Glutamate Transporter Gene SLC1A1 Associated With Obsessive-compulsive Disorder

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Context: There is strong evidence from family and twin studies that genetic determinants play an important role in the etiology of obsessive-compulsive disorder (OCD). In the only genome scan of OCD to date that we are aware of, suggestive linkage was reported to the chromosomal region 9p24, a finding that was subsequently replicated. This region contains the gene encoding the neuronal glutamate transporter, SLC1A1. SLC1A1 represents an excellent candidate gene for OCD based on evidence from neuroimaging and animal studies that altered glutamatergic neurotransmission is implicated in the pathogenesis of this disorder.

Objective: To determine whether sequence variants in SLC1A1 are associated with transmission of the OCD trait.

Design: A family-based candidate gene association study.

Setting: A specialized anxiety disorders outpatient clinic.

Participants: One hundred fifty-seven white probands with DSM-IV OCD recruited from consecutive referrals and their first-degree relatives (476 individuals in total).

Intervention: Nine single nucleotide polymorphisms spanning SLC1A1 were genotyped. Single-locus and haplotype analyses were performed using the Family-Based Association Test and the Transmission Disequilibrium Test. Traits examined included DSM-IV OCD diagnosis and highest lifetime symptom severity as measured using the Yale-Brown Obsessive-Compulsive Scale. Correction for multiple comparisons was performed using permutation tests.

Results: After correction for multiple comparisons, 2 variants, rs301434 ($\chi^2 = 12.04; P = .006$) and rs301435 ($\chi^2 = 9.24; P = .03$), located within a single haplotype block were found to be associated with transmission of OCD. Furthermore, a specific 2-marker haplotype within this block was significantly associated with OCD ($\chi^2 = 12.60; P = .005$). This haplotype association was statistically significant in transmissions to male but not female offspring.

Conclusions: Although requiring replication in larger samples, these findings provide preliminary evidence that sequence variation in SLC1A1 is associated with susceptibility to OCD, particularly in males. Furthermore, these results provide support for the role of altered glutamatergic neurotransmission in the pathogenesis of OCD.

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See also pages 717 and 778
leagues. Both Hanna et al and Willour et al achieved their strongest results based on a "narrow phenotype" model (definite OCD based on DSM-IV criteria) in samples ascertained largely through probands with early-onset disorder. Willour and colleagues also noted substantial heterogeneity within their sample, with only 59% of their families showing evidence for linkage with 9p24.

The linkage peak identified in the Hanna et al and Willour et al studies spans the entire 9p24 region of 7.5 megabases and contains many known and predicted genes. However, this region contains only one gene known to be expressed in the brain: the neuronal glutamate transporter gene SLC1A1 (Online Mendelian Inheritance in Man 133550), which codes for the neuronal glutamate transporter excitatory amino acid carrier 1 (EAAC1). This gene is highly expressed within the cerebral cortex, striatum, and thalamus, brain regions that are connected in functional cortico-striatal-thalamic-cortical circuits implicated in OCD. Given their strategic location near postsynaptic glutamate receptors, it is thought that EAAC1 transporters may facilitate more efficient neurotransmission through fine-tuning local glutamate concentrations and preventing spillover to neighboring synapses.

SLC1A1 is a strong, functional candidate gene for OCD given the mounting evidence for a role of altered glutamate neurotransmission within the cortico-striatal-thalamic circuits in the pathogenesis of OCD. Indirect support for this hypothesis is provided by an animal model in which transgenic mice with increased cortico-striatal glutamate output exhibit a phenotype reminiscent of OCD and "OCD spectrum" disorders including generalized behavioral perseveration, compulsive leaping, grooming-associated pulling and biting of skin and hair (similar to trichotillomania), and tics. More direct support for the role of glutamate in OCD is provided by recent investigation using proton magnetic resonance spectroscopy, suggesting a pharmacologically reversible, glutamatergically mediated thalamocortical-striatal dysfunction in OCD. In addition to its effects on glutamate levels, EAAC1 and other glutamate transporters are crucial for γ-aminobutyric acid (GABA) synthesis in presynaptic terminals. Significant reductions in GABA synthesis have been demonstrated directly through knockdown of SLC1A1 in adult mice and indirectly through application of glutamate transporter antagonists resulting in reduced inhibitory postsynaptic currents in hippocampal slice preparations. This glutamate-GABA interaction has potential implications for OCD given the recent finding by our group that the GABA type B receptor 1 (GABBR1) gene may be a susceptibility factor in this disorder.

