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Metabotropic Glutamate Receptor 3 Genotype May Predict *N*-Acetylaspartate Measures in the Dorsolateral Prefrontal Cortex

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Abstract

Objective—This study was carried out to confirm prior evidence of an effect of a single nucleotide polymorphism (SNP) in the metabotropic glutamate receptor 3 (GRM3) gene (a putative risk factor for schizophrenia) on measures of *N*-acetylaspartate in healthy comparison subjects.

Method—Fifty-four carefully screened healthy volunteers genotyped at SNP rs6465084 underwent magnetic resonance spectroscopic imaging (MRSI) at 3 T and selected neuropsychological testing.

Results—The A/A genotype group exhibited a significant reduction of *N*-acetylaspartate/creatine levels in the right dorsolateral prefrontal cortex compared to the G carriers. A tendency in the same direction was seen in the left dorsolateral prefrontal cortex and in the white matter adjacent to the prefrontal cortex.

Conclusions—These findings provide further evidence that GRM3 affects prefrontal function and that variation in GRM3, monitored by SNP rs6465084, affects GRM3 function.

Introduction

The metabotropic glutamate receptor 3 (GRM3) gene is a key molecule in regulating synaptic glutamate concentrations. Single nucleotide polymorphisms (SNPs) in the GRM3 gene have been associated with schizophrenia in several independent reports (1–3). Recently, Egan et al. (4) showed that the A allele of SNP rs6465084 in intron 2 of the GRM3 gene was associated with an increased risk of schizophrenia, reduced verbal fluency (an index of prefrontal function that has been consistently found to be impaired in patients with schizophrenia and in their healthy relatives), and reduced prefrontal cortical levels of *N*-acetylaspartate/creatine, measured in vivo with nuclear magnetic resonance spectroscopy. *N*-Acetylaspartate/creatine levels reflect the abundance of *N*-acetylaspartate, an amino acid believed to be an index of neuronal function. Reductions of *N*-acetylaspartate/creatine levels in the prefrontal cortex of patients with schizophrenia have been demonstrated in a number of studies. The purpose of the present study was to examine the effect of the A allele of SNP rs6465084 of GRM3 on *N*-acetylaspartate/creatine levels in a new cohort of healthy comparison subjects studied with higher spatial resolution on a 3 T scanner. We hypothesized that *N*-acetylaspartate/creatine

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levels in the prefrontal cortex of A allele homozygotes would be reduced compared to G allele carriers.

Method

The authors studied 54 carefully screened healthy Caucasian American comparison subjects of European ancestry. Demographic information is presented in a table available online (Data Supplement 1 at http://ajp.psychiatryonline.org). All of the participants underwent a structured diagnostic interview and a neurological evaluation, and subjects with any current or past psychiatric or neurological diagnosis, current medical illness, or family history of psychosis were excluded. Written informed consent was obtained from all subjects. The subjects were genotyped at rs6465084 in intron two of the GRM3 gene, as described by Egan et al. (4). The subjects completed a battery of tests, including verbal fluency (letters and categories) and an IQ test (WAIS–Revised; see reference 4 for more details). Proton magnetic resonance spectroscopic imaging (MRSI) was performed with a 3 T GE magnet (General Electric Medical Systems, Milwaukee), with a multislice imaging technique similar to that in previous publications by our group (four slices [7.5 mm cubic voxel dimensions]; spin echo slice selection; TR=2300 msec, TE=280 msec, no water suppression, and lipid signal from the scalp suppressed with outer volume saturation pulses) (5). The MRSI acquisition was conducted on a plane parallel to the main axis of the hippocampi. Regions of interest were drawn on the left and right dorsolateral prefrontal cortex, the cingulate cortex, the white matter of the centrum semiovale, the left and right hippocampal formation, and the occipital cortex on structural magnetic resonance imaging (MRI) scans registered to the MRSI slices. Metabolite signals were calculated as the integral of the magnitude spectrum in a range of 0.25 ppm surrounding peaks for *N*-acetylaspartate, creatine, and choline and were reported as metabolite ratios averaged over the voxels in the regions of interest. Extensive quality control procedures were undertaken to eliminate voxels with obvious artifacts. A low number of voxels in the regions of interest (less than three voxels in slice one and less than seven voxels in slice four) resulted in rejection of that particular region of interest for further statistical analysis. All quality control procedures were performed blind to genotype status. We collapsed the A/G (N=21) and G/G (N=4) genotype subjects into a G carrier group in the analysis. The differences in metabolite ratios and letter and category fluency task scores between the two genotype groups were analyzed by unpaired t tests. One-tailed statistics with a significance level set at $p<0.05$ were used for the dorsolateral prefrontal cortex because of the directional hypothesis based on prior findings by our group (4) (no correction for multiple comparisons). Potential confounding factors, such as age, sex, handedness, education, and IQ were also examined.

