Original Investigation | META-ANALYSIS

Nature of Glutamate Alterations in Schizophrenia
A Meta-analysis of Proton Magnetic Resonance Spectroscopy Studies

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IMPORTANCE Alterations in glutamatergic neurotransmission may be fundamental to the pathophysiology of schizophrenia, and the glutamatergic system is a target for novel therapeutic interventions in the disorder.

OBJECTIVE To investigate the nature of brain glutamate alterations in schizophrenia by conducting a meta-analysis of glutamate proton magnetic resonance (MRS) spectroscopy studies.

DATA SOURCES The MEDLINE database was searched for studies published from January 1, 1980, to April 1, 2015. Search terms included *magnetic resonance spectroscopy*, *schizophrenia*, *psychosis*, *clinical* or *genetic high risk*, and *schizoaffective*. Inclusion criteria were single voxel 1H-MRS studies reporting glutamate, glutamine or Glx values for a patient or risk group in comparison to a healthy volunteer group.

STUDY SELECTION Fifty-nine studies were identified, which included 1686 patients and 1451 healthy individuals serving as controls.

DATA EXTRACTION AND SYNTHESIS A random-effects, inverse-weighted variance model was used to calculate the pooled effect size. Mean values were extracted and verified independently. Effect sizes were determined for glutamate, glutamine, and Glx in brain regions that had been examined in at least 3 different studies. A secondary analysis grouped studies into those examining patients at different stages of illness (high risk, first-episode psychosis, or chronic schizophrenia). Effects of age, antipsychotic dose, and symptom severity were determined using meta-regression.

RESULTS In schizophrenia, there were significant elevations in glutamate in the basal ganglia (Hedges g = 0.63; 95% CI, 0.15-1.11), glutamine in the thalamus (g = 0.56; 95% CI, 0.02-1.09), and Glx in the basal ganglia (g = 0.39; 95% CI, 0.09-0.70) and medial temporal lobe (g = 0.32; 95% CI, 0.12-0.52). No region showed a reduction in glutamate metabolites in schizophrenia. Secondary analyses revealed that elevated medial frontal Glx levels were evident in individuals at high risk for schizophrenia (g = 0.26; 95% CI, 0.05-0.46) but not in those with first-episode psychosis or chronic schizophrenia, whereas elevated Glx in the medial temporal lobe was seen with chronic schizophrenia (g = 0.40; 95% CI, 0.08-0.71) but not in the high-risk or first-episode groups. Meta-regression found no association with age, symptom severity, or antipsychotic dose.

CONCLUSIONS AND RELEVANCE Schizophrenia is associated with elevations in glutamatergic metabolites across several brain regions. This finding supports the hypothesis that schizophrenia is associated with excess glutamatergic neurotransmission in several limbic areas and further indicates that compounds that reduce glutamatergic transmission may have therapeutic potential.

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Editorial

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Several lines of evidence have implicated alterations of the glutamatergic system in the cause of schizophrenia. The *N*-methyl-D-aspartate receptor (NMDAR) hypofunction model[1] proposes that schizophrenia is related to dysfunction of NMDARs on parvalbumin-containing γ-aminobutyric acid-ergic interneurons, leading to excess glutamate release.[2] Administration of NMDAR antagonists, such as ketamine, induce a psychotic state in healthy volunteers and exacerbate psychotic symptoms in patients with schizophrenia.[3] More recently, an autoimmune disorder associated with autoantibodies to the NMDARs has been associated with psychotic symptoms, and NMDAR autoantibodies may be evident in a small proportion of patients with schizophrenia.[4] Several genes associated with schizophrenia code for proteins involved in glutamatergic neurotransmission.[5] The main technique for assessing central glutamate function in man in vivo is proton magnetic resonance spectroscopy (MRS).