Association studies of SLC1A1 and the surrounding genetic region have produced mixed findings. Veenstra-VanderWeele and colleagues failed to find any evidence for biased transmission in a family-based association analysis of a haplotype consisting of 2 single nucleotide polymorphisms (SNPs) in intron 3 of SLC1A1 (P = .42). Interpretation of these data are limited given the small sample size (40 parent-child trios). Willour and colleagues found modest associations between 2 microsatellite markers flanking SLC1A1, GATA62F03 (P = .02) and D9S288 (P = .05). With the recent acceleration of SNP identification arising from the International HapMap Project, many more polymorphisms within SLC1A1 that have not been tested in OCD are available in public databases. In addition, further evidence that genetic variants affecting glutamate neurotransmission may be implicated in OCD has been provided by positive candidate gene findings for the glutamate receptor ionotropic N-methyl-D-aspartate subunit 2B gene (GRIN2B) and the glutamate receptor ionotropic kainate receptor 2 gene (GRK2).

We hypothesized that SLC1A1 represented a candidate susceptibility gene for OCD. In this investigation, our objective was to test the association between SLC1A1 variants and transmission of OCD using a family-based design.

METHODS

SAMPLE CHARACTERISTICS AND CLINICAL ASSESSMENT

The study was approved by the Research Ethics Board of the Centre for Addiction and Mental Health, Toronto, Ontario, where the research was conducted. After complete description of the study to participants, written informed consent was obtained. The 157 probands (138 adults 18 years or older, 19 children or adolescents) were recruited from consecutive referrals to the Anxiety Disorders Clinic and the Children’s Mood and Anxiety Disorders Service at the Centre for Addiction and Mental Health. Families were included in the study only if both biological parents and/or at least 1 sibling were willing to participate in the study. Relatives who agreed to participate were assessed using the same methods. Relatives were deemed affected if they met full DSM-IV criteria for OCD, consistent with the narrow affection model, which produced the strongest linkage findings for 9p24. In the narrow affection model, only relatives with definite OCD are deemed affected as opposed to alternative models in which relatives with subclinical obsessive-compulsive symptoms or “obsessive-compulsive spectrum” disorders (eg, tic disorders) are also coded as affected. All participants were assessed using age-appropriate versions of the Structured Clinical Interview for DSM-IV and probands and affected relatives were assessed using age-appropriate versions of the Yale-Brown Obsessive Compulsive Scale (YBOCS). Lifetime severity of symptoms was estimated using the highest known lifetime YBOCS score, a retrospective estimate of the time when the most severe OCD symptoms were experienced for 2 or more consecutive weeks. In addition, the YBOCS Symptom Checklist was used to determine lifetime history of symptoms within the 4 symptom dimensions first identified by Leckman and colleagues and subsequently confirmed by our group using factor analytic methods. Affected individuals were coded as to whether they endorsed target symptoms within the following symptom dimensions: (1) factor 1 (aggressive, sexual, religious, and somatic obsessions; checking compulsions), (2) factor 2 (symmetry obsessions; repeating, counting, and ordering compulsions), (3) factor 3 (contamination obsessions, washing compulsions), and (4) factor 4 (hoarding obsessions and compulsions).

