram dose, and race	334.1	23.8	4	<0.0001
3	rs2518224 (CC genotype), maximum citalopram dose, rs4825476 (G allele), and race	327.8	30.1	5	<0.0001
4	rs2518224 (CC genotype), rs4825476 (G allele), maximum citalopram dose, remission, and race	323.0	34.8	6	<0.0001

^a Race was forced into each model.

^b N=70 cases, 1,067 controls. The c index for this model is 0.64.

^c N=49 cases, 670 controls. The c index for this model is 0.73.

cidal ideation in this sample, we next investigated the impact of nongenetic variables on the observed genetic association. By means of stepwise logistic regression, we tested the impact of race and the three clinical variables that had shown significant differences in those with and without treatment-emergent suicidal ideation in our initial analysis (see Table 2).

The best-fitting model was achieved with a combination of both markers, maximum citalopram dose, and remission by QIDS-C (Table 3). Race was not a significant covariate in this model. Adjusted odds ratios were close to those in the unadjusted models. The Hosmer-Lemeshow test was nonsignificant for both genders, indicating a good model fit.

Population Structure

The association p values were uniformly distributed, with no excess of small values (Kolmogorov-Smirnov D=0.032, p=0.11). In the white subset, STRAT detected no evidence of mismatch between cases and controls ($\chi^2=303.36$, df=310, p=0.6).

Robustness to Varying Case Definition

The optimal case definition for treatment-emergent suicidal ideation is unknown. As a secondary analysis to assess the impact of varying case definition on the observed association findings, we examined treatment-emergent suicidal ideation as defined by the clinician-rated QIDS-C. The overall Pearson correlation of the QIDS-SR and QIDS-C scores on item 12 was low but highly significant ($r=0.37$, $p=0.0001$). The 144 cases of suicidal ideation defined by the QIDS-C alone showed association with markers in both GRIK2 and GRIA3 at the nominal $p<0.01$ level, although different single-nucleotide polymorphisms were involved. Participants who met the case definition for suicidal ideation on both the QIDS-SR and the QIDS-C (N=55) were significantly more likely to carry exactly the same marker alleles identified in our primary analysis than those who denied all suicidal thoughts on both instruments ($\chi^2=15.42$, df=2, $p=0.0004$).

Since our primary case definition included individuals who scored only 1 (“I feel that life is empty or wonder if it is worth living”) on the “thoughts of death or suicide” item, we compared allelic and genotypic frequencies of the GRIK2 and GRIA3 markers in those with treatment-emergent suicidal ideation who scored 1 and those who scored >1. There were no significant differences, which suggests that all patients who scored over 0 are similar with respect to allele frequencies at the GRIK2 and GRIA3 markers.

Combined Effect of Risk Alleles and Genotypes

Of the six combinations of high-risk alleles and genotypes tested, the highest odds ratio was observed in patients carrying both the high-risk allele of marker rs4825476 and the high-risk genotype of marker rs2518224 (odds ratio=14.98, 95% CI=3.7–60.674). Consistent with this, there was significant evidence of interaction between the two markers by the likelihood ratio test ($\chi^2=12.3$, df=1, $p=0.0004$). The combined impact of both markers on the risk of treatment-emergent suicidal ideation appears to be at least additive, but sample size limitations preclude any precise estimates of the mode of interaction.

Discussion

To our knowledge, this is the first study to detect a significant overall association between a genetic marker and treatment-emergent suicidal ideation. These data suggest that this uncommon but potentially dangerous adverse event may have a genetic component. Until functional alleles are demonstrated or replication is shown in an independent sample, these findings should be viewed as preliminary. However, they may shed light on the biological basis of suicidal ideation that emerges during antidepressant treatment and provide an initial step toward developing markers with clinically meaningful predictive value.

The validation of these findings through replication will be difficult, since treatment-emergent suicidal ideation is so uncommon (9). Several thousand patients may need to

be studied before a sufficiently large replication sample can be obtained. To our knowledge, the STAR*D sample is the only such sample available at this time. However, the association findings we report here show several hallmarks of causal associations (33). The overall sample is large and is representative of major depression in the outpatient setting. The effect sizes are relatively large, and the statistical significance levels stand up to correction for the number of tests carried out. There is an apparent dose-response relationship between these markers and treatment-emergent suicidal ideation: more participants who carried both risk alleles reported suicidal ideation than those who carried only one allele. The implicated genes are biologically plausible candidates with closely related functions. Furthermore, association between these genes and treatment-emergent suicidal ideation persists across alternative case definitions.

