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Glutamate receptor gene (GRIN2B) associated with reduced anterior cingulate glutamatergic concentration in pediatric obsessive-compulsive disorder

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Abstract

In this preliminary study, 16 psychotropic-naïve pediatric OCD patients were studied using magnetic resonance spectroscopy (MRS) and genotyped for six candidate polymorphisms in two glutamate system genes. A significant association was identified between the rs1019385 polymorphism of glutamate receptor, ionotropic, N-methyl-d-aspartate 2B (*GRIN2B*) and decreased anterior cingulate cortex (ACC) glutamatergic concentration (Glx, p=0.02) but not with occipital Glx. These results suggest that *GRIN2B* may be associated with Glx in ACC, a region consistently implicated in OCD.

Keywords

glutamate transporter; magnetic resonance spectroscopy; N-methyl-d-aspartate

1. Introduction

OCD is a complex genetic disorder, likely involving multiple genes of small effect and environmental factors (Pauls 2008). C andidate gene studies have been conducted based on *a priori* etiological hypotheses, one of which postulates altered glutamate neurotransmission (MacMaster et al., 2008). Strong support for this hypothesis comes from proton magnetic resonance spectroscopy (1H-MRS) studies revealing greater glutamatergic concentrations (Glx) in caudate (Rosenberg et al., 2000) and lower Glx in anterior cingulate cortex (ACC)

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(Rosenberg et al., 2004) in pediatric OCD patients. This finding was replicated in adults with OCD (Yucel et al., 2008). Recently, we reported associations between OCD and two glutamate system genes: the glutamate subunit receptor *GRIN2B* (glutamate receptor, ionotropic, N-methy1-D-aspartate 2B), (Arnold et al., 2004), and the glutamate transporter *SLC1A1* (solute linked carrier, family 1, member 1) (Arnold et al., 2006; Dickel et al., 2006). These associations have been replicated in independent samples (Arnold, 2007; Stewart et al., 2007).

Intermediate phenotypes derived from neuroimaging may increase power to identify genetic effects (closer link between genotype and phenotype) and delineate pathways linking risk genes to disorders (Meyer-Lindenberg et al., 2006). To our knowledge, no previous genetic studies of OCD have utilized neuroimaging phenotypes. Therefore, we set out to conduct a proof-of-principle study of an imaging genetics paradigm in which we test associations between glutamate system genes and ACC Glx as a proposed intermediate phenotype for OCD. We hypothesized that putative risk alleles identified in previous candidate gene studies of *GRIN2B* and *SLC1A1* would be associated with reduced ACC Glx in pediatric OCD patients.

2. Methods

2.1. Subjects

Participants included 16 (11 male, 5 female) medication-naïve pediatric OCD patients 7 to 18 years (mean = 11.0 years, S.D. = 3.1). Eight patients overlap with the sample from a previous investigation measuring ACC Glx, with detailed assessment procedures and inclusion/exclusion criteria described previously (Rosenberg et al., 2004). Written informed consent was obtained from parents/guardians, and written assent from pediatric participants. Patients were administered the Schedule for Affective Disorders and Schizophrenia – School-Age Children (Kaufman et al., 1997) and the following clinician-administered instruments (mean ± S.D. in parentheses): Children's Yale-Brown Obsessive Compulsive Scale (CYBOCS, Scahill et al., 1997) (24.0 ± 7.8),, Hamilton Anxiety Rating Scale (HAM-A, (Hamilton, 1959)) (6.76 ± 3.90), Hamilton Depression Rating Scale (HAM-D; (Hamilton, 1967)) (6.47 ± 4.47), and the Yale Global Tic Severity Scale (YGTSS, Leckman et al, 1989) (3.69 ± 8.72).

2.2. Procedures

2.2.1. Imaging Procedures—Imaging data were collected using a Sigma 1.5-Tesla unit (Horizon LX software, General Electric Medical Systems, Milwaukee, WI). MRS scanning, image acquisition and analysis procedures are described elsewhere (Rosenberg et al., 2004). Briefly, 1H-MRS spectra were acquired from a 2 × 1.5 × 1cm (3cc) voxel centered in ACC and a 2 × 2 × 2cm (8cc) voxel centered in OCC (see figure 1) with a short-echo single voxel double spin-echo point resolved spectroscopy (PRESS) pulse sequence (Bottomley, 1987). The anterior cingulate voxel contained predominantly bilateral anterior cingulate gray matter (Brodmann areas 32 and 12) with minor contributions from midline CSF and cingulum white matter. Using LCModel (Provencher, 2001), we acquired data for: Glx (includes glutamate and glutamine), N-acetyl-aspartate (NAA), choline compounds (Cho), creatine/phosphocreatine (Cr), and myo-inositol (mI). We measured ACC volume with boundaries used previously (Szeszko et al., 2004).

