Family-Based Association Testing of OCD-Associated SNPs of *SLC1A1* in an Autism Sample

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Reports identified the neuronal glutamate transporter gene, *SLC1A1* (OMIM 133550, chromosome 9p24), as a positional and functional candidate gene for obsessive–compulsive disorder (OCD). The presence of obsessions and compulsions similar to OCD in autism, the identification of this region in a genome-wide linkage analysis of individuals with autism spectrum disorders (ASDs), and the hypothesized role of glutamate in ASDs make *SLC1A1* a candidate gene for ASD as well. To test for association between *SLC1A1* and autism, we typed three single nucleotide polymorphisms (SNPs, rs301430, rs301979, rs301434) previously associated with OCD in 86 strictly defined trios with autism. Family-Based Association Tests (FBAT) with additive and recessive models were used to check for association. Additionally, an rs301430–rs301979 haplotype identified for OCD was investigated. FBAT revealed nominally significant association between autism and one SNP under a recessive model. The G allele of rs301979 was undertransmitted (equivalent to overtransmission of the C allele under a dominant model) to individuals with autism ($Z = -2.47$, $P = 0.01$). The G allele was also undertransmitted in the T–G haplotype under the recessive model ($Z = -2.41$, $P = 0.02$). Both findings were also observed in the male-only sample. However, they did not withstand correction for multiple comparisons.

Keywords: autism; *SLC1A1*; OCD; association

Introduction

Autism is a neurodevelopmental disorder characterized by deficits in reciprocal social interaction, communication, and presence of restricted and repetitive behaviors with an onset of symptoms by 3 years of age (*Diagnostic and Statistic Manual*, DSM-IV). Twin studies indicate that autism is a predominately genetic disorder with estimates of concordance for autism being much higher among monozygotic twins, 64–91%, than dizygotic twins and siblings, 0–9% [Bailey et al., 1995; Bolton et al., 1994; Smalley, Asarnow, & Spence, 1988; Steffenburg et al., 1989]. A number of genetic variants, including copy number variants, interact together to contribute to autism risk with common variants at certain loci acting as ''hits'' contributing to susceptibility to the disorder [Veenstra-VanderWeele & Cook, 2004]. One strategy for selecting those genes that may be candidate genes is to draw on genetic association findings from disorders that share a phenotype similar to autism. The heterogeneity of autism spectrum disorders (ASDs) [for a review see Geschwind & Levitt, 2007] makes this a difficult task, but also supports the validity of the model, which requires more than one genetic variant to contribute to the full syndrome of autism.

Many individuals with ASDs manifest obsessions and compulsions similar to those seen in individuals with obsessive–compulsive disorder (OCD) [Bodfish, Symons, Parker, & Lewis, 2000; Leyfer et al., 2006; McDougle et al., 1995; Russell, Mataix-Cols, Anson, & Murphy, 2005]. In fact, some types of obsessive–compulsive behaviors, including ordering, hoarding, touching, and tapping, occur more often in individuals with autism than in age- and sex-matched controls with OCD (without tics) [McDougle et al., 1995]. These behaviors fall under the restricted and repetitive behavior domain of autism, which includes restricted interests, unusual preoccupations, rituals, and insistence on sameness.

For an individual to be diagnosed with OCD, many of these same traits are present. According to DSM-IV, the patient must have either recurrent thoughts (obsessions) that cause marked distress that he or she attempts to suppress or neutralize with another thought or action, or

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repetitive behaviors or mental acts (compulsions) that the person feels driven to perform in response to an obsession or rules that must be applied rigidly and that are aimed at preventing or reducing distress. The persistent thoughts are not simply excessive worries about real-life problems. For a diagnosis to be made, the obsessive–compulsive symptoms must cause marked distress, take more than 1 hr a day, or significantly interfere with the person's daily activities or relationships. Like autism, OCD has been characterized as a heterogeneous disorder with three to five symptom factors that may differ in their etiology [Mataix-Cols, Rosario-Campos, & Leckman, 2005; Stewart et al., 2007b]. Recently, it has been suggested that there may be an autistic subtype of individuals with OCD who are more resistant to treatment due to less insight into their disorder [Bejerot, 2007]. These findings build on previous reports of self-reported and observed autistic-like behaviors, including social deficits, in individuals with OCD [Bejerot, Nylander, & Lindstrom, 2001].