Instruments were administered by trained interviewers blind to the genotypes of the probands and then reviewed by psychiatrists (M.A.R. and P.D.A.) experienced in the diagnosis and treatment of OCD and related conditions to ensure diagnostic accuracy using DSM-IV criteria. Only probands with a confirmed diagnosis of OCD were included. Exclusion criteria for probands included lifetime history of neurologic disease (other than Tourette disorder or other tic disorders) or metabolic diseases. Individuals with bipolar disorder, psychotic disorder, or sub-
Genotyping

Using a nonenzymatic, high–salt extraction method, genomic DNA was extracted from 20 mL of venous whole blood of probands with OCD and family members. For our initial study, we selected 6 informative (minor allele frequency >20%) SNPs spanning SLCA1 based on information from the International HapMap Project. We selected SNPs based on location, choosing 2 variants located in coding regions and 4 additional variants sampled from different intronic regions and different haplotype blocks (haplotype block structure based on data from the HapMap Project analyzed using Haploview 3.2). The reference sequence numbers and locations of these 6 SNPs were as follows: SNP1 (rs1980043, intron 1), SNP2 (rs3780415, intron 2), SNP3 (rs7856209, exon 4), SNP4 (rs3780412, intron 7), SNP5 (rs3014340, exon 10), and SNP7 (rs301434, intron 10). To narrow down the association signal indicated by our initial positive findings at SNP7, we genotyped the following 3 additional SNPs: SNP6 (rs301979, intron 10), SNP8 (rs301435, intron 10), and SNP9 (rs3087879, 3′-untranslated region [UTR]).

Six of the 9 SNPs (SNP2, SNP4, SNP5, SNP6, SNP7, and SNP9) were identified as potential tag SNPs using the HapMap data. Genotyping was performed using Assays-on-Demand (Applied Biosystems Inc, Foster City, Calif), which are ready-to-use, TaqMan, probe-based SNP genotyping assays. Genotypes were read using the Applied Biosystems 7000 sequence detection instrument. For each 10-µL reaction, we used 1 µL of 20 ng/µL DNA, 5.0 µL of TaqMan reaction mix, 0.5 µL of Applied Biosystems assay, and 3.3 µL of water.

Statistical Analysis

We tested for association between the 9 selected polymorphisms of the SLCA1 gene and OCD using 2 complementary methods: (1) the Family-Based Association Test (FBAT) and (2) the Transmission Disequilibrium Test (TDT) as implemented in the Haploview 3.2 program. The FBAT is a unified approach to family-based association testing, which was used because it enables analysis of a variety of family structures, both qualitative and quantitative traits, and different models of inheritance using the same framework. The version of FBAT used in this study, version 1.5.5, is available online (www.bioostat.harvard.edu/~fbat).

Single-locus and haplotype analyses were performed in FBAT based on the categorical phenotype of OCD diagnosis and the quantitative phenotype of highest known lifetime YBOCS score. The models of inheritance that were examined are based on the number of copies of an allele required for increased susceptibility and included the additive, dominant, and recessive models, defined as follows: (1) additive, 1 or 2 copies of a risk allele increase the likelihood of possessing a trait in an additive fashion (ie, risk with 2 alleles >1 allele >0 alleles), (2) dominant, 1 or 2 copies of an allele are associated with an equal likelihood of having a trait, and (3) recessive, 2 copies of an allele are necessary to increase the likelihood of having a trait. All 3 models were examined because segregation analyses indicate that OCD is likely due to at least 1 gene of major effect on a polygenic background, with the mode of inheritance unclear. However, our a priori hypothesis was that our strongest results would be achieved under the dominant model, based on the fact that this model was associated with the strongest results in the aforementioned linkage studies of 9p24.5,9

Prior to haplotype testing, the Tagger subroutine on Haploview 3.2 was implemented to select tag SNPs. Tag SNPs are SNPs that predict the variation in other SNPs within the same haplotype block with a high degree of certainty. To minimize the redundancy resulting from testing highly correlated SNPs and the potential loss of power resulting from testing multiple low-frequency haplotypes, we only tested haplotypes consisting of tag SNPs within the same haplotype block. As a further check against multiple testing, we used a 2-stage procedure when performing haplotype analysis using FBAT: (1) the global test was performed followed by (2) testing individual haplotypes if the global test was statistically significant. In the global test, all haplotypes are analyzed in a single multivariate analysis, which circumvents the problem of multiple comparisons but tends to have reduced power in comparison with single-haplotype testing.

