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Glutamate system genes associated with ventral prefrontal and thalamic volume in pediatric obsessive-compulsive disorder

Paul Daniel Arnold^a, Frank P. MacMaster^b, Gregory L. Hanna^c, Margaret A. Richter^d, Tricia Sicard^e, Eliza Burroughs^d, Yousha Mirza^b, Phillip C. Easter^b, Michelle Rose^b, James L Kennedy^e, and David R Rosenberg^b

^a Program in Genetics and Genomic Biology and Department of Psychiatry, Hospital for Sick Children, Toronto, ON, Canada

^b Wayne State University and the Children's Hospital of Michigan, Detroit, MI

^c University of Michigan, Ann Arbor MI

^d Anxiety Disorders Clinic, Centre for Addiction and Mental Health and University of Toronto, Toronto, ON, Canada

^e Neurogenetics Section, Centre for Addiction and Mental Health, University of Toronto, Toronto, ON, Canada

Abstract

This pilot study was undertaken to determine if there was a significant association between specific glutamate system genes and regional volumes of interest implicated in the pathogenesis of obsessive-compulsive disorder (OCD). Volumetric magnetic resonance imaging (MRI) and genotyping of 7 polymorphisms in two genes, glutamate receptor, ionotropic, N-methyl-D-aspartate 2B (*GRIN2B*) and solute linked carrier, family 1, member 1 (*SLC1A1*) were conducted in 31 psychotropic-naïve pediatric OCD patients. The rs1805476 variant of *GRIN2B* was associated with left but not right orbital frontal cortex (OFC) ($p=0.04$) and right but not left anterior cingulate cortex (ACC) volume ($p=0.02$). The *SLC1A1* rs3056 variant was associated with increased total ($p=0.01$), left ($p=0.02$) and right ($p=0.02$) thalamic volume. These results suggest that *GRIN2B* and *SLC1A1* may be associated with regional volumetric alterations in OFC, ACC, and thalamus in children with OCD.

Keywords

Obsessive-compulsive disorder; genetic association; glutamate; orbitofrontal cortex; anterior cingulate cortex; thalamus

INTRODUCTION

Obsessive-compulsive disorder (OCD) is a debilitating neuropsychiatric condition affecting an estimated 1 to 3% of the population (Rasmussen & Eisen, 1994). The National Comorbidity Survey Replication found a median age of onset in OCD of 19 years, with 21% of cases having onset by age 10 (Kessler et al. 2005). Evidence from large controlled family studies of both adult (Nestadt et al. 2000; Pauls et al. 1995), and pediatric probands (do Rosario-Campos et al. 2005; Hanna et al. 2005) suggest that OCD aggregates in families, particularly in families of early onset probands. In addition, twin studies indicate a high additive genetic contribution to dimensionally measured obsessive-compulsive symptoms, ranging from 27–47% in adults to 45–65% in children (as reviewed in (van Grootheest et al. 2005)). Taken together, the family and twin studies suggest a robust genetic contribution to the disorder.

Candidate gene studies in OCD, largely focused on serotonin receptor and transporter genes, have been inconclusive. This has prompted a search for new candidate genes based on novel etiological hypotheses. One hypothesis points to altered glutamate neurotransmission in OCD (Carlsson, 2000; Pittenger et al. 2006; Rosenberg & Keshavan, 1998; Rosenberg et al. 2001). Glutamate is the primary excitatory neurotransmitter within cortico-striatal-thalamic circuits (CSTCs) implicated in OCD (Bronstein & Cummings, 2001). Evidence for altered glutamate activity is accruing from multiple studies using proton magnetic resonance spectroscopy (1H-MRS), cerebral spinal fluid (CSF), animal models and application of medications that modulate glutamate. First, a 1H-MRS study found greater left striatal glutamatergic (Glx) concentrations in psychotropic-naïve, non-depressed pediatric patients with OCD than in age and sex matched healthy controls (Rosenberg et al. 2000a). Left striatal Glx concentrations normalized after 12 weeks of paroxetine therapy (Rosenberg et al. 2000a) but not with cognitive-behavioral therapy (Benazon et al. 2003). The decrease in left striatal Glx with SSRI treatment was positively correlated with reduction in OCD symptom severity. Furthermore, greater Glx to creatine (Cr) ratios have been noted in orbital frontal white matter in OCD patients as compared to controls (Whiteside et al. 2006). In contrast, lower Glx concentrations were observed in the anterior cingulate cortex of non-depressed children with OCD compared with healthy pediatric controls (Rosenberg et al. 2004). This indicates that changes in glutamate activity are regional within the CSTC. Second, CSF concentrations of glutamate were also noted to be elevated in OCD as compared to controls (Chakrabarty et al. 2005). Third, mouse models have indicated alterations in regional glutamate activity that correlate with behavior analogous to obsessive-compulsive symptoms (Nordstrom & Burton, 2002; Welch et al. 2007). Finally, the application of novel glutamate modulating agents, such as riluzole, has shown promise in alleviating OCD symptoms in a case report (Coric et al. 2003) and open label trials in adults (Coric et al. 2005) and children (Grant et al. 2007). Therefore, converging lines of evidence from neuroimaging, genetics, animal models and clinical trials suggest that a glutamatergic abnormality may play a critical role in the pathogenesis of OCD.