Depending on the proton MRS approach and field strength, both glutamate and the glutamate metabolite, glutamine, may be reported separately or in combination (Glx).[6] Glutamatergic metabolites are usually measured in a predetermined voxel of interest. Concentration estimates reflect both intracellular and extracellular glutamate and glutamate involved in metabolism as well as in neurotransmission.[7] Over the past 2 decades, several studies have used proton MRS to investigate regional glutamate concentrations in patients with schizophrenia compared with those in healthy volunteers. However, findings have not been consistent across studies, including reports of elevations,[8-26] no differences,[27-51] and reductions[52-63] in the patient group across a variety of brain regions. These differences may relate to regional effects, proton MRS methodologic differences, stage[24,57-59] or severity[64] of illness, or treatment effects.[17,20] The first meta-analysis[65] of these studies reported decreases in glutamate and increases in glutamine in the medial frontal cortex of patients compared with controls. The total number of publications has more than doubled since the first meta-analysis, which included studies up until 2011. These more recent reports include studies on regions of interest that had previously been examined too infrequently to be included in a meta-analysis. Moreover, the field now includes substantial numbers of studies in individuals at high risk (HR) for schizophrenia and those with first-episode psychosis (FEP), in addition to studies in patients with chronic schizophrenia, permitting separate meta-analyses of these different groups.

The primary aim of this study was to conduct an updated case-control meta-analysis of all published reports of regional glutamatergic measures in those at HR for schizophrenia, with FEP, and with schizophrenia. The second aim was to conduct case-control meta-analyses in clinical subgroups separately (HR, FEP, and chronic schizophrenia [referred to as *schizophrenia* hereinafter]). The third aim was to assess the influences of age, symptom severity, and antipsychotic treatment.

The first hypothesis of the study was that, on the basis of preclinical schizophrenia models showing increases in glutamatergic transmission,[66] glutamatergic metabolites would be increased in cases compared with controls. The second hypothesis was that there would be higher glutamatergic metabolite concentrations in FEP and HR individuals compared with those who had schizophrenia, in line with previous studies comparing patient groups.[23,24,57-59] The third hypothesis was that glutamate and glutamine levels would become lower with antipsychotic treatment[17,20,21] as well as with age in cases relative to controls,[65] but that symptom severity would be associated with higher glutamatergic metabolite concentrations.[64]

Key Points

Question What is the nature of glutamate alterations in schizophrenia as revealed by studies using proton magnetic resonance spectroscopy?

Findings This meta-analysis evaluated 59 studies reporting on regional glutamate, glutamine, or their combined Glx signals. There were significant elevations in glutamate in the basal ganglia, glutamine in the thalamus, and Glx in the basal ganglia and medial temporal lobe but no associations with age, symptom severity, or antipsychotic medication dose.

Meaning Schizophrenia is associated with elevations in glutamate-related metabolites across several brain regions consistent with the hypothesis that there is excess glutamatergic neurotransmission in this condition.

Methods

Study Selection

The MEDLINE database was searched to identify journal articles published between January 1, 1980, and April 1, 2015, using the following search terms: *MRS* or *magnetic resonance spectroscopy* and (1) *schizophrenia* or (2) *psychosis* or (3) *UHR* or (4) *ARMS* or (5) *ultra high risk* or (6) *clinical high risk* or (7) *genetic high risk* or (8) *prodrom** or (9) *schizoaffective*. All single-voxel proton MRS studies reporting glutamate, glutamine, or Glx values for a patient or risk group in comparison with a healthy volunteer group were included in the analysis. In the case of longitudinal studies,[17,43,52] only the values given for the first time point were included. If the same sample or partially overlapping samples were included in more than 1 report, data from the study with the largest sample were included (References 9, 10, 17, 24, 38, 43, 45, 52, 54, 62).

Meta-analysis

Mean values of proton MRS glutamate, glutamine, or Glx concentrations were extracted by one of us (K.M.) and verified by another (A.E.) independently and categorized into the following brain regions of interest: (1) medial frontal cortex, including studies with voxels in the medial prefrontal cortex and in the anterior cingulate cortex since these voxels often spatially overlap; (2) dorsolateral prefrontal cortex (DLPFC); (3) frontal white matter; (4) thalamus; (5) medial temporal lobe (MTL) (including hippocampus); (6) basal ganglia (including caudate, putamen, and globus pallidus); and (7) cerebellum.