All tests were performed based on the compound null hypothesis of no linkage and no association between the phenotype and the genetic variant. The asymptotic variance option was used in FBAT for calculation of z scores from which the P values were derived, with a set to .05. Because construction of the standardized z score is based on a normal approximation, analyses in which there were fewer than 10 informative families were excluded from consideration to minimize violation of normality due to small sample sizes. A secondary FBAT analysis was performed based on the (1) sex of the proband and (2) presence of an affected proband and/or sibling with early onset of symptoms. Sex-specific analysis was performed since there is evidence of sex dimorphism in the clinical phenotype of OCD and sex-specific associations with other candidate gene variants. Our data were split according to sex with the aid of the PedSplit program developed by our group. Early onset was defined as younger than 15 years, consistent with the cutoff used for the linkage study of Hanna and colleagues in which suggestive linkage to the 9p24 region containing SLCA1 was previously reported.

Genetic associations were also tested using the TDT option as implemented in Haploview. In contrast to FBAT, only complete trios (including transmissions to affected siblings) are analyzed in this version of TDT. However, Haploview was used in addition to FBAT because this program includes an option for performing permutation tests for both single markers and haplotypes. Single marker and haplotypes within blocks were tested for 100 000 permutations, resulting in a corrected P value based on the number of permutations in which the χ² value exceeded the observed χ² value. Linkage disequilibrium information was obtained from Haploview, including D′ values and the haplotype block structure. Hardy–Weinberg equilibrium was also analyzed using the Haploview program.

Results

A total of 476 individuals in 157 families was genotyped. There were 157 probands (97 females and 60 males) and 49 affected relatives (including 34 females and 15 males) in our sample, for a total of 206 affected individuals. A total of 270 unaffected family members was genotyped. Family structures were varied and included 72 simple proband-parent trios, 39 sibships containing a proband plus 1 or more siblings (19 containing at least 1 affected individual), 21 nuclear families with a parent-proband trio plus 1 or more siblings (4 containing at least 1 affected individual), and 23 sibships plus 1 parent (5 containing at least 1 affected sibling). The ethnic background of the families was 96% white.

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The mean (SD) age at onset of probands and affected siblings was 14.4 (9.20) years. A history of clinically significant tics was present in 24.9% of affected individuals. Of 152 participants for whom we had information regarding target symptoms, the proportion of individuals endorsing target symptoms within the 4 symptom dimensions was as follows: factor 1 (obsessions/checking, 71.4%), factor 2 (symmetry/ordering, 54.6%), factor 3 (contamination/cleaning, 48.0%), and factor 4 (hoarding, 17.1%).

Genotype frequencies in probands did not differ significantly from Hardy-Weinberg equilibrium for any of the 9 SNPs. The degree of linkage disequilibrium between the 9 polymorphisms is depicted using $D'$ in Table 1. The highest $D'$ values were between SNPs 3 and 4 ($D'=0.99$) and between SNPs 7, 8, and 9 ($D'=0.96-1.00$). Analysis of our data using Haploview 3.2 indicated that these 2 clusters of SNPs constitute 2 distinct haplotype blocks. However, results from running the Tagger subroutine indicated that SNP3 adequately covered the allelic variation in block 1 and that SNP7 and SNP9 were tag SNPs for block 2. Therefore, haplotype analyses were only performed using combinations of SNP7 and SNP9 since haplotype analysis of SNPs 3 and 4 would be expected to provide no more information than analysis of SNP3 alone.