OCD-like traits are part of the broad autism phenotype (i.e. a cluster of behaviors similar to those in individuals with autism often observed in family members of autistic individuals who lack a diagnosis), which suggests that these behaviors may be heritable [for reviews see Bailey, Palferman, Heavey, & Le Couteur, 1998; Murphy et al., 2000]. Relatives of individuals with autism are more likely than relatives of individuals with other developmental disorders to have OCD [Bolton, Pickles, Murphy, & Rutter, 1998; Micali, Chakrabarti, & Fombonne, 2004]. Among affected sibling pairs, two sub-domains of the Autism Diagnostic Interview—Revised [ADI-R, Lord, Rutter, & Le Couteur, 1994], which relate to OCD (''encompassing preoccupations/circumscribed interests'' and ''apparently compulsive adherence to nonfunctional compulsions/rituals''), show strong familiality [Silverman et al., 2002]. Further, genetic studies have shown greater evidence for linkage in families with autism with higher insistence on sameness scores on chromosome 15q11–q13 (*GABRB3*) [Shao et al., 2003], greater obsessive–compulsive behaviors on chromosome 1 at the marker D1S1656 [Buxbaum et al., 2004], and increased rigid–compulsive behavior at chromosome 17q11.2 [Sutcliffe et al., 2005].

Recently, *SLC1A1* was identified as a candidate gene for both OCD and autism. Located on chromosome 9p24, *SLC1A1* is a neuronal glutamate transporter expressed in the brain. This chromosomal region demonstrated suggestive linkage in a genome-wide scan of seven, large extended pedigrees of probands with early onset OCD [Hanna et al., 2002]. This finding was replicated in 42 pedigrees with early onset OCD [Willour et al., 2004]. Subsequently, three studies have reported an association between markers at *SLC1A1* and OCD [Arnold, Sicard, Burroughs, Richter, & Kennedy, 2006; Dickel et al., 2006; Stewart et al., 2007a]. The Autism Genome Project, a large-scale, collaborative autism genetics research project, identified *SLC1A1* in a genome-wide linkage scan of autism due to its proximity to a linkage peak at 9p24.1, and its role in glutamate function [Szatmari et al., 2007]. Both genome-wide analyses of OCD and autism detected these findings in individuals with ''narrow'' or stricter diagnostic criteria, and in families that contained female probands with autism and OCD.

In addition to being a positional candidate for OCD and autism, *SLC1A1* is a functional candidate due to its role in coding for the neuronal glutamate transporter excitatory amino acid carrier 1. There is suggestive evidence that glutamate plays a role in the pathology of autism [McDougle, Erickson, Stigler, & Posey, 2005]. For example, when compared to controls male adults with autism show an increase in glutamate, but not other amino acids (i.e. glutamine, glycine, D-serine, and L-serine) in serum [Shinohe et al., 2006]. In addition, for probands with ASDs and their first-degree relatives, increased serum glutamic acid has also been found [Aldred, Moore, Fitzgerald, & Waring, 2003]. Other studies have reported increased amygdala–hippocampal glutamate in individuals with ASDs when compared to controls using in vivo proton-magnetic resonance spectroscopy [Page et al., 2006], and abnormalities in the mRNA expression of members of the glutamate system in postmortem brains of individuals with autism [Purcell, Jeon, Zimmerman, Blue, & Pevsner, 2001].

Taken together, these reports suggest that *SLC1A1* is a viable positional and functional candidate gene for autism, especially given the phenotypic similarities between autism and OCD. To test whether *SLC1A1* is associated with autism, we typed three single nucleotide polymorphisms (SNPs, rs301430, rs301979, and rs301434) in the 3′ region of the gene. The aim of the study was to examine if previously associated SNPs in *SLC1A1* were associated with autism. Of note, these three SNPs were associated with OCD in one or more of the previous association studies [Arnold et al., 2006; Dickel et al., 2006; Stewart et al., 2007a]. We tested each marker and a previously identified haplotype (rs301430–rs301979) for association in a strictly defined autism sample. Because each of the previous reports found a stronger association among male than female probands for at least one marker tested, we also repeated the analyses in a male-only sample. However, an alternative strategy would be to study *SLC1A1* in autism from a wider, less hypothesis-driven approach.

Method

Subjects and Assessment

This study was approved by the University of Illinois at Chicago Institutional Review Board. After complete description of the study to the parents, written informed consent was obtained.