2.2.2. Genotyping procedures—Genomic DNA was extracted from blood using standard methods. Polymorphisms were selected based on our Toronto family-based association study (Arnold, 2007), from *GRIN2B* (rs1019385, rs890, rs1805476 and rs1805502) and *SLC1A1* (rs3087879 and rs3056). Another polymorphism in *SLC1A1*, rs301434 was genotyped but excluded from analysis since there were less than 4 individuals in the homozygote groups. Genotyping was performed using Applied Biosystems Inc. (Foster City, CA) Taqman® assays described elsewhere (Arnold, 2007).

2.3. Statistical Analysis

ANCOVA analyses were performed (Statistical Package for Social Sciences (SPSS), version 15) with genotypes as independent variables and ACC metabolites as dependent variables. We hypothesized *a priori* that candidate gene associations would be specific to Glx. Covariates included age and ACC volume (see Rosenberg & Keshavan, 1998). As a control, we analyzed for associations with occipital Glx, with age and gray matter volume as covariates. To directly contrast effects of brain region, two-way repeated measures ANCOVA was performed with brain region (ACC or OCC) as the within-subjects factor, genotype as the between-subjects factor, and age as a covariate. When there were less than five individuals per genotype group these subjects were grouped together with heterozygotes (see Papassotiropoulos et al., 2006). Normality and homogeneity of variance between groups were checked through boxplots and Levene's test.

3. Results

The mean absolute concentration of Glx was 9.94 millimoles (mM) for ACC and 10.38 mM for OCC. The mean SD for Glx was 6.53 mM (range 5 to 31 mM) for ACC and 7.20 mM (range 4 to 31 mM) for OCC. The only statistically significant association was between *GRIN2B*-rs1019385 and ACC Glx. Decreased ACC Glx was seen with G/G genotype compared with T/G or T/T (F=6.2 (1, 13), P=0.03). ACC Glx values for rs1019385 genotype groups were normally distributed. Repeated analysis after removing a single outlier (Glx level =13.4) maintained a strong trend for association (F=4.26 (1, 12), P=0.06). Data for ACC and OCC Glx by genotype are illustrated in Table 1. Adding ACC volume to age as a covariate did not change results. Since Glx may change with menstrual cycle (Batra et al., 2008) we repeated analyses after removing the only post-menarchal female, with no effect on results.

CYBOCS was not different between *GRIN2B*-rs1019385 genotype groups (F=0.31 (1,13), P=0.59). The association between*GRIN2B* rs1019385 and ACC Glx remained statistically significant when controlling for CYBOCS score (F=5.37 (1, 12), P=0.046), HAM-D score (F=6.38 (1, 12), P=0.03) or HAM-A score (F=10.38 (1, 12), P=0.008).

No associations were found with occipital Glx. No region by genotype interaction was identified using two-way repeated measures ANCOVA for *GRIN2B*-rs1019385 (F=0.28 (1, 13), P=.87). The only significant region by genotype interaction was with *GRIN2B*-rs1805476 (F=5.66 (1, 12), P=0.04) due to a greater difference between genotype groups for OCC (Table 1) compared with ACC. No associations were found with ACC NAA, Cho, Cr, or mI).

4. Discussion

In this preliminary study, we found a significant association between ACC but not OCC Glx and the *GRIN2B*-rs1019385 polymorphism, with GG individuals exhibiting decreased Glx compared with carriers of the T allele. Our family-based studies have found the G allele (GG genotype specifically) to be associated with OCD (Arnold, 2007). The G allele of rs1019385 is a promoter region variant that leads to reduced transcription (Miyatake et al., 2002), which could affect glutamate neurotransmission. Association between the risk G/G genotype and ACC Glx is consistent with previous studies implicating ACC in OCD (Szeszko et al., 2004) and is in the expected direction (lower Glx) (Rosenberg et al., 2004). The same pattern was observed in the other 5 polymorphisms, in that the putative risk genotype (e.g. the G/G genotype for rs890, Arnold et al., 2004) was correlated with lower Glx, although these associations were not statistically significant and may not have been detected due to low power.