Only analyses for which at least 3 independent data sets were available were included. When more than 1 clinical group was reported in a single study, the values were treated as independent data sets and the number of healthy volunteers was adjusted by dividing by the number of clinical groups. When data were reported bilaterally, only those for the left hemisphere were included because the left hemisphere was examined in most studies.

The ability of proton MRS to resolve the overlapping resonances of glutamate and glutamine increases with field strength. Previous estimates[6] of the degree of contamination of glutamate and glutamine signals at different field strengths using optimized sequences indicated that it would be appropriate to include studies reporting glutamate if the data were acquired at field strengths of 3 T or above and studies reporting glutamine at 4 T or above. A secondary analysis included data acquired at all field strengths.

The proton MRS measures of glutamate, glutamine, or Glx were analyzed separately, which was accounted for by applying a Bonferroni-corrected threshold for statistical significance of $P < .017$. The effect size statistic Hedges *g*, which incorporates a correction for bias from small sample sizes, was calculated by subtracting the mean glutamate, glutamine, or Glx values reported in cases by the mean value reported in the control group divided by the pooled SD across groups.[67] If means or SDs were not reported, authors were contacted for this information. A Hedges *g* value of 0 indicates no difference between cases and controls, negative values indicate lower glutamatergic metabolite levels in cases than controls, and positive values denote higher glutamatergic metabolite levels in cases than controls.

A random-effects, inverse-weighted variance model[68] was used to calculate the pooled effect size since the studies were expected to display high heterogeneity as different correction methods and clinical samples were used. Study effect size was weighted according to sample size. Heterogeneity was measured using the I^2 value, with higher percentages denoting higher variation across studies in the meta-analysis. The meta-analysis for each brain region was performed using meta-analytical equations entered into Excel (Microsoft Corp) (http://www.depressiondatabase.org). These equations are identical to the METAN command in Stata (StataCorp LP), which is commonly used in meta-analyses publications. In terms of validation, the method has been used in parallel with Stata in previous meta-analyses[69] and produced the same results.

Effect sizes were initially calculated for all patients and controls and then for each clinical group (HR, FEP, and schizophrenia) separately. Separate analysis was also performed of patient groups (FEP and schizophrenia) since most HR individuals will not develop psychosis.

Meta-regression

To explore the relationship between glutamate, glutamine, and Glx effect sizes for each study and selected demographics or clinical variables, random effects meta-regressions were conducted using the metareg command in Stata, version 11.2 2009. The variables investigated were age; Positive and Negative Syndrome Scale (PANSS)[70] total; positive, negative, and general subscale scores; chlorpromazine-equivalent dose; and duration of illness. In studies that used the Brief Psychiatric Rating Scale, the scores were converted to PANSS scores.[71] When these measures were not reported, study authors were contacted to request the data. Publication bias was examined using the Egger regression test for regions including at least 10 studies[72] and meta-regressions of study effect size and year of publication. A leave-one-out jackknife sensitivity analysis was conducted for regions with at least 4 studies in which significant between-group differences were found.

Results

The literature search identified 59 studies, with a total of 1686 cases and 1451 controls (PRISMA flow diagram presented in eFigure 1 in the Supplement). The sample sizes ranged from 5 to 84 for cases and 4 to 81 for healthy volunteers (eTable 1 in the Supplement). Two studies[73,74] reporting multivoxel data were excluded.

Fourteen studies (References 18, 25, 26, 29, 31, 37, 49, 51-53, 56, 57, 62, 75) examined participants at HR for psychosis. Eighteen studies (References 9, 12, 17-19, 23, 24, 28, 30, 32, 33, 45, 48, 57-59, 63, 76) examined patients experiencing a first episode of psychosis (FEP), all with an onset of illness within the last 2½ years. Thirty-six studies (References 8, 10, 11, 13-16, 20-24, 27, 31, 34-36, 38-44, 46, 47, 50, 53-55, 57-61, 77) examined patients with established (chronic) schizophrenia (eResults in the Supplement provide detailed patient information).