This evidence has led to the search for glutamate related genes that may be associated with OCD. We recently reported variants within two glutamate system genes to be associated with OCD. First, an association between OCD and variants of the glutamate subunit receptor gene *GRIN2B* (glutamate receptor, ionotropic, N-methyl-D-aspartate 2B), a gene encoding a subunit of the NMDA receptor was reported (Arnold et al. 2004). A positive association between variants in the 3'-untranslated region (3'-UTR) of *GRIN2B* was identified in three independently ascertained samples consisting of a total of 255 small OCD families of both adult and pediatric probands. In addition, rs1019385, a variant in the promoter region was found to be associated with OCD in the total sample (Arnold, 2007). This same variant was also associated with decreased anterior cingulate glutamatergic concentration (Glx) in a preliminary study of 18 psychotropic-naïve pediatric patients with OCD (Arnold et al. 2007). In a genome scan based on large multigenerational families with OCD ascertained through pediatric probands, suggestive linkage was found in chromosome 9p24 (Hanna et al. 2002), a finding which was subsequently replicated (Willour et al. 2004). The 9p24 region contains the neuronal glutamate transporter gene *SLC1A1* (solute linked carrier, family 1, member 1). An association between *SLC1A1* variation and OCD, particularly in males, was reported simultaneously in two independent samples using family-based association methods (Arnold et al. 2006; Dickel et al. 2006) and more recently replicated in a third sample (Stewart et al. 2007). Both *GRIN2B* and *SLC1A1* are highly expressed within the fetal compared with the adult human brain, and in mature brain tissue these genes are highly expressed in brain regions implicated in the pathogenesis of OCD (Su et al. 2002). What remains unknown is the influence these genetic variants have on the CSTC in OCD patients.

The findings of association with sequence variation in *GRIN2B* and *SLC1A1* were based on the phenotype of DSM-IV OCD diagnosis. However, OCD is a complex, etiologically

heterogeneous genetic disorder that is believed to involve multiple interacting genetic and environmental factors. One approach to minimize this complexity is to study biologically salient intermediate phenotypes, quantitative traits that are correlated with the disorder in question but may be more closely linked to the action of genes compared with complex behaviors (Gottesman & Gould, 2003; Meyer-Lindenberg & Weinberger, 2006; Turner et al. 2006). Brain imaging profiles represent particularly promising intermediate phenotypes (Meyer-Lindenberg & Weinberger, 2006; Rosenberg & Hanna, 2000), as exemplified by studies in healthy volunteers demonstrating large effect size for candidate gene variants on structural (e.g. (Szeszko et al. 2005) and functional (Hanna et al. 2002; Hariri et al. 2005) neuroimaging measures. Neuroimaging findings of differences in cortico-striatal thalamic circuits between OCD individuals and controls, therefore, may provide valuable intermediate phenotypes for identification of susceptibility genes.

Given the mounting evidence for glutamate involvement in OCD pathogenesis, we were interested in testing the *GRIN2B* and *SLC1A1* polymorphisms we had previously identified in family-based candidate gene studies of OCD for association with neuroimaging phenotypes. Here we focused on three brain regions previously implicated in both volumetric magnetic resonance imaging (MRI) and 1-H MRS studies of OCD, including the orbital frontal cortex (OFC) (Szeszko et al. 1999, Whiteside et al. 2006), anterior cingulate cortex (Rosenberg and Keshavan, 1998; Szeszko et al. 2004, Rosenberg et al. 2004), and caudate (Rosenberg et al. 1997, and Rosenberg et al. 2000a). We also examined a fourth region, the thalamus based on strong evidence of volumetric differences in our own MRI studies of pediatric probands compared with healthy controls (Gilbert et al. 2000; Smith et al. 2003; Mirza et al. 2006). In this pilot study, we report on our initial findings based on volumetric magnetic resonance imaging (MRI) in pediatric psychotropic-naïve probands.

