Association Testing of the Positional and Functional Candidate Gene SLC1A1/EAAC1 in Early-Onset Obsessive-compulsive Disorder

Diane E. Dickel, BA; Jeremy Veenstra-VanderWeele, MD; Nancy J. Cox, PhD; Xiaolin Wu, MD, PhD; Daniel J. Fischer, MSW; Michelle Van Etten-Lee, PhD; Joseph A. Himle, PhD; Bennett L. Leventhal, MD; Edwin H. Cook, Jr, MD; Gregory L. Hanna, MD

Context: The first 2 independent linkage studies for obsessive-compulsive disorder (OCD) identified a region on 9p24 with suggestive evidence for linkage. The glutamate transporter gene solute carrier family 1, member 1 (SLC1A1) is a promising functional candidate in this region because altered glutamatergic concentrations have been found in the striatum and anterior cingulate in neuroimaging studies of pediatric OCD.

Objective: To determine whether genotypes at polymorphisms in the SLC1A1 gene region are associated with early-onset OCD.

Design: Family-based analysis of association using the transmission disequilibrium test, confirmed using the family-based association test.

Setting: Anxiety disorders program in an academic medical center.

Participants: Seventy-one probands with DSM-III-R or DSM-IV OCD and their parents.

Methods: Nine single nucleotide polymorphisms spaced throughout the SLC1A1 gene region were genotyped.

Results: Significant association was detected at rs3780412 (P = .04) and rs301430 (P = .03), 2 common adjacent single nucleotide polymorphisms in the 3’ region of SLC1A1. Analysis by sex revealed that association at rs3780412 was limited to male probands (P = .002). Significant association was also detected for the T/C haplotype at rs301430-rs301979 (P = .03), the only haplotype block identified among the 9 single nucleotide polymorphisms. Analysis by sex also revealed that the haplotype association was limited to male probands (P = .003). A deletion in the 3’ flanking region of SLC1A1 was also detected that imperfectly segregated with OCD in a large, multigenerational family with multiple affected individuals.

Conclusions: The 3’ region of SLC1A1 may contain a susceptibility allele for early-onset OCD, with differential effects in males and females. The results also provide further support for the involvement of a glutamatergic dysfunction in the pathogenesis of early-onset OCD.

Arch Gen Psychiatry. 2006;63:778-785
is strongly associated with a more familial form of OCD. Recent controlled studies using pediatric probands found that the lifetime prevalence of OCD was significantly higher in case relatives vs control relatives: Hanna et al., 22.5% vs 2.6%, and do Rosario-Campos et al., 22.7% vs 0.9%.

The only published genome scan of OCD used families ascertained through pediatric probands to identify a region on chromosome 9p24 with suggestive evidence for linkage. A parametric linkage analysis using a dominant Mendelian model of inheritance yielded a logarithm of odds score of 2.25 that declined to 1.97 with additional participants and markers in that region. In an effort to replicate this finding, Willour and colleagues genotyped 13 microsatellite markers from chromosome 9p24 in 50 families. Their study identified an overlapping region with suggestive evidence for linkage centered only 0.5 centimorgan from the original finding. Furthermore, Taylor and colleagues reported a 9p monosomy in a patient with Tourette syndrome and OCD. These findings indicate that 9p24 is a strong candidate region for early-onset OCD. Association studies in this region have been mixed, however, with 1 study finding modest association at 2 microsatellite markers flanking the solute carrier family 1, member 1 gene (SLC1A1), GATA62F03 (P = .02) and D9S288 (P = .05), and 1 study finding no evidence of association at 2 single nucleotide polymorphisms (SNPs) in SLC1A1 intron 3 (P = .42).

The SLC1A1 gene, which codes for the glutamate transporter EAAC1 (EAAT3), is the most promising candidate gene in the region shared by the 9p24 linkage and association findings and the reported 9p monosomy. EAAC1 is a high-affinity glutamate transporter primarily expressed in neurons, intestine, kidney, liver, and heart. EAAC1 couples the transport of L-glutamate into cells with the cotransport of sodium and hydrogen and the countertransport of potassium, an important step in protecting neurons from glutamate excitotoxicity (for a review see Kanai and Hediger). The SLC1A1 knockout mice show decreased spontaneous locomotor activity and dicarboxylic aminoaciduria.

The SLC1A1 gene is a plausible functional candidate and a strong positional candidate. Pediatric patients with OCD have been shown to have reduced glutamatergic concentrations in the anterior cingulate cortex and significantly increased concentrations in the caudate compared with unaffected controls. Caudate glutamatergic concentrations and OC symptoms decrease concurrently with paroxetine treatment. Cerebrospinal fluid glutamate levels were also found to be higher in adults with OCD compared with adult control subjects.