Meta-analysis

In the medial frontal cortex, there were no significant findings for glutamate (HR group, 3; FEP group, 3; and schizophrenia group, 11), Glx (HR group, 8; FEP group, 3; and schizophrenia group, 13) or glutamine (HR group, 0; FEP group, 2; and schizophrenia group, 3) (**Table** and eFigure 2 in the Supplement). Analysis of each clinical group separately revealed higher Glx concentrations in HR individuals ($g = 0.26$; 95% CI, 0.05-0.46; $P = .01$). There were no significant between-group differences in glutamate or glutamine levels.

In the frontal white matter, there were no significant effects overall for glutamate (HR group, 1; FEP group, 1; schizophrenia group, 1; and FEP + schizophrenia group, 1) or Glx (HR group, 0; FEP group, 2; and schizophrenia group, 7) in cases compared with controls, and only 1 study[76] examined glutamine (Table). The schizophrenia group showed elevated Glx levels compared with controls ($g = 0.42$; 95% CI, 0.18-0.66; $P = .001$).

In the DLPFC, there were no significant effects for Glx in cases (HR group, 2; FEP group, 2; and schizophrenia group, 8) or in the schizophrenia group. There were insufficient data above 1.5 T for glutamate and at 4 T for glutamine in the DLPFC.

In the basal ganglia, both glutamate (HR group, 1; FEP group, 2; and schizophrenia group, 1; $g = 0.63$; 95% CI, 0.15-1.11; $P = .01$) and Glx concentrations (HR group, 4; FEP group, 3; and schizophrenia group, 2; $g = 0.39$; 95% CI, 0.09-0.70; $P = .01$) (**Figure 1** and **Figure 2**) were higher in cases than in controls. Subgroup analysis of Glx found elevations in the FEP group ($g = 0.66$; 95% CI, 0.28-1.03; $P < .001$) but not in the HR