This study has several limitations. Treatment-emergent suicidal ideation was defined on the basis of items in a depression rating scale, and neither the instrument nor the STAR*D study was designed to address this adverse event. Since there was no placebo group, we cannot determine what fraction of suicidal ideation in this study sample is directly attributable to antidepressant treatment. Moreover, the case definition we used is arbitrary. Given the lack of a widely accepted definition, we used one similar to that seen in the literature as well as that adopted by the FDA, which instituted the black box warning. Alternative case definitions are possible. We tested one alternative definition based on the QIDS-C, which yielded genetic association results similar to those we obtained with the definition based on QIDS-SR, and our results would remain significant at the $p < 0.05$ level even if we doubled the permutation p values to account for a second possible phenotype definition. These limitations are characteristic of all existing large antidepressant treatment samples and highlight the need for large, placebo-controlled studies of treatment-emergent suicidal ideation in the future.

We observed that participants who developed suicidal ideation experienced a less favorable response to treatment overall, which could confound the interpretation of genetic association results. To address this limitation, we controlled for citalopram dose and remission rates in the secondary regression analysis. This analysis showed that the genetic markers we report were significant independent predictors of treatment-emergent suicidal ideation in this sample.

Another limitation of this particular study is that all participants were treated only with citalopram, so these findings may not be generalizable to other antidepressant medications. Citalopram is similar to other SSRIs and is one of the most widely prescribed antidepressants, so these findings have clinical relevance even if confined to citalopram and related compounds.

This study interrogated 68 genes. Although these genes were considered likely candidates for outcomes related to

antidepressant treatment, there may be additional genes related to treatment-emergent suicidal ideation that were not studied here. For example, one previous study (34) found that markers in CREB1 were associated with treatment-emergent suicidal ideation in men. Moreover, the markers used did not cover the selected genes completely. Additional markers could detect additional association signals. Since markers were selected on the basis of low intermarker LD, haplotype tests would be expected to have poor power and thus were not performed. Still, this is the most comprehensive genetic study of treatment-emergent suicidal ideation conducted to date. More comprehensive studies will not likely emerge until after genome-wide association studies have been performed with this sample. As is true for most large studies, additional phenotypes have been and will continue to be tested over time in this sample. Our experiment-wide p value corrections, which are based on the hypotheses tested in this study, should be considered in this light.

Cryptic population stratification is always a risk in case-control genetic association studies. To address this, we showed that 1) there is no relationship between race and treatment-emergent suicidal ideation in this sample; 2) association p values were uniformly distributed within the largest (white) subset under the assumption of one population; and 3) there is no evidence of mismatch between participants with and without suicidal ideation within the white subset. Thus, these association results are not likely to be the result of population stratification.

While treatment-emergent suicidal ideation is an understandable cause for concern and has fueled widespread reassessment of antidepressant prescribing practices, it is not clear how it is related to suicidal behavior (9). Evidence suggests that the risk of suicide attempt is higher in depressed persons before the prescription of antidepressants than afterward (35). Only two participants attempted suicide while undergoing treatment in the STAR*D study, and one of them participated in DNA collection. Although this patient consistently denied suicidal ideation, he did carry the high-risk alleles for both GRIK2 and GRIA3.

The markers we have identified do not appear to be related to a general tendency toward suicide but rather to suicidal thoughts specifically emerging during antidepressant treatment. To our knowledge, neither of these genes has been previously associated with suicidal ideation. There was no difference in allele or genotype frequencies at either marker rs2518224 or marker rs4825476 in participants who had a history of suicide attempts or reported suicidal ideation at their initial visit before the start of treatment (data not shown).

Both markers implicated in this study lie within genes that encode ionotropic glutamate receptors. This is consistent with prior evidence that antidepressants affect glutamate signaling. Agonists to both ionotropic and metabotropic glutamate receptors may have an antidepressant

sant-like effect (36). Chronic antidepressant treatment increases membrane expression of AMPA receptors in rat hippocampus (37) in a time frame consistent with therapeutic effects. In addition, chronic treatment with SSRIs increases phosphorylation of the active sites of AMPA receptors in extracts of cortex, hippocampus, and striatum (38). A recent study demonstrated specific and regionally discrete changes in the expression and editing of AMPA and kainate glutamate receptors, along with selective reduction of conductance for GluR3-containing receptors, after treatment with antidepressants (39). That study also showed that prolonged exposure to antidepressants produced site-selective and area-specific effects on this particular posttranscriptional regulation.

In summary, we identified markers in two genes within the glutamate signaling pathway that may shed light on the biological basis of treatment-emergent suicidal ideation and have the potential to help identify patients at high risk of having suicidal ideation emerge during citalopram treatment. Such patients may benefit from closer monitoring, alternative treatments, or specialty care. Further work is needed to replicate these findings and uncover the functional variation that underlies the association signals we observed.

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