Although a previous study by our group found no linkage disequilibrium (LD) between OCD and SLC1A1, that study examined only 2 SNPs, both in intron 3 of the gene. We expand this study to examine the association between SLC1A1 and early-onset OCD by genotyping more SNPs that more comprehensively cover the gene and by using a larger patient sample. We use a family-based approach to detect association, the transmission disequilibrium test (TDT), which has less power than methods using case and hypernormal controls; however, the TDT avoids the possibility of population stratification bias that may be present even in relatively homogeneous populations. We report significant association with 2 common adjacent SNPs in the 3’ region of SLC1A1. We also describe a deletion in the 3’ flanking region of SLC1A1 in a large, multigenerational family with multiple affected individuals.

**METHODS**

**PARTICIPANT ASCERTAINMENT**

Participants were ascertained in 2 separate groups for earlier genome scan and candidate gene studies. The first group was ascertained as described previously. Briefly, this group consisted of 21 parent-child trios from singleplex families and 19 parent-child trios from 7 multiplex families. All the probands were directly interviewed and met the DSM-IV criteria for OCD. (The DSM-IV had not yet been published when subject ascertainment began.) Proband ranged in age from 6 to 17 years (mean ± SD, 14.4 ± 3.8 years), and all had onset of OC symptoms at 3 to 14 years of age (mean ± SD, 8.8 ± 3.9 years). The exclusion criteria were (1) a diagnosis of a chronic neurologic disorder other than tic disorder; (2) a lifetime DSM-III-R diagnosis of mental retardation, autistic disorder, bipolar disorder, or schizophrenia; (3) currently living away from both biological parents; and (4) adoption.

The second group of participants was recruited for the purpose of an expanded genome scan and candidate gene studies. The probands in the second group were recruited from clinics in the University of Michigan Health System and from local chapters of the Obsessive Compulsive Foundation. All the probands were directly interviewed to determine whether they met the DSM-IV criteria for a lifetime diagnosis of OCD. The second group of probands consisted of 11 males and 15 females ranging in age from 7 to 64 years (mean ± SD, 28.4 ± 17.3 years). Age at onset of OC symptoms in these probands ranged from 3 to 18 years (mean ± SD, 8.0 ± 3.7 years). The exclusion criteria for the second group of probands were (1) the onset of OC symptoms after age 18 years; (2) a lifetime DSM-IV diagnosis of autistic disorder, schizophrenia, or bipolar I disorder; (3) adoption; (4) if younger than 18 years, currently living away from both biological parents; and (5) a first-degree relative with a lifetime DSM-IV diagnosis of autistic disorder, schizophrenia, or bipolar I disorder. The study was approved by the institutional review board of the University of Michigan Medical School.

After providing informed consent and assent, probands and relatives younger than 18 years were interviewed using the Schedule for Affective Disorder and Schizophrenia for School-Age Children—Epidemiologic Version—5. This interview was completed independently with a parent of the participant and with the participant. Proband and relatives 18 years and older were interviewed using the Structured Clinical Interview for DSM-IV Axis I Disorders. Both interviews were supplemented with sections on OCD and tic disorders derived from the Schedule for Tourette and Other Behavioral Syndromes. The section on OCD included a series of questions modified to cover all the criteria for a lifetime DSM-IV diagnosis of OCD and a checklist from the Yale-Brown Obsessive Compulsive Scale modified to obtain information about the lifetime occurrence of specific obsessions and compulsions. All interviews were audiotaped and coded on paper to assess reliability, maintain quality control, and achieve diagnostic consensus. All the interviewers had at least a master’s degree and clinical training in either child or adult psychopathology. They were trained to at least 90% diagnostic agreement with the individual instruments. After completion of all
the p-terminus of chromosome 9. The rs10758629 SNP was not included in Genome Browser, May 2004 Assembly, and are measured in base pairs from regions. The positions are from the University of California at Santa Cruz were reached with the assistance of a third diagnostician fol-
ments occurred between 2 diagnosticians, consensus diagnoses
pants were interviewed again if necessary to clarify incom-
lected or contradictory information. When major disagree-
ences occurred between 2 diagnosticians, consensus diagnoses were reached with the assistance of a third diagnostician fol-
ceries from each consenting individual and were frozen at either –20°C or –70°C until DNA extraction. DNA was extracted from
Peripheral whole blood samples were obtained by venipunc-
ments were analyzed by capillary electrophoresis using an ABI 3730
µL of 5-U/µL AmpliTaq Gold polymerase, 0.1 µL of each 10µM
triphosphate mix, 1.2 µL of 25mM magnesium chloride, 0.08
µL of 5-U/µL AmpliTaq Gold polymerase, 0.1 µL of each 10µM
primer, and 5.52 µL of water for a total reaction volume of 10
µL of 5-U/µL AmpliTaq Gold polymerase, 0.1 µL of each 10µM
primer, and 5.52 µL of water for a total reaction volume of 10
µL. All the reactions were performed using a PerkinElmer 9700 Thermocycler (Applied Biosystems) under the following conditions: 1 AmpErase step at 50.0°C for 2 min-
utes, 1 enzyme activation step at 95.0°C for 10 minutes, and 40
alternating cycles of denaturation at 92.0°C and reanneal-
ing and extension at 58.0°C for 1 minute. The fluorescence in-
tensity of the final reaction product was measured using an L JL
Analyst AD fluorescence microplate reader and L JL Criterion
Host Software (L JL Biosystems, Sunnyvale, Calif).