Table. Meta-analysis Results Summary for All Patient Groups in All Brain Regions

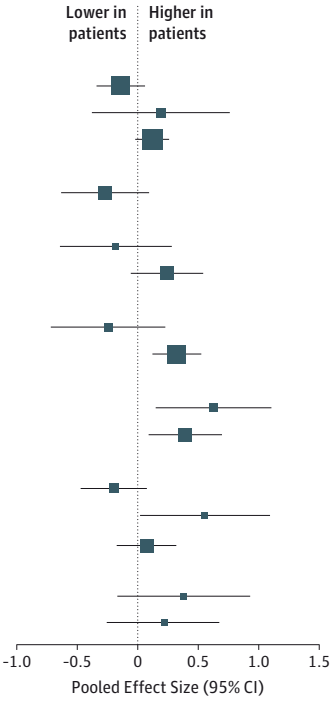
Brain Region by Metabolite	Group	Studies	Cases	Healthy Controls	Effect Size		Heterogeneity	
					95% CI	*P* Value	I^2, %	*P* Value
Medial frontal cortex								
Glutamate	All cases (3 HR, 3 FEP, 11 SZ)	17	411	381	−0.14 (−0.34 to 0.06)	.17	39.7	.05
	HR	3	102	80	−0.09 (−0.68 to 0.50)	.77	61.3	.08
	FEP (0 medicated, 3 unmedicated)	3	34	28	−0.09 (−0.46 to 0.29)	.64	12.8	.32
	SZ (11 medicated, 0 unmedicated)	11	242	233	−0.16 (−0.44 to 0.12)	.24	18.7	.04
Glutamine	All cases (0 HR, 2 FEP, 3 SZ)	5	84	78	0.19 (−0.38 to 0.76)	.52	67.7	.02
	SZ (3 medicated, 0 unmedicated)	3	50	50	−0.19 (−0.78 to 0.40)	.53	51.7	.13
Glx	All cases (8 HR, 3 FEP, 13 SZ)	24	487	440	0.12 (−0.02 to 0.27)	.10	13.1	.28
	HR	8	203	183	0.26 (0.05 to 0.46)	.01[a]	0.0	.51
	FEP (1 medicated, 2 unmedicated)	3	49	49	0.03 (−0.37 to 0.42)	.90	0.0	.37
	SZ (12 medicated, 1 unmedicated)	13	235	208	0.02 (−0.21 to 0.24)	.89	20.5	.24
DLPFC								
Glutamate	All cases (0 HR, 0 FEP, 1 SZ)	1	NA	NA	NA	NA	NA	NA
Glutamine	All cases (0 HR, 0 FEP, 0 SZ)	0	NA	NA	NA	NA	NA	NA
Glx	All cases (2 HR, 2 FEP, 8 SZ)	12	262	184	−0.27 (−0.63 to 0.09)	.15	68.6	<.001
	SZ (7 medicated, 1 unmedicated)	8	172	132	−0.32 (−0.85 to 0.21)	.23	77.6	<.001
Frontal WM								
Glutamate	All cases (1 HR, 1 FEP, 1 SZ, 1 FEP + SZ)	4	57	48	−0.18 (−0.64 to 0.28)	.44	25.6	.26
Glutamine	All cases (0 HR, 1 FEP, 0 SZ)	1	NA	NA	NA	NA	NA	NA
Glx	All cases (0 HR, 2 FEP, 7 SZ)	9	261	135	0.24 (−0.06 to 0.54)	.11	46.2	.06
	SZ (4 medicated, 3 unmedicated)	7	200	110	0.42 (0.18 to 0.66)	.001[a]	0.0	.58
MTL								
Glutamate	All cases (3 HR, 0 FEP, 3 SZ)	6	83	91	−0.24 (−0.71 to 0.24)	.33	55.3	.05
	HR	3	47	49	−0.34 (−0.86 to 0.17)	.19	30.7	.24
	SZ (2 medicated, 1 mixed)	3	36	43	−0.08 (−1.02 to 0.86)	.87	75.0	.02
Glutamine	All cases (0 HR, 0 FEP, 0 SZ)	0	NA	NA	NA	NA	NA	NA
Glx	All cases (5 HR, 5 FEP, 8 SZ)	18	441	350	0.32 (0.12 to 0.52)	.002[a]	42.0	.03
	HR	5	112	78	0.36 (−0.14 to 0.86)	.16	56.7	.06
	FEP (4 medicated, 1 unmedicated)	5	132	94	0.12 (−0.16 to 0.40)	.39	2.2	.39
	SZ (5 medicated, 3 unmedicated)	8	197	179	0.40 (0.08 to 0.71)	.01[a]	51.3	.04
Basal ganglia								
Glutamate	All cases (1 HR, 2 FEP, 1 SZ)	4	89	83	0.63 (0.15 to 1.11)	.01[a]	57.2	.07
Glutamine	All cases (0 HR, 0 FEP, 0 SZ)	0	NA	NA	NA	NA	NA	NA
Glx	All cases (4 HR, 3 FEP, 2 SZ)	9	216	182	0.39 (0.09 to 0.70)	.01[a]	51.5	.04
	HR	4	116	102	0.23 (−0.34 to 0.80)	.43	73.7	.01
	FEP (1 medicated, 2 unmedicated)	3	59	56	0.66 (0.28 to 1.03)	<.001[a]	0.0	.99
Thalamus								
Glutamate	All cases (1 HR, 2 FEP, 1 SZ)	4	125	103	−0.20 (−0.47 to 0.08)	.16	4.5	.37
Glutamine	All cases (0 HR, 2 FEP, 1 SZ)	3	50	48	0.56 (0.02 to 1.09)	.04	40.5	.19
Glx	All cases (3 HR, 2 FEP, 2 SZ)	7	240	159	0.07 (−0.17 to 0.32)	.56	24.1	.24
	HR	3	120	101	0.16 (−0.39 to 0.71)	.57	71.7	.03
Cerebellum								
Glutamate	All cases (1 HR, 2 FEP, 0 SZ)	3	59	58	0.38 (−0.17 to 0.93)	.17	54.2	.11
Glutamine	All cases (0 HR, 0 FEP, 0 SZ)	0	NA	NA	NA	NA	NA	NA
Glx	All cases (1 HR, 2 FEP, 0 SZ)	3	59	58	0.22 (−0.25 to 0.68)	.36	37.5	.20

Abbreviations: DLPFC, dorsolateral prefrontal cortex; FEP, first-episode psychosis; Glx, combined glutamate and glutamine signal; HR, high risk; MTL, medial temporal lobe; NA, not applicable; SZ, chronic schizophrenia; WM, white matter.