The FBAT analyses under the additive model were first performed on SNPs 1 to 5 and SNP7, resulting in a significant association with SNP7 ($P=0.0077$ under the additive model), leading us to genotypes SNP6, SNP8, and SNP9 to obtain more information regarding this region of SLC1A1. The results of FBAT single-locus analyses for all 9 SNPs are presented in Table 2 for the additive model, based on analysis of both the total sample and after stratification of the sample based on sex of the affected individual (proband or affected sibling). In the total sample, there was a significant association with OCD diagnosis for SNP7 (rs3014343, SNP8 (rs3014343), and SNP9 (rs3087879). As in our initial analysis, the most highly significant association was with SNP7, with increased transmission of allele C under the additive model ($z=3.39; P=0.0007$). Increased transmission of allele C was also found under the recessive model ($z=3.68; P=0.0002$), whereas significantly decreased transmission of allele T was seen under the additive ($z=-3.39; P=0.0007$) and dominant ($z=-3.58; P=0.0002$) models. Omnibus haplotype testing for SNP7 and SNP9 (block 2) indicated that this haplotype block was significantly associated with OCD. With respect to individual haplotypes, increased transmission of the C-G haplotype was found under both the additive ($z=3.43; P=0.0006$) and recessive ($z=3.53; P=0.0004$) models, whereas there was a weaker yet still statistically significant association with decreased transmission of the T-C haplotype under the additive ($z=-2.23; P=0.03$) and dominant ($z=-2.03; P=0.04$) models (Table 3).

Analysis of the quantitative trait of highest lifetime symptom severity (total YBOCS score) under the additive model also resulted in a statistically significant association with SNP7 (allele C, $z=2.58; P=0.01$). Furthermore, omnibus testing using haplotype analysis using FBAT resulted in a statistically significant association for this block. Lifetime YBOCS scores were associated with increased transmission of the C-G haplotype under the additive ($z=2.81; P=0.005$) and dominant ($z=2.26; P=0.02$) models, whereas decreased transmission of the T-G haplotype was found under both the additive ($z=-2.36; P=0.02$) and dominant ($z=-2.07; P=0.04$) models.

We also performed separate analyses of transmissions to male (83 families) and female (118 families) probands or siblings (Table 2). These analyses revealed an association with OCD diagnosis for SNP7 ($z=3.09; P=0.002$), SNP8 ($z=3.24; P=0.001$), and SNP9 ($z=3.09; P=0.002$) in families of male affected offspring (under the additive model). Haplotype testing of transmissions to male offspring resulted in a significant result for omnibus testing of the SNP7 to SNP9 block, with increased transmission of the C-G haplotype ($z=3.29; P=0.001$) and decreased transmission of the T-C haplotype ($z=-3.14; P=0.002$) to male offspring. There was a trend toward increased transmission of SNP7 ($z=1.66; P=0.10$) and the C-G haplotype ($z=-1.65; P=0.099$) to female offspring but no statistically significant single-locus or haplotype associations (Table 3). There were no significant differences between male and female participants with respect to age at onset ($t=0.26; P=0.79$), history of tics ($\chi^2=0.52; P=0.47$), or symptom dimensions (factor 1, $\chi^2=0.14; P=0.71$; factor 2, $\chi^2=0.87; P=0.31$; factor 4, $\chi^2=0.12; P=0.73$).

Secondary analyses were performed on the subsets of families in which the probands had an age at symptom onset known to be younger than 15 years (early onset)
or older than or equal to 15 years (late onset). We only had reliable age-at-onset data in 117 of our 157 nuclear families. Analysis of the 77 families containing offspring with early-onset symptoms resulted in no statistically significant findings.

Finally, TDT analysis was performed using Haploview followed by completion of a permutation test. Results from the whole sample and analysis of transmissions to male offspring are reported in Table 4. For the whole sample, the strongest result was for the SNP7 to SNP9 haplotype block, in which only 510 of 100,000 permutations of the data resulted in a $\chi^2$ value greater than the observed $\chi^2$ of 12.60 ($P = .005$), and for SNP7 ($\chi^2 = 10.49; P = .006$). For transmissions to males, empirical $P$ values were statistically significant for SNP7 ($\chi^2 = 10.31; P = .001$), the C-G haplotype ($\chi^2 = 9.39; P = .02$), and SNP9 ($\chi^2 = 8.76; P = .03$). Our finding of a positive association with the SNP7 C allele in the families of probands with early-onset disorder was not significant following correction for multiple comparisons using the permutation test implemented in Haploview.