STATISTICAL ANALYSIS

Departures from Hardy-Weinberg equilibrium were assessed using the HWE program from the LINKUTIL package provided by Dr Jurg Ott (ftp://linkage.rockefeller.edu/software/utilities/). PedCheck was used to identify genotype incom-
patibilities. The HaploView program (http://www.broad.mit.
edu/mpg/haplovie/) was used to identify potential haplotype blocks and to calculate intermarker LD, including LD with the 2 SNPs from the previous study of SLC1A1, rs10974625 and rs1805311, that had been genotyped in a subset of the present sample. HaploView was also used to calculate family-based association using the TDT for individual markers and for the only identified haplotype block (rs301430-rs301979), where the rs301979G allele is present only on chromosomes that con-
tain the rs301430C allele. Because nonindependent trios were present in multiple extended families in our sample and link-
age was previously reported in the 9p24 region, we also used the empirical correction for linkage implemented in the family-
based association test (FBAT) to evaluate the possibility that linkage alone accounted for significant findings using the TDT. The “FBAT-e” and “HBAT-e” functions use a null hy-
pothesis of “no association in the presence of linkage” for geno-
types and haplotypes, respectively. Precise estimates of power for this modification of the FBAT have not been published; however, the number of families informative for the statistic decreased to 0.74 to 0.77 of our complete families.

DELETION CHARACTERIZATION

Primers (sense: FAM-TT TTGGCCAGGGAGATAGAA-3’ and antisense: 5’-GGTCTTCTCATAGTACGACATGCTG-3’) were designed to amplify a region surrounding rs301443 to confirm the presence of the deletion and to determine its size. A PCR kit (AmpliTaq Gold; Applied Biosystems) was used to genotype the deletion. Each PCR contained 1.0 µL of 10-ng/µL genomic DNA, 1.0 µL of 10× PCR Buffer II, 1.0 µL of 10mM deoxynucleoside triphosphate mix, 1.2 µL of 25mM magnesium chloride, 0.08 µL of 5-U/µL AmpliTaq Gold polymerase, 0.1 µL of each 10µM primer, and 5.52 µL of water for a total reaction volume of 10 µL. All the reactions were performed using a PerkinElmer 9700 Thermocycler under the following conditions: 1 enzyme acti-
vation step at 95.0°C for 12 minutes, 36 cycles of 3 alternating steps (denaturation at 94.0°C for 15 seconds, annealing at 55.0°C for 15 seconds, and extension at 72.0°C for 30 seconds), and 1 final extension step at 72.0°C for 10 minutes. The final PCR products were analyzed by capillary electrophoresis using an ABI 3730 (Applied Biosystems), and genotypes were called using Gen
eMapper v3.5 (Applied Biosystems). All the participants were screened for this deletion.

DNA EXTRACTION

Peripheral whole blood samples were obtained by venipunc-
ture from each consenting individual were frozen at either –20°C or –70°C until DNA extraction. DNA was extracted from
300 µL of blood using a DNA purification kit (Puregene; Gen-
tra Systems, Minneapolis, Minn).

GENOTYPING

Single nucleotide polymorphisms, selected for complete cover-
age of the SLC1A1 gene, were chosen from SNP genotyping
assays (TaqMan; Applied Biosystems, Foster City, Calif). Any
SNPs that had minor allele frequencies less than 0.2 in white
populations were excluded. Two pairs of SNPs were expected to be in strong LD based on near-identical allele frequency, and 1 SNP from each pair was excluded. Ten SNPs remained for typing, and a tagging SNP search using a software tool (SNPBrowser version 2.0; Applied Biosystems) showed that these SNPs provide adequate coverage of the SLC1A1 gene and the immediate flanking region (haplotype R²>85%). The average spacing between SNPs across the gene was 12.4 kilobases (kb).