Recruitment, assessment, and inclusion criteria were the same as that outlined in a previously described sample [Kim et al., 2002], but the sample was reduced because only one sibling was randomly selected from each affected sibling pair. The research participants: (1) were at least 3 years old at the time of ADI-R administration, (2) had sufficient blood or DNA available, and (3) met ADI-R [Lord et al., 1994] and ADOS (Autism Diagnostic Observational Schedule) classification for autistic disorder [Lord et al., 2000]. In addition, all participants had a best estimate diagnosis of autistic disorder by a clinical psychologist and child psychiatrist. There were 86 probands, 68 males and 18 females. Parents identified the proband ethnicity as follows: 70 Caucasian, 5 African American, 4 Hispanic/Latino, and 7 Asian. Probands' age at the time of the ADI-R ranged from 38 to 232 months ($M = 83$, SD = 45).

Genotyping of SNPs

Three SNPs were genotyped by TaqMan® SNP genotyping assays (Applied Biosystems, Foster City, CA) (Table IS). The standard TaqMan® SNP genotyping assay protocol was observed for polymerase chain reactions (PCRs), which contained 10 ng of dry DNA, 2.5 μL of 2 × TaqMan Universal Master Mix (Applied Biosystems, Foster City, CA), 0.25 μL of 20 × SNP Genotyping Assay Mix (Applied Biosystems, Foster City, CA), and 2.25 μL of water, for a total volume of 5 μL. All PCRs were performed using a GeneAmp® PCR System 9700 Thermocycler (Applied Biosystems, Foster City, CA) under the following conditions: 1 enzyme activation step at 95.0°C for 10 min, and 50 alternating cycles of denaturation at 92.0°C for 15 s and reannealing and extension at 60.0°C for 90 s. The fluorescence intensity of the final reaction product was measured using a Fluoroskan Ascent microplate fluorometer (Thermo Fisher Scientific, Inc., Waltham, MA).

All genotyping was performed blind to clinical and demographic data as well as family relationships. Three family trios were dropped from the analyses because a sample from each trio (one parent, two probands) repeatedly failed genotyping for all three markers. We also identified Mendelian errors from two family trios (one family had two Mendelian errors for both rs301434 and rs301979 and another family had a Mendelian error for rs301434). We confirmed this finding by independent genotyping. We suspect that the family with two errors may have a possible deletion for at least 3′ region of *SLC1A1* as the affected proband was homozygous for rs301434 (G/G, mother G/G, father A/A), rs301979 (G/G, mother C/G, father C/C), and rs301430 (T/T, mother C/T, father T/T). Although it is possible that this is a case of nonpaternity, this is inconsistent with genotypes from this pedigree reported in other studies. The other proband was heterozygous for rs301434; both parents were homozygous (G/G), which is puzzling since this family also did not have any inconsistency in genotypes from other studies.

Statistical Analyses

The distributions of the genotypes were tested using χ^2 for the Hardy–Weinberg equilibrium (HWE) using the program from the LINKUTIL package (http://linkage.rockefeller.edu/ott/linkutil.htm). Haploview (version 4.0, http://www.broad.mit.edu/mpg/haploview/index.php) was used to identify Mendelian errors [Barrett, Fry, Maller, & Daly, 2005]. Single tests of association under additive and recessive models were assessed using the Family-Based Association Tests (FBAT) program (v. 1.7.1) [Rabinowitz & Laird, 2000]. Recessive models were included because they were used in at least one of the OCD association studies [Arnold et al., 2006]; the additive model of FBAT is presented for comparison. It should be noted that because each SNP is biallelic, the significance of the results for the recessive model is the same as the dominant model (the *Z*-scores for the alleles are flipped and in the opposite direction). In this study, the FBAT empirical variance (''-e'') option was not used to test for association in the presence of linkage because the probands were not related (only one sibling was randomly selected from each affected sibling pair). We also used the ''hbat'' function in FBAT to calculate haplotype estimations of the previously reported rs301430–rs301979 haplotype and pairwise linkage disequilibrium (LD) between the SNPs under additive, dominant, and recessive models. The ''mode a'' option was used to obtain test statistics for the individual haplotypes and as a global test (www.biostat.harvard.edu/~fbat/default.html). The genotypes for the pedigrees with Mendelian errors were set to missing for those markers. α was set at $P < 0.05$.