The following abbreviations are used:
- FBAT: Family-Based Association Test
- NA: not applicable
- SNP: single nucleotide polymorphism
- RE = $(-S - E(S))/(-var(S))$, where $S$ = test statistic for observed number of alleles.
- FG = $z = \left(\frac{S - E(S)}{\sqrt{\text{var}(S)}}\right)$, where $S$ = test statistic for observed number of alleles.
- $z^*$ = test statistic for observed number of alleles.
- $z^\dagger$ = test statistic for observed number of alleles.

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### Table 2. Results of the FBAT Analysis of SLC1A1 Polymorphisms: Additive Model

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<tr>
<th>Polymorphism</th>
<th>Allele (Frequency)</th>
<th>Families*</th>
<th>z Score†</th>
<th>P Value‡</th>
<th>Families*</th>
<th>z Score†</th>
<th>P Value‡</th>
<th>Families*</th>
<th>z Score†</th>
<th>P Value‡</th>
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<td>SNP1 (rs1980943)</td>
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<td></td>
<td>A (0.36)</td>
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<td>28 −1.30 .19</td>
<td>44 0.37 .71</td>
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<td>SNP2 (rs3780415)</td>
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<td>28 1.33 .18</td>
<td>47 −1.64 .10</td>
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<tr>
<td></td>
<td>C (0.43)</td>
<td>84 0.74 .46</td>
<td>28 −1.33 .18</td>
<td>47 1.64 .10</td>
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<td></td>
<td>A (0.42)</td>
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<td>SNP4 (rs3780412)</td>
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<td></td>
<td>C (0.55)</td>
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<td>SNP5 (rs301430)</td>
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<tr>
<td></td>
<td>C (0.26)</td>
<td>76 −0.41 .68</td>
<td>31 −1.33 .18</td>
<td>40 0.23 .82</td>
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<td>SNP6 (rs301979)</td>
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<td>C (0.61)</td>
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<td>27 −3.09 .002§</td>
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<tr>
<td></td>
<td>C (0.57)</td>
<td>84 3.39 .0007§</td>
<td>27 3.09 .002§</td>
<td>51 1.65 .10</td>
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<td></td>
<td>C (0.46)</td>
<td>79 −3.32 .0009§</td>
<td>29 −3.24 .001</td>
<td>45 −1.42 .16</td>
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<tr>
<td></td>
<td>C (0.35)</td>
<td>76 −2.74 .006§</td>
<td>26 −3.09 .002</td>
<td>44 −1.02 .31</td>
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### Abbreviations
- FBAT: Family-Based Association Test
- NA: not applicable
- SNP: single nucleotide polymorphism

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### Table 3. Haplotype Analysis of SNP7 and SNP9 (Haplotype Block 2) Using FBAT

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Total Sample</th>
<th>Male Offspring</th>
<th>Female Offspring</th>
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<td>Additive</td>
<td>Families*</td>
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<td>P Value‡</td>
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<td>C-G (0.52)</td>
<td>T-G (0.34)</td>
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<td>−1.65</td>
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<td>Omarbus test (3 df)</td>
<td>$\chi^2 = 13.8$</td>
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<td>Dominant</td>
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<td>1.87</td>
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<td>T-G (0.34)</td>
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<td>−2.03</td>
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<td></td>
<td>T-G (0.12)</td>
<td>38.9</td>
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<tr>
<td>Omarbus test (3 df)</td>
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<tr>
<td>Recessive</td>
<td>C-G (0.52)</td>
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<td>3.53</td>
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<td></td>
<td>T-G (0.34)</td>
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<td>Omarbus test (2 df)</td>
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<td>.001§</td>
<td></td>
</tr>
</tbody>
</table>