Figure 1 shows the locations of the SNPs used.

Polymerase chain reactions (PCRs) for the SNP genotyping
assays contained 10 ng of dry DNA, 2.5 µL of 2× TaqMan
Universal Master Mix (Applied Biosystems), 0.25 µL of 20×
SNP Genotyping Assay Mix, and 2.25 µL of water, for a total
volume of 5.0 µL. All the reactions were performed using a PerkinElmer 9700 Thermocycler (Applied Biosystems) under the following conditions: 1 AmpErase step at 50.0°C for 2 min-
utes, 1 enzyme activation step at 95.0°C for 10 minutes, and 40
alternating cycles of denaturation at 92.0°C and reannealing
and extension at 58.0°C for 1 minute. The fluorescence in-
tensity of the final reaction product was measured using an L JL
Analyst AD fluorescence microplate reader and L JL Criterion
Host Software (L JL Biosystems, Sunnyvale, Calif).

Figure 1. Solute carrier family 1, member 1 gene (SLC1A1) organization and single nucleotide polymorphism (SNP) locations. The horizontal line represents the genomic sequence; vertical bars, exons, with tall bars representing translated regions and short bars representing untranslated regions. The positions are from the University of California at Santa Cruz Genome Browser, May 2004 Assembly, and are measured in base pairs from the p-terminus of chromosome 9. The rs10758629 SNP was not included in the statistical analysis.
Sequencing was performed to determine the exact location of the deletion. Three participants, 2 heterozygous for the deletion and 1 homozygous without the deletion, were chosen for sequencing. These individuals (IV:1, IV:7, and III:2, respectively) are shown in Figure 2. A region surrounding rs301443 was amplified using the PCR conditions, and the reverse primer is listed in the preceding paragraph. The same forward primer was also used but without the FAM label. After PCR amplification, 0.5 µL of 1-U/µl shrimp alkaline phosphatase (Roche, Indianapolis, Ind), 0.5 µL of 10× shrimp alkaline phosphatase reaction buffer, and 0.25 µL of exonuclease 1 (USB Corp, Cleveland, Ohio) were added to each 10-µL PCR product to degrade unincorporated deoxynucleoside triphosphates and primers. The shrimp alkaline phosphatase reactions were performed using a PerkinElmer 9700 Thermocycler under the following conditions: 1 cycle at 37.0°C for 45 minutes and 1 cycle at 95.0°C for 15 minutes. The previously mentioned PCR primers were diluted and used for sequencing, which was performed in both directions. Sequencing reactions contained 1.2 µL of PCR template, 1.2 µL of 1.6Pm primer, 2.0 µL of ready reaction mix (BigDye v3.1 Terminator; Applied Biosystems), and 2.0 µL of water, for a total reaction volume of 6.4 µL. Sequencing reactions were performed using capillary electrophoresis using an ABI 3730 (Applied Biosystems), and the resulting sequences were read using Sequencher v4.2 (Gene Codes Corp, Ann Arbor).

RESULTS

Before statistical analysis, 1 SNP (rs10758629, designated as C___1459316_10 by Applied Biosystems) was dropped owing to poorly defined genotype clusters. Genotypes for the 9 remaining markers used in this study showed no deviation from Hardy-Weinberg equilibrium. PedCheck detected 6 incompatibilities in the genotyping data. However, 5 of the 6 incompatibilities were found only in SNP rs301443 in 1 large, multigenerational family in a pattern indicating the presence of a deletion. Because of the deletion, this family was not included in the association analysis for rs301443 but was included for the remaining markers. An 11-base pair (bp) deletion downstream of rs301443 in 1 large, multigenerational family in a pattern indicating the presence of a deletion. Because of the deletion, this family was not included in the association analysis for rs301443 but was included for the remaining markers. An 11-base pair (bp) deletion downstream of rs301443 in 1 large, multigenerational family in a pattern indicating the presence of a deletion. Because of the deletion, this family was not included in the association analysis for rs301443 but was included for the remaining markers.