Strengths of this study include minimizing confounding of 1H-MRS results due to chronic illness and pharmacotherapy, but limitations exist. Small sample size resulted in limited power

to detect between-group differences (Type II error), while there was a risk of Type I error from multiple comparisons. The problem of Type I error was mitigated by strong *a priori* hypotheses regarding the SNPs, which have all been associated with OCD in previous studies, however we acknowledge that our results would not hold up to correction for multiple comparisons,. Nonetheless, we believe our results represent a novel proof-of-principle experiment of an imaging genetics paradigm in OCD and therefore are of sufficient interest to be reported. We hope that these findings stimulate further research including replication in independent samples, which is required to confirm genetic association results (Gorroochurn et al., 2007).

Using 1.5 T MRS enabled measurement of Glx, a multi-peak signal including glutamate, glutamine, and gamma-amino butyric acid (GABA) rather than glutamate alone. Although Glx may be a reasonable proxy for glutamate (Provencher, 1993; Rosenberg et al., 2005) there is consensus in the field that the subcomponents of Glx are best resolved at higher field. Although we controlled for intracranial volume, ideally one would control for proportions of grey and white matter and cerebrospinal fluid. Using tissue segmentation to better account for voxel water content allows for the more accurate calculation of absolute concentration. Not employing this technique may contribute to measurement error (Stanley et al., 1995). We have initiated studies at high field (3T) for better resolution of Glx subcomponents and are controlling for grey matter, white matter, and CSF in order to ensure the most accurate possible calculations of absolute concentration.

Future studies should explore other regions implicated in OCD (e.g. caudate and orbital-frontal cortex) and/or employ more comprehensive candidate gene or genome-wide association approaches. As approximately 30% of Caucasians are G/G and most don't have OCD, and most individuals with OCD do not have G/G, variation in rs1019385 is neither necessary nor sufficient for the disorder. This situation is expected with complex genetic traits, in which risk factors are probabilistic rather than deterministic in nature (Page et al., 2003). We did not detect any difference in symptom severity between rs1019385 genotype groups, however other possible genotype-phenotype associations (e.g. family history, drug response, symptoms subtype) should be further explored.

In summary, ACC, but not occipital, Glx was associated with a *GRIN2B* variant that may confer increased risk for OCD. Findings were in the expected direction, associated with decreased ACC Glx. This is the first published report to examine the relationship between genetic variation and a neurochemical phenotype in OCD, and therefore represents a novel proof-of-principle experiment. We hope that our findings will stimulate further research on the genetic basis of OCD, ultimately leading to improved diagnosis and treatment.

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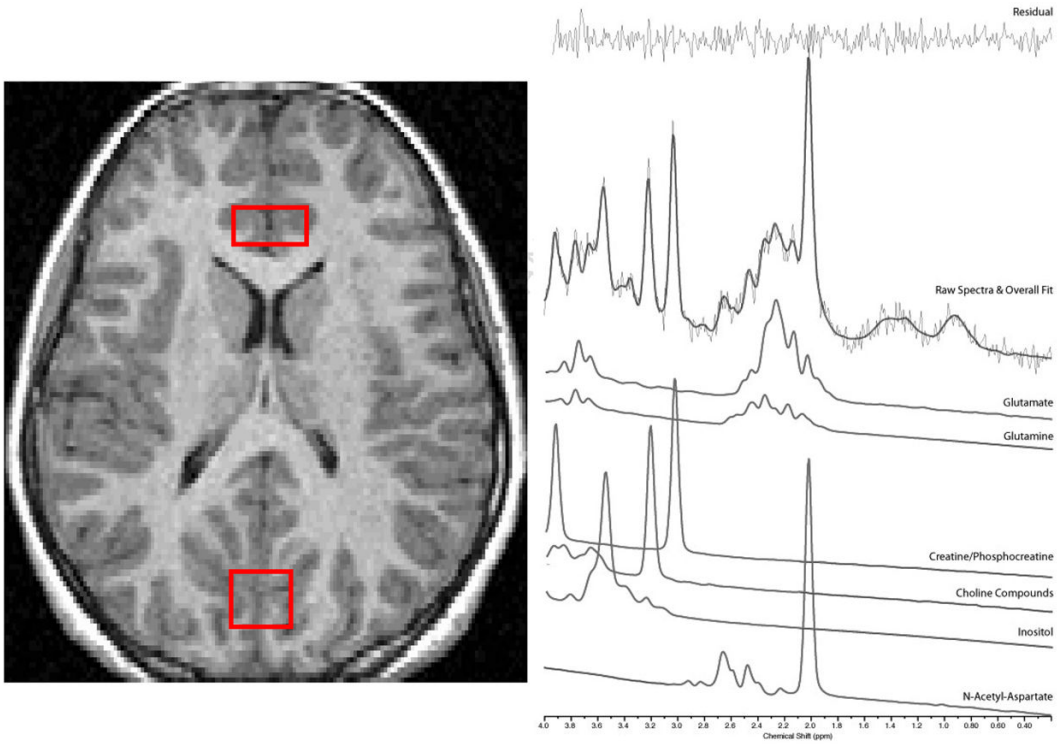