### Abbreviations
- FBAT: Family-Based Association Test
- NA: not applicable
- SNP: single nucleotide polymorphism

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Consistent with our hypothesis, a significant association was observed between 3 tightly linked polymorphisms lying within the same haplotype block of SLC1A1 and OCD. Furthermore, a common haplotype (C-G) of tag SNPs in this block was also positively associated with OCD diagnosis under the additive and recessive models of inheritance in FBAT. When tested using TDT as implemented in Haploview, the association with the C-G haplotype and SNP7 (rs301434) remained highly significant even after correction for multiple comparisons based on permutation tests. This haplotype also appeared to be associated with the quantitative score of lifetime symptom severity in individuals with OCD. Consistent with our findings, Dickel and colleagues have recently found evidence of association to rs3780412 and rs301430 in an independent sample of families derived from probands with early-onset disorder. Although we did not find an association with these 2 SNPs, rs301430 is in modest linkage disequilibrium with SNP9 (D’ = 0.79), lying within the haplotype block associated in our study with OCD.

The fact that our most significant haplotype association was only found under the additive and recessive models is contrary to expectation based on linkage findings in 9p24, in which findings were found only under the dominant model. As expected, there was an increased number of informative families under the additive model as opposed to the dominant and recessive models of inheritance, resulting in more statistical power based on additive assumptions. The authors of the FBAT program have noted that an assumption of an additive model is appropriate under most circumstances unless there is very compelling evidence for a dominant/recessive model (N. Laird, PhD, FBAT course, January 2005), and our results are consistent with this.

Our other major finding, not predicted a priori, was that the association between the C-G haplotype and OCD was highly significant in transmissions to male but not female offspring. This finding occurred despite the smaller sample size of transmissions to male compared with female offspring with OCD. Our findings are consistent with sex-specific genetic effects for complex behavioral traits reported in humans and model organisms and also are consistent with evidence of sex dimorphism of clinical features of OCD. For example, males are believed to have an earlier onset of OCD and a higher likelihood of having comorbid tics or prominent symmetry/ordering symptoms (although these differences were not found when comparing males and females with OCD in our own sample). Furthermore, a segregation analysis of OCD found significant differences in the inheritance of OCD. Our other secondary analysis of transmissions to probands with early- vs late-onset disorder indicated a weak association within only the early-onset group, which was not significant following correction for multiple comparisons using a permutation test implemented in the Haploview program. However, interpretation of these findings is limited both by the missing age-at-onset data in 26% of our families and the relatively small number of families with only offspring with late-onset disorder. Given evidence indicating that early-onset OCD may be genetically distinct from late-onset OCD, further study of the impact of age at onset on genetic associations in OCD is warranted.

The location of SNP7 and SNP8 in intron 10, a considerable distance (approximately 1 kilobase) from the nearest intron-exon boundary, suggests that these polymorphisms are unlikely to directly produce functional effects on SLC1A1 and thereby influence the OCD phenotype. SNP9, on the other hand, is located in the 3’UTR of the gene. Although there are no studies of the functional effects of variants in SLC1A1 to guide our research, we speculate, based on studies of other genes, that variants in the 3’UTR, such as the ones tested in this study, could produce changes in messenger RNA processing and thereby affect the quantity of the EAAC1 protein. We are currently genotyping additional polymorphisms in the 3’UTR region to more clearly define the association signal and to test our hypothesis that this is where the risk variant lies. One possible mechanism by which variants in the 3’UTR may alter gene expression is through alteration of binding sites for microRNAs, regulatory molecules that act through targeting messenger RNA for cleavage or translational repression. Interestingly, a rare sequence variant located within a 3’UTR microRNA binding site in the gene Slit and Trk-like 1 (SLITRK1) was recently identified in patients with Tourette syndrome and obsessive-compulsive symptoms. An alternative hypothesis is that the true risk polymorphism(s) is located in either exon 11 or 12, which appear to lie within the same haplotype block according to the HapMap data. However, no SNPs in the coding regions of exons 11 or 12 are available in public databases and none were identified when SLC1A1 was sequenced in 7 subjects with OCD. More extended sequencing of exons 10 through 12 and the 3’UTR in a larger sample of patients with OCD is indicated as it might lead to the identification of a functional variant in the region.