Two SNPs showed nominally significant association with early-onset OCD in the overall sample. The higher-frequency A allele of rs3780412 and the lower-frequency C allele of rs301430 were overtransmitted (χ²=4.19; P=0.04 and χ²=4.91; P=0.03, respectively). The overtransmission of the A allele at rs3780412 was observed in male probands (χ²=9.53; P=0.002) but not in female probands (χ²=0.03; P=.87). The FBAT-e results paralleled the TDT results, with trends for overtransmission of rs3780412A (P=.09) and rs301430 (P=.06) in the overall sample and significant overtransmission of rs3780412 to male probands (P=.009) but not to female probands (P=.66). Table 1 gives the TDT results for the overall sample and for male and female probands separately. Pairwise LD between rs3780412 and rs301430 was intermediate (r²=0.15, D’=0.64). Intermarker LD for all the markers is given in Table 2. The 2 markers genotyped in the previous study of SLC1A1 in OCD, rs10974625 and rs1805311, were in nearly complete but not perfect LD with rs3780412, rs301430, and rs301979 (r²=0.116-0.696, D’=0.94-1.0) but in only modest LD with the other SNPs.

Only 1 haplotype block was identified, and it comprised 2 markers, rs301430 and rs301979. The T/C haplotype of these 2 markers was undertransmitted in the overall sample (χ²=4.90; P=0.03) (Table 3). The undertransmission of this haplotype was also detected in male probands (χ²=8.91; P=0.003) but not in female probands (χ²=0.00; P=.99). The HBA1-e results paralleled the TDT results, with undertransmission of this haplotype in the overall sample (P=.02) that strengthened in the male probands (P=.004) but that was not detected in the female probands (P=.74).

Two independent linkage studies17,18 and a report of a 9p monosomy in a patient with OCD and Tourette syndrome19 implicate 9q24 as a candidate region for OCD. Previous association studies in this region, however, have been limited and have produced mixed results. One study18 found modest evidence of association at 2 microsatellite markers: GATA62F03 (P=.02), which lies 613 kb 3’ to SLC1A1, and D9S288 (P=.05), which lies 539 kb 5’ to SLC1A1. The only previous study20 to examine association at markers in SLC1A1 found no evidence of association. Our finding of nominally significant association at 2 SNPs, rs3780412 and rs301430, and a haplotype including a third SNP, rs301979, in SLC1A1 supports a role for SLC1A1 in early-onset OCD. Consistent with this finding, researchers have also found evidence of association at rs301434 and rs301435 in a
family-based association study of OCD (Paul Arnold, MD, e-mail communication, 2005). Furthermore, both studies find evidence of association primarily for male probands with OCD.

We also discovered a rare 11-bp deletion in the 3′ flanking region of SLC1A1 in a large, multigenerational family (Figure 2). This deletion is located in a noncoding region of an uncharacterized gene, C9ORF68, that seems to be alternatively spliced. Figure 3 shows the location of the deletion and the genetic organization of C9ORF68. As shown in Figure 2, this deletion does not perfectly cosegregate with OCD in this family: 2 unaffected individuals have the deletion and 1 affected individual does not. It is possible that the deletion disrupts splicing for C9ORF68, assuming that this putative gene makes a functional protein product, or alters a regulatory element for one of the genes in this region. However, given the size of the deletion and the only modest