Results

Parental genotypes at each marker were consistent with the Hardy–Weinberg equilibrium: rs301430 ($\chi^2 = 0.38$, d.f. = 1, $P = 0.54$), rs301979 ($\chi^2 = 0.52$, d.f. = 1, $P = 0.47$), and rs301434 ($\chi^2 = 1.30$, $P = 0.25$). Proband genotypes were consistent with HWE for rs301430 ($\chi^2 = 0.07$, d.f. = 1, $P = 0.79$) and rs301434 ($\chi^2 = 0.01$, d.f. = 1, $P = 0.93$), but not rs301979 ($\chi^2 = 4.17$, d.f. = 1, $P = 0.04$). Table IIS shows the allele and genotype frequencies in the parents and the probands. The lower frequency G allele of rs301979 was undertransmitted to individuals with autism under the FBAT recessive mode ($Z = -2.47$, $P = 0.01$) (Table I). This is equivalent to overtransmission of the C allele under a dominant model. The undertransmission of the G allele was also detected in males only under both the FBAT additive ($Z = -2.18$, $P = 0.03$)

Table I. Single Marker Associations with Autism using FBAT

SNP	Model of inheritance[a]	Allele	Families[b]	S[c]	$E(S)$[d]	Z[e]	P[f]
rs301430	Additive	C	56	43	42	0.24	0.81
	Recessive	C	22	8	7	0.47	0.64
		T	50	21	21	0.00	1.00
rs301979	Additive	C	55	78	71	1.70	0.09
	Recessive	C	50	24	21.75	0.66	0.51
		G	18	1	5.75	−2.47	**0.01**
rs301434	Additive	A	59	65	59.5	1.27	0.20
	Recessive	G	37	11	14.5	−1.22	0.22
		A	38	17	15	0.69	0.49

Bold indicates $P<0.05$.

[a]Note for the additive model, the overtransmitted allele is shown. The test statistic and significance level for the other allele are the same values (Z-score in the opposite direction) because these are biallelic markers. The corresponding Z-score and P-values for each allele of a biallelic marker under a dominant model are the reverse of those of the recessive model (Z-score in the opposite direction).

[b]Number of informative families (i.e. families with a nonzero contribution of the test statistic).

[c]S, test statistics for the observed number of transmitted alleles.

[d]$E(S)$, expected value of S under the null hypothesis (i.e. no linkage or association).

[e]$Z = (S-E(S))/(\text{var}(S))$.

[f]P, two-tailed.

FBAT, Family-Based Association Test; SNP, single nucleotide polymorphism.

(equivalent to overtransmission of the C allele under the additive model) and the recessive ($Z=-2.53$, $P=0.01$) models. Neither of the other SNPs showed significant association in the full or male-only samples (Ps>0.15). LD between rs301430 and rs301979 was $r^2=0.21$ ($D'=1.00$), between rs301430 and rs301434 was $r^2=0.00$ ($D'=0.04$), and between rs301979 and rs301434 was $r^2=0.31$ ($D'=0.87$).

Haplotype testing between rs301430 and rs301979 revealed a trend for the T/G haplotype to be undertransmitted to probands, but only under the recessive model ($\chi^2=7.41$, d.f. = 3, $P=0.06$) (Table IIIS). This was not the case for the additive model ($\chi^2=3.36$, d.f. = 2, $P=0.19$) or dominant model ($\chi^2=1.16$, d.f. = 3, $P=0.76$). Because the sample size was relatively small, we also ran haplotype permutation tests. Using this approach, the recessive model was nominally significant ($P<0.05$), but the additive ($P=0.19$) and dominant ($P=0.90$) were not. In the male-only sample, the same trend appeared for the T/G haplotype to be undertransmitted to probands ($Z=-2.36$, $P=0.02$) under the additive model (global test: $\chi^2=5.68$, d.f. = 2, $P=0.06$). There were too few observations under the recessive model to include the T–G haplotype in the global tests. Global permutation tests in the male-only sample approached significance under the additive model ($P=0.07$) and was significant under the recessive model ($P=0.04$).