As noted earlier, family and twin studies suggest that OCD is a complex genetic trait likely resulting from the interaction of multiple genetic variants as well as non-genetic risk factors. Therefore, future research into the role of variation in SLC1A1 and other glutamate genes

### Table 4. Permutation Tests of SLC1A1 Polymorphisms: Results After 100,000 Permutations

<table>
<thead>
<tr>
<th>Sample</th>
<th>Polymorphism or Haplotype</th>
<th>(\chi^2) (From TDT)</th>
<th>Empirical P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Whole sample</strong></td>
<td>C-G (block 2)</td>
<td>12.60</td>
<td>.005</td>
</tr>
<tr>
<td>SNP7 (rs301434)</td>
<td>12.04</td>
<td>.006</td>
<td></td>
</tr>
<tr>
<td>T-G (block 2)</td>
<td>5.63</td>
<td>.19</td>
<td></td>
</tr>
<tr>
<td>SNP9 (rs3087879)</td>
<td>5.19</td>
<td>.22</td>
<td></td>
</tr>
<tr>
<td>T-C (block 2)</td>
<td>4.35</td>
<td>.32</td>
<td></td>
</tr>
<tr>
<td><strong>Male offspring</strong></td>
<td>SNP7 (rs301434)</td>
<td>10.31</td>
<td>.01</td>
</tr>
<tr>
<td>C-G (block 2)</td>
<td>9.39</td>
<td>.02</td>
<td></td>
</tr>
<tr>
<td>SNP9 (rs3087879)</td>
<td>8.76</td>
<td>.03</td>
<td></td>
</tr>
<tr>
<td>T-C (block 2)</td>
<td>7.22</td>
<td>.05</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: SNP, single nucleotide polymorphism; TDT, Transmission Disequilibrium Test.

*Empirical P value based on number of times \(\chi^2\) values exceed observed \(\chi^2\) in 100,000 permutations. All other results were not statistically significant based on corrected P values <.05.
in OCD should explicitly consider the effects of gene × gene and gene × environment interactions, though relatively large sample sizes and sophisticated statistical approaches will be needed to provide sufficient power for such analyses. Careful consideration of the heterogeneity of the phenotype is also needed. For example, there is increasing evidence from factor analytic studies that OCD consists of a small number of overlapping but distinct symptom dimensions rather than a unitary disorder. Future association studies of OCD should include separate analyses of these symptom dimensions based on the assumption that they represent endophenotypes that are likely to be more etiologically homogeneous and closely linked to the action of genes compared with OCD diagnosis. Structural and functional neuroimaging profiles also represent potential endophenotypes for genetic association studies of OCD and other complex neuropsychiatric disorders.

In summary, we found a positive association between the neuronal glutamate transporter gene SLC1A1 and OCD, a finding that remained statistically significant even after correction for multiple testing based on a permutation test. This association was statistically significant in transmissions to male but not female offspring. Furthermore, an independent group has observed a similar association with 2 additional variants in SLC1A1, which was also specific to male offspring. Further research, including sequencing of the putative susceptibility region and examination for possible microRNA binding sites, is warranted to locate the actual functional variant(s) contributing to the OCD phenotype. The likelihood that variation within SLC1A1 affects risk for OCD is enhanced given our strong a priori hypothesis based on both earlier findings of linkage to effects risk for OCD is enhanced given our strong a priori hypothesis based on both earlier findings of linkage to 9p24 (H11003 gene). The likelihood that variation within SLC1A1 affects risk for OCD is enhanced given our strong a priori hypothesis. The likelihood that variation within SLC1A1 affects risk for OCD is enhanced given our strong a priori hypothesis. The likelihood that variation within SLC1A1 affects risk for OCD is enhanced given our strong a priori hypothesis. The likelihood that variation within SLC1A1 affects risk for OCD is enhanced given our strong a priori hypothesis. The likelihood that variation within SLC1A1 affects risk for OCD is enhanced given our strong a priori hypothesis. The likelihood that variation within SLC1A1 affects risk for OCD is enhanced given our strong a priori hypothesis. The likelihood that variation within SLC1A1 affects risk for OCD

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