---

### Table 1. TDT Results for SNP Markers in the Overall Sample and in Male and Female Probands Separately

<table>
<thead>
<tr>
<th>Polymorphism Name</th>
<th>AB Assay Name</th>
<th>Type of SNP</th>
<th>Transmitted From Heterozygous Parent</th>
<th>Major Allele</th>
<th>Minor Allele</th>
<th>( \chi^2 )</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs10814987</td>
<td>C___3026341_10</td>
<td>G/T</td>
<td>30</td>
<td>27</td>
<td>0.16</td>
<td>.69</td>
<td></td>
</tr>
<tr>
<td>rs10739062</td>
<td>C___3026344_10</td>
<td>C/G</td>
<td>35</td>
<td>46</td>
<td>1.49</td>
<td>.22</td>
<td></td>
</tr>
<tr>
<td>rs1980943</td>
<td>C___1459297_10</td>
<td>A/G</td>
<td>33</td>
<td>31</td>
<td>0.06</td>
<td>.80</td>
<td></td>
</tr>
<tr>
<td>rs10115600</td>
<td>C___1459308_10</td>
<td>C/G</td>
<td>31</td>
<td>23</td>
<td>1.19</td>
<td>.28</td>
<td></td>
</tr>
<tr>
<td>rs3780412</td>
<td>C___11261320_10</td>
<td>A/G</td>
<td>43</td>
<td>26</td>
<td>4.19</td>
<td>.04</td>
<td></td>
</tr>
<tr>
<td>rs301430</td>
<td>C___797982_1_</td>
<td>T/C</td>
<td>24</td>
<td>42</td>
<td>4.91</td>
<td>.03</td>
<td></td>
</tr>
<tr>
<td>rs301979</td>
<td>C___3059669_1_</td>
<td>C/G</td>
<td>29</td>
<td>33</td>
<td>0.26</td>
<td>.61</td>
<td></td>
</tr>
<tr>
<td>rs301454</td>
<td>C___3059680_10</td>
<td>A/G</td>
<td>35</td>
<td>30</td>
<td>0.38</td>
<td>.54</td>
<td></td>
</tr>
<tr>
<td>rs301443</td>
<td>C___3059691_1_</td>
<td>C/G</td>
<td>29</td>
<td>25</td>
<td>0.30</td>
<td>.59</td>
<td></td>
</tr>
<tr>
<td>Male Probands</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs10814987</td>
<td>C___3026341_10</td>
<td>G/T</td>
<td>17</td>
<td>14</td>
<td>0.29</td>
<td>.59</td>
<td></td>
</tr>
<tr>
<td>rs10739062</td>
<td>C___3026344_10</td>
<td>C/G</td>
<td>18</td>
<td>23</td>
<td>0.61</td>
<td>.43</td>
<td></td>
</tr>
<tr>
<td>rs1980943</td>
<td>C___1459297_10</td>
<td>A/G</td>
<td>17</td>
<td>16</td>
<td>0.03</td>
<td>.86</td>
<td></td>
</tr>
<tr>
<td>rs10115600</td>
<td>C___1459308_10</td>
<td>C/G</td>
<td>13</td>
<td>13</td>
<td>0.00</td>
<td>&gt; .99</td>
<td></td>
</tr>
<tr>
<td>rs3780412</td>
<td>C___11261320_10</td>
<td>A/G</td>
<td>26</td>
<td>8</td>
<td>9.53</td>
<td>.002</td>
<td></td>
</tr>
<tr>
<td>rs301430</td>
<td>C___797982_1_</td>
<td>T/C</td>
<td>15</td>
<td>25</td>
<td>2.50</td>
<td>.11</td>
<td></td>
</tr>
<tr>
<td>rs301979</td>
<td>C___3059669_1_</td>
<td>C/G</td>
<td>11</td>
<td>20</td>
<td>2.61</td>
<td>.11</td>
<td></td>
</tr>
<tr>
<td>rs301454</td>
<td>C___3059680_10</td>
<td>A/G</td>
<td>19</td>
<td>17</td>
<td>0.11</td>
<td>.74</td>
<td></td>
</tr>
<tr>
<td>rs301443</td>
<td>C___3059691_1_</td>
<td>C/G</td>
<td>14</td>
<td>13</td>
<td>0.04</td>
<td>.85</td>
<td></td>
</tr>
<tr>
<td>Female Probands</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs10814987</td>
<td>C___3026341_10</td>
<td>G/T</td>
<td>13</td>
<td>13</td>
<td>0.00</td>
<td>&gt; .99</td>
<td></td>
</tr>
<tr>
<td>rs10739062</td>
<td>C___3026344_10</td>
<td>C/G</td>
<td>17</td>
<td>23</td>
<td>0.90</td>
<td>.34</td>
<td></td>
</tr>
<tr>
<td>rs1980943</td>
<td>C___1459297_10</td>
<td>A/G</td>
<td>16</td>
<td>15</td>
<td>0.03</td>
<td>.86</td>
<td></td>
</tr>
<tr>
<td>rs10115600</td>
<td>C___1459308_10</td>
<td>C/G</td>
<td>18</td>
<td>10</td>
<td>2.29</td>
<td>.13</td>
<td></td>
</tr>
<tr>
<td>rs3780412</td>
<td>C___11261320_10</td>
<td>A/G</td>
<td>17</td>
<td>18</td>
<td>0.03</td>
<td>.87</td>
<td></td>
</tr>
<tr>
<td>rs301430</td>
<td>C___797982_1_</td>
<td>T/C</td>
<td>9</td>
<td>17</td>
<td>2.46</td>
<td>.12</td>
<td></td>
</tr>
<tr>
<td>rs301979</td>
<td>C___3059669_1_</td>
<td>C/G</td>
<td>18</td>
<td>13</td>
<td>0.81</td>
<td>.37</td>
<td></td>
</tr>
<tr>
<td>rs301454</td>
<td>C___3059680_10</td>
<td>A/G</td>
<td>16</td>
<td>13</td>
<td>0.31</td>
<td>.58</td>
<td></td>
</tr>
<tr>
<td>rs301443</td>
<td>C___3059691_1_</td>
<td>C/G</td>
<td>15</td>
<td>12</td>
<td>0.33</td>
<td>.56</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AB, Applied Biosystems; SNP, single nucleotide polymorphism; TDT, transmission disequilibrium test.