[a] Results that survived multiple comparisons for each region as glutamate, glutamine, and Glx were investigated.

Figure 1. Summary Effect Sizes for Glutamatergic Differences Between Cases and Controls in Each Brain Region Examined

Area	Hedges *g* (95% CI)	I^2, %
Medial frontal cortex		
Glutamate	-0.14 (-0.34 to 0.06)	40
Glutamine	0.19 (-0.38 to 0.76)	68
Glx	0.12 (-0.02 to 0.27)	13
DLPFC		
Glx	-0.27 (-0.63 to 0.09)	69
Frontal WM		
Glutamate	-0.18 (-0.64 to 0.28)	26
Glx	0.24 (-0.06 to 0.54)	46
MTL		
Glutamate	-0.24 (-0.71 to 0.24)	55
Glx	0.32 (0.12 to 0.52)	42
Basal ganglia		
Glutamate	0.63 (0.15 to 1.11)	57
Glx	0.39 (0.09 to 0.70)	52
Thalamus		
Glutamate	-0.20 (-0.47 to 0.08)	5
Glutamine	0.56 (0.02 to 1.09)	41
Glx	0.07 (-0.17 to 0.32)	24
Cerebellum		
Glutamate	0.38 (-0.17 to 0.93)	54
Glx	0.22 (-0.25 to 0.68)	38



Negative Hedges *g* values denote lower glutamatergic metabolite concentrations in cases than controls; positive values denote higher glutamatergic metabolite concentrations in cases than controls. The size of the data markers is proportional to the total number of individuals. DLPFC indicates dorsolateral prefrontal cortex; Glx, combined glutamate and glutamine signal; MTL, medial temporal lobe; and WM, white matter.

group; there were insufficient studies in patients with schizophrenia to determine the results.

In the MTL, Glx was increased in cases compared with controls (HR group, 5; FEP group, 5; and schizophrenia group, 8; g = 0.32; 95% CI, 0.12-0.52; P = .002) (Figures 1 and 2) but not glutamate (HR group, 3; FEP group, 0; and schizophrenia group, 3). Subgroup analysis found significantly higher Glx only in the schizophrenia group (g = 0.40; 95% CI, 0.08-0.71; P = .01) (Figure 2). There were no between-group differences in glutamate levels. The same results were found after excluding patients with 22q11 deletion.[16] There were insufficient data at 4 T for glutamine.

In the thalamus, glutamine concentrations were higher in cases than controls (FEP group, 2; schizophrenia group, 1; g = 0.56; 95% CI, 0.02-1.09; P = .04) (Figure 2). There were no between-group differences in glutamate (HR group, 1; FEP group, 2; and schizophrenia group, 1) or Glx (HR group, 3; FEP group, 2; and schizophrenia group, 2). No significant effects were present for glutamate or Glx in the cerebellum (HR group, 1; FEP group, 2; and schizophrenia group, 0) (Table).

Analysis Limited to Patient Groups

When analysis was limited to patients by excluding the HR group, no additional significant findings were apparent in any region (eTable 2 in the Supplement). Elevated Glx levels in MTL (g = 0.31; 95% CI, 0.09-0.53; P = .007) and basal ganglia (g = 0.57; 95% CI, 0.26-0.88; P < .001), as well as elevated glutamine levels in the thalamus (g = 0.56; 95% CI, 0.02-1.10; P = .04), remained significant; however, glutamate in the basal ganglia (P = .08) was no longer significant.

Meta-analysis Including Studies at Low Field Strength

Meta-analyses were repeated including studies that measured glutamate or glutamine at low field strengths (<3 T for glutamate[12,23,28,32] or <4 T for glutamine (References 10, 12, 13, 16, 23, 32, 35, 55, 62, 77)). Inclusion of these studies did not change the finding of no difference in glutamate levels overall in the medial frontal region, although glutamine levels were significantly elevated in cases (HR group, 1; FEP group, 3; and schizophrenia group, 7; g = 0.35; 95% CI, 0.02-0.67; P = .04; I^2 = 63%) and in the FEP group (g = 0.84; 95% CI, 0.38-1.30; P < .001; I^2 = 0%).