MATERIALS AND METHODS

1. Subjects

The study group consisted of 31 (18 male, 13 female) medication-naïve children and adolescents with OCD, ranging from 7 to 18 years of age (mean = 11.8 years, s.d. = 3.1). Medication-naïve, pediatric subjects were selected to avoid confounding of MRI results due to chronic illness and pharmacotherapy. None of the patients were receiving cognitive-behavioral therapy at the time of participation. Patients with a history of significant debilitating medical or neurologic conditions, major depressive disorder, bipolar disorder, psychosis, substance use or dependence, eating disorders, attention deficit hyperactivity disorder, IQ < 80, pervasive developmental disorder, learning disorders or tic-related conditions were excluded. Written informed consent was obtained from all subjects. All patients were administered the Schedule for Affective Disorders and Schizophrenia – School-Age Children (Kaufman et al. 1997) and the Children's Yale-Brown Obsessive Compulsive Scale (CYBOCS) (mean = 25.6, s.d. = 7.3) (Scahill et al. 1997). All patients with OCD had a CYBOCS score of at least 17 consistent with significant dysfunction. Other clinician-administered instruments included (mean ± S.D. in parentheses): Hamilton Anxiety Rating Scale (HAM-A, (Hamilton, 1959)) (7.72 ± 4.71), Hamilton Depression Rating Scale (HAM-D; (Hamilton, 1967)) (7.93 ± 5.52), and the Yale Global Tic Severity Scale (YGTSS, Leckman et al, 1989) (2.50 ± 6.73). In order to determine IQ, the following subscales of the WISC-III (Wechsler Intelligence Scale for Children-III, Wechsler 1991) are administered to all patients: Block Design, Vocabulary and Digit Span. If a child receives a score of less than 6 on any of these scales, the entire WISC-III is administered. An overall IQ of 80 was required for participation in this study.

2. Procedures

2.1. Imaging—Imaging data were collected at the Children's Hospital of Michigan Imaging Center using a Sigma 1.5-Tesla unit (Horizon LX software, General Electric Medical Systems, Milwaukee, WI). Scanning methods, image acquisition and analysis procedures used to obtain structural magnetic resonance imaging (MRI) have been described in detail elsewhere (Benazon et al. 2003; Mirza et al. 2004; Rosenberg et al. 2000a; Rosenberg and Keshavan 1998; Rosenberg et al. 1997; Rosenberg et al. 2000b; Rosenberg et al. 2004; Szeszko et al. 2004). A 3-dimensional spoiled gradient echo pulse sequence obtained 124 1.5-mm-thick contiguous coronal images. Parameters were: echo time=5 milliseconds, repetition time=25 milliseconds, acquisition matrix=256 × 256 pixels, field of view=24 cm, and flip angle=40°. Well-trained and reliable operators, blinded to the subject's group membership and other identifying information, measured regional brain volumes in the coronal plane using the MEDx program using a manual tracing technique. Briefly, region of interest definitions are outlined below.

Orbital Frontal Cortex (OFC): The anterior boundary was the last appearance of the anterior horizontal ramus. The posterior boundary was the last appearance of the olfactory sulcus. The lateral boundary was the anterior horizontal ramus or circular sulcus of insula. The medial boundary was the olfactory sulcus (Szeszko et al. 1999). Intraclass correlation coefficient (ICC) values were 0.98 for the right OFC and 0.92 for the left OFC.

Anterior Cingulate Cortex: The boundaries of the anterior cingulate gyrus were as follows: The anterior boundary of the cingulate was the tip of the cingulate sulcus and the posterior border the connection of the superior and precentral sulci. The superior boundary was the cingulate sulcus and the inferior border was the callosal sulcus (Rosenberg and Keshavan, 1998; Szeszko et al. 1999). ICC values were 0.98 for the right ACC and 0.97 for the left ACC.

Caudate: For the anterior boundary, measurement of the caudate began when it was first visible rostrally. The posterior boundary was the point at which the tail of the caudate was no longer clearly visible. Special care was taken to exclude cerebrospinal fluid medially and to exclude the nucleus accumbens ventrally. The internal capsule, the nucleus accumbens, and the globus pallidus separated the putamen from the caudate (Rosenberg et al. 1997; Szeszko et al. 1999). The ICC values for measurement of the caudate were 0.91 for the right caudate, and 0.90 for the left caudate.

Thalamus: Right and left thalami were measured separately. The anterior boundary was the coronal slice with the mamillary bodies and interventricular foramen present. The posterior boundary was the coronal slice where the thalamus merged under the crux fornix. The internal capsule was considered the lateral boundary and the third ventricle the medial boundary. The superior boundary was the main body of the lateral ventricle and the inferior boundary was the hypothalamus (Gilbert et al. 2000). The ICC for right thalamus was 0.95 and 0.96 for left thalamus.

2.2. Genotyping—Genomic DNA was extracted from venous blood lymphocytes using a non-enzymatic, high salt method (Lahiri & Nurnberger, 1991). Four *GRIN2B* polymorphisms were selected for genotyping based on significant association findings in an ongoing Toronto family-based association study: rs1019385 (Arnold, 2007), rs890, rs1805476 and rs1805502 (Arnold et al. 2004). Similarly, three polymorphisms were selected for genotyping from *SLC1A1* based on significant association findings in the same Toronto sample: rs301434, rs3087879 (Arnold et al. 2006) and rs3056 (unpublished results).