### Table 2. Intermarker Linkage Disequilibrium*

<table>
<thead>
<tr>
<th>Marker</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (rs10814987)</td>
<td>0.00</td>
<td>0.02</td>
<td>0.01</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.01</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>2 (rs10739062)</td>
<td>0.11</td>
<td>0.11</td>
<td>0.11</td>
<td>0.01</td>
<td>0.00</td>
<td>0.02</td>
<td>0.02</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>3 (rs1980943)</td>
<td>0.24</td>
<td>0.39</td>
<td>0.10</td>
<td>0.01</td>
<td>0.02</td>
<td>0.01</td>
<td>0.01</td>
<td>0.00</td>
<td>0.01</td>
</tr>
<tr>
<td>4 (rs10115600)</td>
<td>0.16</td>
<td>0.75</td>
<td>0.63</td>
<td>0.01</td>
<td>0.02</td>
<td>0.01</td>
<td>0.01</td>
<td>0.00</td>
<td>0.04</td>
</tr>
<tr>
<td>5 (rs3780412)</td>
<td>0.02</td>
<td>0.12</td>
<td>0.18</td>
<td>0.15</td>
<td>0.01</td>
<td>0.20</td>
<td>0.19</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>6 (rs301430)</td>
<td>0.04</td>
<td>0.03</td>
<td>0.14</td>
<td>0.34</td>
<td>0.64</td>
<td>0.18</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>7 (rs301979)</td>
<td>0.04</td>
<td>0.36</td>
<td>0.18</td>
<td>0.29</td>
<td>0.87</td>
<td>1.00</td>
<td>0.28</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>8 (rs301434)</td>
<td>0.11</td>
<td>0.19</td>
<td>0.07</td>
<td>0.14</td>
<td>0.47</td>
<td>0.06</td>
<td>0.93</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>9 (rs301443)</td>
<td>0.04</td>
<td>0.06</td>
<td>0.08</td>
<td>0.45</td>
<td>0.27</td>
<td>0.01</td>
<td>0.53</td>
<td>0.55</td>
<td></td>
</tr>
</tbody>
</table>

* \( D^2 \) values are shown below the blank cells and \( r^2 \) values are shown above the blank cells.
conservation found at its location, it is unlikely that it is contributing to OCD susceptibility in this family. Further study is needed to determine whether this deletion is altering the function or expression of C9ORF68 or other genes in the region.

Caution should be exercised in interpreting the nominally significant association findings at rs3780412, rs301430, and rs301979. First, use of the TDT, which tests for association in the presence of linkage, in multiplex families in a genomic region with evidence of linkage raises the possibility that linkage may confound a significant finding in the absence of true association. To assess whether this may be the case, we applied the FBAT with the exclusion of linkage effects. The resulting decrease in significance were proportional to the loss of power, suggesting that linkage does not solely account for the finding. Second, because of the strong evidence for SLC1A1 as a positional and functional candidate gene for OCD, a correction for multiple testing was not performed. The most conservative correction at this locus, the Bonferroni method, would eliminate the significant findings in the overall sample but leave significant findings in the group of male probands. Correcting for association testing across the genome, which has not been pursued in this sample, would eliminate any significant findings whatsoever. However, these nominally significant findings must be considered in the context of low power to detect the relatively weak association expected at a marker in a complex genetic disorder. The parallel findings in the accompanying study by Arnold and colleagues also support the case for SLC1A1 as a primary candidate gene in OCD.

These association findings in 2 studies present a challenge for additional research on the possible involvement of SLC1A1 in OCD. None of the 5 SNPs associated in one or the other study seems likely to itself confer susceptibility. Four of the SNPs (rs3780412, rs301979, rs301434, and rs301435) are located in introns, and 1 SNP (rs301430) is a synonymous coding SNP. In addition, no single SNP was significantly associated in both studies, despite both studies genotyping at least 1 SNP that was associated in the other study. Therefore, to account for these findings, functional variation in this region must be in varying degrees of LD with these SNPs in different populations or multiple functional variants must be present and account for the association with different SNPs. Similar patterns of association findings at different markers across populations have been noted at other genes implicated in psychiatric disorders, such as the serotonin transporter gene in autism, where multiple common and uncommon functional alleles have been reported. Compared with previous studies, the present study and that of Arnold and colleagues achieved improved coverage of the SLC1A1 gene region; however, the low intermarker LD in portions of this genomic region (Table 2) makes it difficult to clarify the extent of association. It may eventually be necessary to genotype all the common variation in this region to achieve adequate coverage. Further screening of the coding region and surrounding gene regulatory regions for uncommon variation is also warranted, although an initial screen in the probands from the original genome scan revealed no amino acid variation. Finally, expression in the renal tubules may allow assessment of potentially functional variation in SLC1A1 by correlation with urinary levels of aspartate and glutamate.