Discussion

We tested three SNPs (rs301430, rs301979, rs301434) in *SLC1A1* previously identified for association with OCD in an autism sample. One SNP (rs301979) was associated with autism under a recessive model. The lower frequency G allele was undertransmitted to individuals with autism. This is equivalent to overtransmission of the C allele under the dominant model. A haplotype previously identified for association with OCD [Dickel et al., 2006], including this SNP (rs301430–rs301979), was also significant under a recessive model. The T–G haplotype type was undertransmitted to individuals with autism. These findings were found in the male-only sample. Although there was a departure from HWE for rs301979 in the probands, we do not think this contributed to the association findings as the parental genotypes did not differ from HWE. This departure was likely due to the undertransmission of the G allele of this SNP [Wittke-Thompson, Pluzhnikov, & Cox, 2005].

Overall, our findings are similar to the recent reports of association between *SLC1A1* and OCD in terms of observed allele frequencies, number of informative families, and strength of findings. Dickel et al. [2006] reported that two of the nine SNPs they tested, including rs3780412 from the Arnold paper ($P=0.04$) and rs301430, which showed significant overtransmission of the C allele ($P=0.03$), were significantly associated with OCD. Only rs3780412 was associated with OCD when they restricted their sample to males only. The effect of the rs301430–rs301979 haplotype was stronger in the male-only ($P=0.003$) than in the combined sample ($P=0.03$). The T–C haplotype, in contrast to the T–G haplotype was undertransmitted in these samples. In fact, the T–G haplotype showed a trend towards overtransmission in the male-only sample (16 transmitted vs. 7 untransmitted, $P=0.06$). Stewart et al. [2007a] reported no significant association with OCD for any single marker when both sexes were combined (Ps>0.05). However, two other SNPs (including rs3780412) showed association in the male-only sample (Ps$=0.045$), and a significant 3-marker haplotype including rs301430 was reported for both the combined and male-only sample (Ps<0.01).

Arnold et al. [2006] reported that three of the nine SNPs they tested were significantly associated with OCD including rs301434, which showed overtransmission of the G allele under the additive and recessive models (Ps$<.01$). A haplotype with this marker was also associated with OCD. These findings replicated in the male-only sample, which also showed that the G allele of rs301979 was marginally overtransmitted ($P=0.07$). One limitation in comparison of the OCD studies and this study is that the OCD studies did not report population origin of samples.

Given the trends for association in this region, one limitation of this study is that we typed only three SNPs in *SLC1A1*. Notably, we typed three of the four SNPs that were common in the previous OCD studies. It is possible that a susceptibility locus for autism lies within this region that may or may not be in high LD with one of our SNPs. This possibility may be further explored by genotyping additional markers in this region. Future efforts may also be directed at exploring the possible copy number variations (CNVs) observed in one trio using other methods, such as Quantitative PCR or Multiplex Ligation-dependent Probe Amplification.

A second limitation of this study is the sample size, especially with regard to the endophenotype. It would have been ideal to select a sample in which all the probands showed OCD-like behaviors. Half of high-functioning individuals with autism have symptoms of OCD [Russell et al., 2005], and OCD was the second most prevalent (37%) co-morbid disorder in children and adolescents with autism assessed using an adaptation of the Kiddie Schedule for Affective Disorders and Schizophrenia [Leyfer et al., 2006]. Nevertheless, it is likely that some of our sample of individuals with autism did not show any symptoms of OCD. In addition, the study of a larger sample (e.g. Autism Genome Project samples) would allow testing of possible effects of population differences in allele frequencies or associated variants.

It is unlikely that all individuals with autism share all of the same genetic variants. A plausible alternative is that individuals with similar phenotypes share similar genetic risks for the disorder. This idea is supported by behavioral evidence that parents of individuals with autism who score high on repetitive behaviors, as measured by the restricted interests and compulsive behavior (D1, D2) subdomains on the ADI-R, have higher rates of OCD than parents of individuals with ASDs with lower scores on these domains [Hollander, King, Delaney, Smith, & Silverman, 2003]. Although the ADI-R is a valid instrument for diagnosing autism, it was not designed as a rating scale and its ability to capture specific symptoms of OCD is limited [Lord et al., 1994]. Using other measures such as the Yale–Brown Obsessive Compulsive Scale, which has been validated with both OCD [Scahill et al., 1997] and autism [Scahill et al., 2006] samples, or the Repetitive Behavior Scale—Revised [Lam & Aman, 2007], to select individuals with high OCD symptoms may strengthen the association between *SLC1A1* and ASDs.

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