Information regarding SNPs including position and minor allele frequency is shown in Table 1. Genotyping of *GRIN2B* polymorphisms was performed using Taqman® assays-by-design from Applied Biosystems Inc. (Foster City, CA), whereas genotyping of *SLC1A1* polymorphisms was performed using Applied Biosystems Inc. (Foster City, CA) assays-on-demand (ready-to-use TaqMan® probe-based SNP genotyping assays). For each reaction, 20ng genomic DNA was amplified as per manufacturer's directions in a total volume of 10ul in an MJ Research thermocycler (Bio-Rad Laboratories, Hercules, CA).

The post-read allelic discrimination option was used for analysis of plates on the ABIPrism 7000 Sequence Detection System (software v1.2.3). Genotypes were assigned automatically, following which visual inspection was performed for the purpose of confirmation and error checking. Probes and primer sequences are available by request. The genotyping methods used were identical to those used in our family-based association studies (Arnold et al. 2004, Arnold et al. 2006, Arnold 2007).

3. Statistical Analysis

For each variant, analyses were performed to test for differences in structural phenotypes including total volumes of the orbitofrontal cortex, anterior cingulate, caudate, and thalamus. Only total volume was initially analyzed, and the threshold for significance was set at $p < .05$. However, if a p value of $< .10$ was identified for a brain region, more detailed analysis was performed to determine laterality (left vs. right volumes). The number of tests was therefore minimized in order to reduce Type I error due to multiple comparisons, but no formal statistical correction was applied. Analysis was performed using Analysis of Covariance (ANCOVA) with age and total brain volumes as the covariates, since our prior published reports have found age-related alterations in intracranial volume (ICV) in children and adolescents with OCD vs. healthy pediatric controls (Szeszko et al. 2004, 2008a). Effect sizes (d) for significant analyses were calculated according to the guidelines outlined by Cohen (1988).

Given the small sample size and variable minor allele frequencies of the candidate polymorphisms tested, in many cases one of the homozygote groups had a small number of individuals, below that usually recommended for ANOVA-based methods. Therefore, when there were less than five individuals in a genotype group for a given analysis these subjects were grouped together with the heterozygote in the analysis. The approach of combining genotype groups due to small sample size is consistent with other studies examining intermediate phenotypes, (e.g. Papassotiropoulos et al. 2006; Szeszko et al. 2008b). Boxplots were constructed and inspected to ensure normal distribution of outcome variables and check for the presence of extreme values. As an added precaution, Levene's test was performed to evaluate the equality of error variances of independent variables between genotype groups. These checks were performed since normality and homogeneity of variance between groups are assumptions underlying ANCOVA (Tabachnik & Fidell, 2001). Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) package, version 15.

RESULTS

Data for genotype and regional brain volumes are presented in Tables 2 through 5 for orbitofrontal cortex, ACC, caudate, and thalamus. All results are covaried for age, and data are shown with and without covarying for total brain volume.

Orbitofrontal Cortex (OFC)

As shown in Table 2, there was a trend towards an association between *GRIN2B*-rs1805476 and OFC which did not reach statistical significance ($F=2.92$, $p=.10$). Separate analysis of left and right OFC revealed a significant association between left OFC volume and rs1805476

covaried by age ($F=4.85, p=0.04$), intracranial volume ($F=4.57, p=0.04$), but not when covaried for both age and intracranial volume ($F=3.57, p=0.07$). Specifically, having one or two copies of the A allele was associated with increased left OFC volume compared with the homozygote CC genotype (see Figure 1). The effect size (d) of this association between *GRIN2B*-rs1805476 and left OFC volume was 0.97. Results remained significant if outliers were removed. There was no significant association with right OFC volume ($F=.14, p=.71$). Genotype groups for rs1805476 did not differ with regard to OCD symptom severity (CYBOCS total, obsessions or compulsions scores), anxiety, depression, or tic severity. There were no significant associations between any of the other polymorphisms tested and OFC volume (Table 2).

Anterior Cingulate Cortex (ACC)

As shown in Table 3, there was a trend towards an association between *GRIN2B*-rs1805476 and total ACC volume which did not reach statistical significance ($F=3.30, p=0.08$). Separate analysis of left and right ACC resulted in a significant association between right ACC volume and rs1805476 covaried by age ($F=5.79, p=0.02$), and by age and intracranial volume ($F=4.47, p=0.04$). Specifically, having one or two copies of the A allele was associated with increased right ACC volume compared with the homozygote CC genotype (see Figure 2). The effect size (d) of this association between *GRIN2B*-rs1805476 and right ACC volume was 0.68. Results remained significant if outliers were removed. However, Levene's test for homogeneity of variance was statistically significant for the analysis of right ACC volume and rs1805476 genotype, indicating that the variance within the two genotype groups was significantly different. There was no significant association between rs1805476 and left ACC volume ($F=0.01, p=0.90$) and no significant associations between any of the other polymorphisms tested and ACC volume (Table 3).