Association findings at SLC1A1 add to existing genetic, neuroimaging, and neurochemical evidence of glutamatergic dysfunction in OCD. Identification of functional variation is necessary to clarify the relationship between SLC1A1 and the glutamate abnormalities in OCD; however, the regional distribution and function of EAAC1 in the brain match neuroimaging and neurochemical findings. The glutamate transporter EAAC1 shows particularly high expression in the striatum but is also expressed postsynaptically on glutamatergic and γ-aminobutyric acidergic neurons throughout the cortex, including the cingulate cortex. Antisense RNA to SLC1A1 substantially decreases γ-aminobutyric acid synthesis in the rat hippocampus, suggesting that EAAC1 uptake may provide a pool of glutamate for conversion to γ-aminobutyric acid. Some evidence suggests that tonic glutamatergic signaling from the ventral prefrontal cortex...
nal cortex, which includes the cingulate cortex, decreases phasic activity in the striatum, which may explain the inverse relationship in pediatric OCD between the glutamatergic signal in the cingulate cortex, where it is decreased, and in the caudate, where it is increased.23,24,49 The role of EAAC1 in glutamate reuptake and γ-aminobutyric acid synthesis makes it a natural candidate protein as part of the putative tonic-phasic dysregulation in OCD. Altered SLC1A1/EAAC1 function could also be involved in perturbation of the glutamate system in other psychiatric disorders frequently comorbid with OCD, especially mood disorders,50 where changes in the glutamate system have been observed.23,51,52

Our finding of association confined to male probands supports a body of literature on sex differences in OCD. Males with OCD differ from females with OCD in terms of clinical presentation and course. Males are more likely to have a childhood onset, a chronic course of disease, and comorbid tic disorder or attention-deficit/hyperactivity disorder.9,33-37 Consistent with clinical differences, segregation analyses suggest that the inheritance of OCD is affected by sex effects.38,39 A variety of previous genetic association studies60-64 have identified association in one chromosome in disease, but it could also reflect general hormonal or developmental sex differences.

In conclusion, we genotyped 9 SNPs spanning the glutamate transporter gene SLC1A1 in a family-based association study of early-onset OCD and identified nominal evidence of association at 2 of the markers examined, rs3780412 and rs301430, and a haplotype that includes a third marker, rs301979. The association was stronger in males, supporting a sex-specific contribution to OCD susceptibility. This evidence supports a role for SLC1A1 in the etiology of early-onset OCD and adds further support to a role for glutamate dysfunction in the pathogenesis of this disorder.23-25 Analyses of possible associations with the examined markers in subtypes of OCD are warranted. We also discovered an uncommon 11-bp deletion in the 3′ flanking region of the gene in 10 members of a multigenerational family. Further work is needed to determine whether this deletion is contributing to OCD susceptibility in this family.

Submitted for Publication: August 4, 2005; final revision received December 12, 2005; accepted December 29, 2005.

Correspondence: Jeremy Veenstra-VanderWeele, MD, Center for Molecular Neuroscience, Medical Research Building III, Vanderbilt University Medical Center, 465 21st Ave S, Nashville, TN 37232 (jeremy.veenstra-vanderweele@vanderbilt.edu).

Funding/Support: This work was supported in part by grants K02 MH01389 (Dr Cook), K20 MH01065 (Dr Hanna), and R01 MH58376 (Dr Hanna) from the National Institutes of Health; the Jean Young and Walden W. Shaw Foundation (Dr Leventhal); the Harris Foundation (Dr Leventhal); the Brain Research Foundation (Dr Cook); and the Obsessive Compulsive Foundation (Drs Cook and Hanna).

Acknowledgment: We thank Kathy Hennessy and Cheryl Roe, MS, for their expert technical assistance and the families who participated in this study.


34. Pauls DL, Hurst CR. Schedule for Tourette and Other Behavioral Syndromes. New Haven, Conn: Child Study Center, Yale University School of Medicine; 1991.


52. McCullumsmith RE, Meador-Woodruff JH. Brainlactal excitatory amino acid trans- porter transcript expression in schizophrenia, bipolar disorder, and major de- pressive disorder. Neuropsychopharmacology. 2002;26:368-375.