Figure 1.
1H-MRS voxel placement (A) and Sample Spectra from the Anterior Cingulate and Occipital Lobe

Table 1

Glutamatergic Concentration (Glx) and Genotype

					***F* value[1](df)**	***P* value**	***F* value (df)**	***P* value**
Region-Gene Association	**Polymorphism**	**Genotype**	***N***	**Mean Glx in mM (S.D.)[2]**	**Controlled for Age**		**Controlled for Age, Volume[3]**	
ACC – *GRIN2B*	rs1019385	G/G	6	9.60 (1.39)	6.20(1,13)	**0.03**	5.86(1,12)	**0.03**
		G/T or T/T	10	10.44 (1.19)				
	rs890	T/T	4	10.41 (0.36)	0.06 (1,12)	0.81	0.07 (1,11)	0.80
		T/G or G/G	11	10.07 (1.55)				
	rs1805476	A/A or A/C	8	10.11 (1.75)[4]	1.70 (1.12)	0.22	2.76 (1,11)	0.12
		C/C	7	10.00 (0.65)				
	rs1805502[5]	T/T	9	10.03 (1.72)	0.25 (1,12)	0.63	0.23 (1,11)	0.64
		T/C	6	10.36 (0.40)				
ACC – *SLC1A1*[4]	rs3087879	G/G	6	9.66 (1.17)	0.70 (1,13)	0.42	0.67 (1,12)	0.43
		G/C or C/C	10	10.40 (1.34)				
	rs3056	G/A	5	10.26 (0.44)	0.25 (1,12)	0.62	0.21 (1,11)	0.66
		A/A	10	10.08 (1.64)				
OCC – GRIN2B	rs1019385	G/G	6	8.45 (1.46)	0.89 (1,13)	0.36	0.94 (1,12)	0.35
		G/T or T/T	10	9.22 (1.81)				
	rs890	T/T	4	9.29 (1.09)	0.10 (1,12)	0.75	0.11 (1,11)	0.74
		T/G or G/G	11	8.75 (1.94)				
	rs1805476	A/A or A/C	8	9.53 (0.91)[4]	3.05 (1.12)	0.11	2.80 (1,11)	0.12
		C/C	7	7.95 (1.93)				
	rs1805502[5]	T/T	9	9.07 (1.92)	0.54 (1,12)	0.48	0.34 (1,11)	0.57
		T/C	6	8.65 (1.54)				
OCC – *SLC1A1*	rs3087879	G/G	6	8.65 (1.60)	0.12 (1,13)	0.73	0.08 (1,12)	0.79
		G/C or C/C	10	9.10 (1.79)				
	rs3056	G/A	5	8.74 (1.64)	0.16 (1,12)	0.69	0.15 (1,11)	0.70
		A/A	10	8.90 (1.82)				

ACC = Anterior Cingulate Cortex; OCC = Occipital Cortex

[1] F and p values are for tests of between-subjects effects.

[2] Mean Glx = absolute concentration in millimoles (mM) with standard deviation in parentheses.

[3]Volume = Total ACC volume for ACC, Total grey matter volume for OCC.

[4]Levene's test violated, indicating that error variances are not equal across genotype groups.

[5]None of the subjects had the least common C/C genotype

Significant p values ($p<0.05$) are indicated in **bold** text.

N.B. Trend for significance ($F=4.26$, $p=0.06$) for *GRIN2B*-rs1019385 after removal of outlier