Caudate

The *GRIN2B*-rs1019385 variant exhibited a non-significant trend towards association with caudate volume ($F=3.52, p=0.07$), and follow-up analyses revealed a trend towards association between this polymorphism and left ($F=3.17, p=0.08$ when both covariates included) but not right caudate volume. Caudate volume was not associated with any of the other polymorphisms tested (Table 4).

Thalamus

No significant differences were detected for thalamic volume based on *GRIN2B* genotype. For *SLC1A1*, a single variant (rs3056) was found to be associated with thalamic volume after correcting for age and total intracranial volume. Specifically, increased thalamic volume was significantly associated with the A/A genotype compared with carriers of the G allele (mostly G/A with a single G/G individual) ($F=7.25, p=0.01$ covarying for both age and intracranial volume). Figure 3 illustrates the differences in thalamic volume between *SLC1A1* genotype groups. The effect size (d) of this association between rs3056 in *SLC1A1* and total thalamic volume was 0.81. Results remained significant if outliers were removed. Secondary analysis based on laterality revealed that both left ($F=6.16, p=0.02$) and right ($F=6.18, p=0.02$) thalamic volume was significantly increased with the A/A genotype. Genotype groups for rs3056 did not differ with regard to OCD symptom severity (CYBOCS total, obsessions or compulsions scores), anxiety, depression, or tic severity. No significant associations were identified between the *SLC1A1* rs301434 and rs3087879 polymorphisms and thalamic volume (Table 5).

Given that three previous association studies of *SLC1A1* in OCD found the strongest effects in males (Arnold et al. 2006; Dickel et al. 2006; Stewart et al. 2007) we performed an exploratory analysis of the possible effects of gender on thalamic volume. A two-way ANCOVA was performed with gender and genotype as the two factors, age as the covariate, and thalamic volume (whole, right, or left) as the dependent variable. Analysis of whole and

left thalamic volume revealed a non-significant trend for a gender effect on volume ($p=0.09$ and $p=0.07$ respectively) with increased volume in females compared with males. However, there was no significant genotype by gender interaction and genotype main effects remained significant.

DISCUSSION

In this study, we found preliminary evidence that variants within glutamate system genes are associated with volumetric differences in brain regions implicated in this disorder. Firstly, the rs1805476 variant is located within the 3'-untranslated region of *GRIN2B*. Although we have not found a significant association between this variant and OCD, we have previously demonstrated increased transmission of the C-C haplotype of rs1805476 and nearby rs1805502 to OCD probands under the recessive model of inheritance (i.e. requiring two copies of the C allele) (Arnold et al. 2004; Arnold et al. 2007). In the current study, we identified an association between this variant and OFC volume which was specific to the left OFC ($F=4.85$, $p=0.04$). Specifically, CC homozygotes had decreased left OFC volume compared with carriers of the A allele (AA or AC genotype). A number of MRI studies have shown decreased OFC volume in OCD patients compared with controls, using either region-of-interest or voxel-based morphometry (VBM) methods (Atmaca et al. 2007; Choi et al. 2004; Kang et al. 2004; Pujol et al. 2004; Szeszko et al. 1999; Szeszko et al. 2004). This includes one recent study of treatment-naïve OCD adults in which bilateral reduction of OFC volume was reported (Atmaca et al. 2007). Another report implicated decreased left OFC volume (Kang et al. 2004), and the same group has also reported more specifically that left anterior OFC volume was decreased in OCD and that this volumetric decrease correlated with impaired organizational strategies (Choi et al. 2004). Based on these studies, our finding of decreased left OFC volume in CC homozygotes is biologically plausible in that the risk CC genotype is associated with decreased OFC volume, identified by most structural neuroimaging studies as being associated with OCD. However, a smaller number of structural MRI studies have resulted in contradictory findings, including: increased grey matter in the left OFC of medication-naïve OCD adults (Kim et al. 2001), increased posterior OFC volume (Valente et al. 2005), and no volumetric difference for OFC (Riffkin et al. 2005). Interestingly, the location of rs1805476 in the 3'-untranslated region (3'-UTR) of *GRIN2B* suggests that this variant or a variant in tight linkage disequilibrium with it could influence translational control, possibly through altering a microRNA binding site (Bartel, 2004; John et al. 2004), and thereby affect the quantity of the NMDA 2B receptor subunit that is produced (de Moor et al. 2005). There is a possible microRNA target site lying between rs1805476 and rs1805502 (identified as binding human miRNA-224 using <http://www.microrna.org>, (John et al. 2004)).