Results

The main results are presented in the table. The A/A genotype group exhibited a significant reduction of *N*-acetylaspartate/creatine levels in the right dorsolateral prefrontal cortex compared to the G carriers, a finding that would survive corrections for six multiple comparisons. There were tendencies in the same direction in the left dorsolateral prefrontal cortex and the adjacent white matter of the centrum semiovale. No other metabolite ratios or regions of interest were significantly different between the groups. The genotype groups were well matched for age, handedness, years of education, and IQ (see table); however, there tended to be more women in the A/A genotype group. When the authors used gender as a covariate in an analysis of variance, the effect of genotype remained significant in the right dorsolateral prefrontal cortex (effect of gender: $F=0.33$, $df=(1,51)$, $p=0.28$; effect of genotype: $F=5.80$, $df=(1,51)$, $p=0.01$). There were no significant differences in verbal fluency performance between the A/A carriers and the G carriers.

Discussion

The authors found that the GRM3 genotype at SNP rs6465084 predicted *N*-acetylaspartate/creatine levels in the dorsolateral prefrontal cortex in a new group of normal subjects, thus confirming an earlier report from our group (4) that had used a less highly resolved and sensitive MRI technique (the current scans were performed with a 1.5 T scanner at half the resolution of the original report). The A/A homozygotes (i.e., those with a genotype associated with an increased risk for schizophrenia) had lower *N*-acetylaspartate/creatine levels in the dorsolateral prefrontal cortex, possibly indicating decreased neuronal function. It is remarkable that a genotype effect was detected on this phenotype with such a small group. Based on evidence that GRM3 modulates synaptic glutamate and that it is a receptor for *N*-acetyl-aspartyl-glutamate and that *N*-acetylaspartate is related to mitochondrial activity and to glutamate levels (6,7), the prefrontal *N*-acetylaspartate reduction may reflect an alteration in genetically regulated glutamate neurotransmission or innervation patterns. Our data represent further evidence that glutamate system dysfunction may play a role in the prefrontal functional abnormalities seen in schizophrenia. Our in vivo data also add support to evidence that SNP rs6465084 monitors a functional variation in GRM3. Our group has shown that the same genotype is associated with reduced expression of presynaptic vesicular markers (8) and of the glial glutamate transporter in postmortem human brain tissue (4), but conclusive effects on GRM3 mRNA or protein levels have not been found. Although it is possible that this intronic SNP may cause altered splicing, we cannot infer from the currently available data that this is a functional locus. We did not observe an expected difference in verbal fluency between the genotypes. This may be due to a lack of power in this rather small group or possibly to a covariate that was not identified in this study (e.g., undetected ethnic stratification). The limitations of our study were that we did not correct for the respective contribution of gray and white matter to our regions of interest, nor did we obtain “absolute” measures of *N*-acetylaspartate levels, which are made significantly more imprecise at 3 T for long times of echo because of the effects of T 2 relaxation and B 1 inhomogeneities.

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Table 1

Demographic and Clinical Characteristics and *N*-Acetylaspartate/Creatine Levels for Two Genotype Groups[a]

Characteristic	A/A Carriers			G Carriers			Analysis		
	N	%		N	%		χ^2	df	p
Gender							3.2	1	0.06
Female	19	66		10	40				
Male	10	34		15	60				
	N	Mean	SD	N	Mean	SD	t	df	p
Age	29	32.8	8.2	25	31.4	10.5	0.56	52	0.58
Education (years)	29	16.3	2.2	25	16.7	3.1	−0.59	52	0.55
Handedness	29	81.0	51.0	25	85.7	26.4	−0.41	52	0.68
IQ	29	105.5	7.0	25	107.4	7.4	−0.96	52	0.34
Verbal fluency									
Letter	29	44.9	14.1	25	44.8	9.1	0.03	52	0.98
Category	29	52.1	8.7	25	53.1	9.4	−0.41	52	0.68
Centrum semiovale	26	2.27	0.19	21	2.41	0.27	−2.06	45	0.04
Anterior cingulate cortex	26	1.84	0.14	22	1.89	0.15	−1.08	46	0.29
Hippocampus									
Right	17	1.70	0.23	13	1.67	0.24	0.28	28	0.78
Left	17	1.66	0.26	18	1.73	0.22	−0.79	23	0.43
Occipital	24	1.98	0.22	21	1.92	0.26	0.77	43	0.45
	N	Mean	SD	N	Mean	SD	t (one-tailed)	df	p
Dorsolateral prefrontal cortex									
Right	29	2.01	0.19	25	2.16	0.23	−2.66	52	0.005
Left	25	2.04	0.21	22	2.12	0.22	−1.38	45	0.09

[a] Some of the groups overlap.