In addition to being associated with left OFC, the rs1805476 variant of *GRIN2B* was found to be associated with the ACC. It is interesting to note that the association was with right ACC in contrast to the left-sided finding for OFC. In the current study the CC genotype of the rs1805476 genotype previously associated with risk for OCD was associated with *decreased* right ACC volume. The direction of the association differed from what we would have predicted from three previous reports by our group which found increased anterior cingulate volume (Rosenberg & Keshavan, 1998; Szeszko et al. 2004) or increased ACC total and right grey matter (Szeszko et al. 2008a) in pediatric psychotropic-naïve OCD patients compared with healthy controls. If the genetic association is replicated in larger sample sizes, one possible explanation for this discrepancy is that perturbation of ACC volume from normal is important rather than the direction of change. Consistent with our findings of an association between ACC volume and *GRIN2B*, we have elsewhere reported an association between rs1019385, a variant in the promoter region of *GRIN2B* and decreased anterior cingulate glutamatergic concentration (Glx) in a preliminary study of 18 psychotropic-naïve pediatric patients with

OCD (Arnold et al. 2007). Overall, our preliminary findings add to the growing literature on the importance of the ACC for OCD pathogenesis.

Our third major finding was the association of an *SLC1A1* variant and thalamic volume. Specifically, individuals with the A/A genotype of rs3056 were found to have increased thalamic volume compared to carriers of the G allele. Recently, we reported the two marker C-G haplotype of the rs301434 and rs3087879 polymorphisms to be associated with OCD in our family-based sample (Arnold et al. 2006) and more recently we have found that the A allele of rs3056 is part of a common, extended C-G-A haplotype of all three variants included in this study ($p=.0008$, unpublished results). Interestingly, only the rs3056A allele appears to be associated with thalamic volume, not the rs301434 or rs3087879 polymorphisms. Like rs1805476, the location of rs3056 in the 3'-UTR suggests that this variant could have functional effects mediated by microRNA repression or other mechanisms of translational control (de Moor et al. 2005). There are two possible microRNA target sites lying less than 100 base pairs from rs3056 (human microRNA-96) and rs3087879 (human micro-RNA-132) respectively (identified using <http://www.microrna.org>). Sequencing of this region might identify additional variants that could influence microRNA binding.

Our group has previously reported that thalamic volume is increased in pediatric medication-naïve OCD patients compared with controls, and that thalamic volume normalizes with effective treatment with SSRI medication (Gilbert et al. 2000). This treatment effect appears to be specific to medication, since thalamic volume did not normalize with cognitive-behavioral therapy (Rosenberg et al. 2000b). Two other studies of adult medication-naïve OCD patients also found increased thalamic volume compared with controls (Atmaca et al. 2007; Kim et al. 2001). Although no published studies have reported decreased thalamic volume compared with controls, a single study of adults using VBM methods reported decreased thalamic volume to be directly correlated with increased OCD symptom severity (Valente et al. 2005). Taken together, the neuroimaging evidence suggests an association between increased thalamic volume and OCD. Therefore, the direction of our findings seems plausible; with the putative risk allele of the *SLC1A1* rs3056 variant associated with the putative risk phenotype of increased thalamic volume.

Little is known regarding the functional consequences of the candidate variants examined in the current study and their effects on glutamate activity. However, there is increasing evidence that changes in glutamatergic neurotransmission can influence the density of dendritic spines (Alvarez and Sabatini 2007). Plasticity of dendritic spines is particularly important during early development, but also continues in the adult brain. Therefore, one might speculate that changes in the expression of *GRIN2B* and *SLC1A1* early in development influence dendritic plasticity. Alterations in dendritic spine density could be in turn reflected in volumetric differences between individuals with OCD and normal controls. Should our findings be confirmed in larger studies, further research would be needed to test this speculative model linking these DNA sequence variations to changes in dendritic spine density resulting in changes in regional brain volumes.

This study had significant strengths, including its focus on medication-naïve, pediatric subjects that limited confounding of MRI results due to chronic illness and pharmacotherapy. The effect sizes for the associations identified were large (Cohen, 1988), ranging between 0.68 and 0.97, and similar to effect sizes for the serotonin transporter linked polymorphic region (5HTTLPR) and amygdala activation, the most studied association in the new field of imaging genetics (Munafò et al. 2008). However, as reviewed by Glahn et al (2007) there are a number of limitations inherent to imaging genetics studies that are applicable to this project. First, there was limited power to detect between-group differences due to the small sample size and need to focus on a restricted number of brain regions and SNPs. In our statistical analyses, we took

precautions to counter possible errors resulting from the small sample size including checking normality of distribution and homogeneity of variance. However, this would certainly not eliminate the possibility of a false positive. There was also risk of Type I error due to multiple testing in this exploratory study, although we attempted to limit this by focusing on brain regions and SNPs selected based on strong *a priori* hypotheses. Overall, we acknowledge our findings should be interpreted with caution given the small sample size but feel that the effects were of large enough magnitude, and the results of sufficient interest to warrant a preliminary report while we continue to collect a larger sample in order to confirm these findings. Second, reliability of MRI methods may be limited by the determination of regional volumes based on manual tracing which may result in operator dependent error or bias. However, standardization of methods and the high level of training of raters should mitigate such user effects. Third, future studies should include since diagnosis of tic disorder was an exclusionary criterion for this study, the findings may not be generalizable to children with significant tic comorbidity.

Finally, this study was limited by the lack of a healthy comparison group. Inclusion of a normal control group would: 1) establish whether the regions of interest (ROI) are abnormal in the patients, and 2) reveal whether the genetic effects on regional brain volumes are limited to individuals with OCD or also relate to the normal range of brain structural variation seen in the general pediatric population. With regard to volumetric differences between OCD children and controls, we feel confident that the ROIs studied would be abnormal in our patient group since they were selected *a priori* for examination of gene-ROI association based on multiple previous studies (reviewed in Introduction) demonstrating robust differences between OCD patients and controls. However, we have no evidence regarding the effects of our selected candidate genetic variants on brain structure in healthy children and adolescents. We speculate that these variants influence regional brain volumes in normal individuals, but that this effect will be greater in children with OCD, possibly due to co-occurring genetic and environmental risk factors which disrupt the same neurodevelopmental processes. This hypothesis is consistent with previous imaging genetics studies which have found candidate gene effects which were present in both normal control and affected individuals but were more pronounced in the latter (Szeszko et al. 2005; Szeszko et al. 2008b). Expanding the current research to test these genetic effects in healthy controls is therefore a priority for future research, and we are therefore applying for funding to conduct a larger imaging genetics study including both psychotropic-naïve OCD children and healthy age- and sex-matched controls.

In summary, three neuroimaging phenotypes – left OFC volume, right ACC volume, and thalamic volume – were associated with glutamate system variants previously identified in our family-based studies as conferring risk to OCD. Associations with left OFC volume and thalamic volume were in the expected direction with the putative risk variant associated with a neuroimaging phenotype previously described in OCD, whereas the association with right ACC volume was in the opposite direction from what we would have predicted from earlier volumetric studies. This study is the first to our knowledge examining the relationship between candidate genetic polymorphisms and neuroimaging phenotypes in OCD. Given that neuroimaging phenotypes may be closer to the action of genes than complex behavioral phenomena, it is hoped that further research in this area will shed light on the genetic basis of this neuropsychiatric disorder and lead to improved diagnosis and treatment.

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Figure 1.
Left Orbital Frontal Cortex Volume and GRIN2B rs1805476

Figure 2.
Right Anterior Cingulate Cortex Volume and GRIN2B rs1805476

Figure 3.
Total Thalamic Volume and SLC1A1 rs3056

Table 1Single Nucleotide Polymorphisms Genotyped in *GRIN2B* and *SLC1A1*

SNP Name	Position ¹	Minor Allele (Frequency) ²	Gene region
Glutamate Receptor, Ionotropic, N-methyl-d-aspartate 2B (<i>GRIN2B</i>), Chromosome			
12p12			
Rs1019385	14026110	T (0.44)	Promoter
Rs890	13606575	T (0.48)	3'-UTR
Rs1805476	13605630	A (0.38)	3'-UTR
Rs1805502	13605448	C (0.16)	3'-UTR
Solute Linked Carrier, Family 1, Member 1 (<i>SLC1A1</i>), Chromosome 9p24			
Rs301434	4572082	T (0.43)	Intron
Rs3087879	4576808	C (0.34)	3'-UTR
Rs3056	4577146	G (0.10)	3'-UTR

¹The positions are from the University of California at Santa Cruz Genome Browser, March 2006 Assembly

²The minor allele frequencies are based on our previous family-based association studies (Arnold et al. 2004; Arnold et al. 2006; Arnold, 2007 and unpublished results)

Table 2

Orbitofrontal Cortex Volume and Genotype

Polymorphism	Genotype	N	Mean volume in cm ³ (s.d.)	F value	P value	F value	P value	F value	P value
<i>GRIN2B</i>									
rs1019385	G/G	10	18.80 (2.52)	0.05	0.82	0.07			0.79
	G/T or T/T	18	18.83 (3.47)						
rs890	T/T	7	19.85 (2.50)	0.40	0.68	0.57			0.58
	T/G	12	18.85 (3.06)						
	G/G	8	17.83 (3.87)						
rs1805476	A/A or A/C	15	19.96 (3.07)	2.92	0.10 ^T	1.90			.18
	C/C	12	17.41 (2.79)						
<i>Left OFC</i> ¹	A/A or A/C	16	10.64 (2.14)	4.85	0.04	3.57			0.07
	C/C	13	8.65 (1.97)						
<i>Right OFC</i>	A/A or A/C	15	9.35 (1.64)	0.14	0.71	0.05			0.83
	C/C	12	8.71 (1.57)						
rs1805502 ²	T/T	18	18.83 (3.48)	0.19	0.67	0.12			0.74
	T/C	9	18.79 (2.63)						
<i>SLC1A1</i>									
rs301434	T/T	5	20.13 (2.00)	1.00	0.33	0.41			0.53
	T/C or C/C	22	18.58 (3.34)						
rs3087879	G/G	13	18.74 (3.24)	0.00	0.95	0.15			0.70
	G/C or C/C	15	18.96 (3.21)						
rs3056	G/G or G/A	10	20.13 (3.18)	1.78	0.20	2.24			0.15
	A/A	16	18.09 (3.09)						

¹Secondary analyses (left vs. right) performed for OFC because $p < .10$.

²There were no C/C individuals in the sample.

^TTrend towards statistical significance (defined as $p < .10$).
Significant p values ($p < .05$) are indicated in **bold** text.

Table 3

Anterior Cingulate Cortex Volume and Genotype

Polymorphism	Genotype	N	Mean volume in cm ³ (s.d.)	F value	P value	F value	P value	F value	P value
<i>GRIN2B</i>									
rs1019385	G/G	11	11.90 (1.94)	0.01	0.93	0.44			0.51
	G/T or T/T	19	11.81 (2.19)						
rs890	T/T	8	12.76 (1.92)	0.87	0.43	1.32			0.29
	T/G	13	11.89 (1.74)						
	G/G	8	11.37 (2.30)						
rs1805476	A/A or A/C	16	12.57 (1.83)	3.30	0.08 ^T	2.16			0.15
	C/C	13	10.92 (2.12)						
<i>Left ACC</i> ¹	A/A or A/C	16	6.09 (1.82)	0.01	0.90	0.02			0.88
	C/C	13	5.47 (1.44)						
<i>Right ACC</i>	A/A or A/C	16	6.48 (1.01)	5.79	0.02	4.47			0.04 ²
	C/C	13	5.45 (1.88)						
rs1805502 ³	T/T	19	12.01 (2.00)	0.05	0.83	0.50			0.48
	T/C	10	11.94 (1.97)						
<i>SLC1A1</i>									
rs301434	T/T	5	12.16 (2.29)	0.07	0.79	0.03			0.87
	T/C or C/C	24	11.91 (2.01)						
rs3087879	G/G	13	11.71 (2.14)	0.02	0.90	0.05			0.82
	G/C or C/C	17	11.95 (2.07)						
rs3056	G/G or G/A	10	12.38 (1.80)	0.54	0.47	0.55			0.46
	A/A	18	11.61 (2.13)						

¹ Secondary analyses (left vs. right) performed for OFC because $p < .10$.

² Levene's test was significant ($F=4.47$, $p=.01$) consistent with departure from homogeneity of variance.

³ There were no C/C individuals in the sample.

^T Trend towards statistical significance (defined as $p < .10$).

Significant p values ($p < .05$) are indicated in **bold** text.

Table 4

Caudate Volume and Genotype

Polymorphism	Genotype	N	Mean volume in cm ³ (s.d.)	F value	P value	F value	P value	Controlled for Age	Controlled for Age, Total Intracranial volume
<i>GRIN2B</i>									
rs1019385	G/G	11	6.71 (0.64)	3.52	0.07	2.60			0.12
	G/T or T/T	19	7.24 (0.80)						
<i>Left Caudate</i> ¹	G/G	11	3.29 (0.37)	4.28	0.05	3.17			0.08
	G/T or T/T	19	3.59 (0.37)						
<i>Right Caudate</i>	G/G	11	3.41 (0.31)	2.28	0.14	1.60			0.22
	G/T or T/T	19	3.65 (0.48)						
rs890	T/T	8	6.67 (0.68)	1.41	0.26	1.49			0.25
	T/G	13	7.00 (0.69)						
	G/G	8	7.35 (0.80)						
	A/A or A/C	16	6.94 (0.85)	0.00	0.98	0.12			0.73
	C/C	13	7.15 (0.74)						
	T/T	19	7.15 (0.74)	2.40	0.13	1.22			0.28
	T/C	10	6.68 (0.66)						
<i>SLC1A1</i>									
rs301434	T/T	5	6.82 (0.60)	0.70	0.41	1.26			0.27
	T/C or C/C	24	7.13 (0.81)						
rs3087879	G/G	13	7.05 (0.83)	0.04	0.84	0.00			0.99
	G/C or C/C	17	7.04 (0.77)						
rs3056	G/G or G/A	10	6.82 (0.82)	1.12	0.30	1.21			0.28
	A/A	18	7.21 (0.76)						

¹Secondary analyses (left vs. right) performed for Caudate because p<.10.

²There were no C/C individuals in the sample.

^TTrend towards statistical significance (defined as p<.10).

Significant p values (p<.05) are indicated in **bold** text.

Thalamus Volume and Genotype

²Secondary analysis of left and right thalamus volumes performed since total thalamus volume significantly different between genotype groups. Significant p values ($p<.05$) are indicated in **bold** text