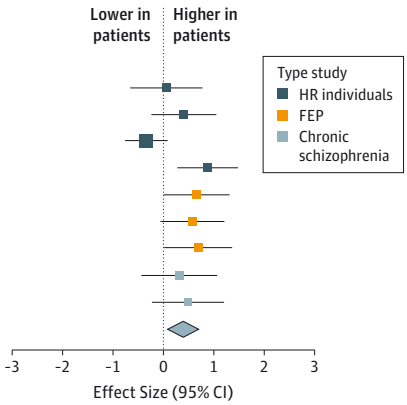
In the DLPFC, there were no significant effects for glutamate (HR group, 0; FEP group, 3; and schizophrenia group, 3) or glutamine (HR group, 0; FEP group, 2; schizophrenia group, 4) in cases compared with controls. The schizophrenia group showed elevated glutamine levels in the DLPFC (g = 0.46; 95% CI, 0.06-0.86; P = .02; I^2 = 25%), which was no longer significant when 1 study[16] in patients with a 22q11 deletion was excluded. There were no between-group differences in glutamate levels.

In the MTL, glutamine levels were increased in cases compared with controls (HR group, 1; FEP group, 1; and schizophrenia group, 2; g = 0.41; 95% CI, 0.02-0.80; P = .04; I^2 = 0%). There remained no differences in glutamate for cases or separate clinical groups (HR group, 3; FEP group, 1; and schizophrenia group, 4). The same results were found after excluding patients with a 22q11 deletion.[16]

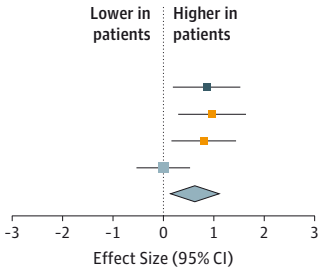
All studies of glutamate in the frontal white matter, basal ganglia, thalamus, and cerebellum were above 1.5 T. All studies of glutamine in the thalamus were at 4 T.

Figure 2. Study Effect Sizes in Brain Regions Showing Significant Glutamatergic Differences Between Cases and Controls

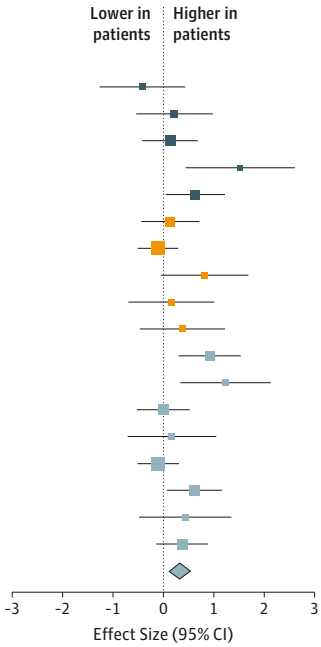
Source	Hedges *g* (95% CI)
Basal ganglia: Glx	
Block et al,[31] 2000	0.06
de la Fuente-Sandoval et al,[18] 2011	0.40
Keshavan et al,[37] 2009	-0.34
Tandon et al,[25] 2013	0.88
de la Fuente-Sandoval et al,[18] 2011	0.66
de la Fuente-Sandoval et al,[17] 2013	0.63
Goto et al,[19] 2012	0.69
Block et al,[31] 2000	0.31
Yamasue et al,[50] 2003	0.49
Summary: $g = 0.39$ (95% CI, 0.09-0.70); $I^2 = 52\%$	



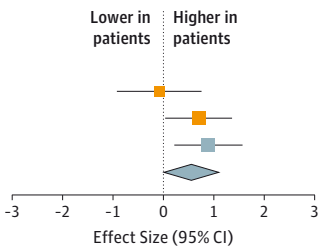
Source	Hedges *g* (95% CI)
Basal ganglia: glutamate	
de la Fuente-Sandoval et al,[18] 2011	0.86
de la Fuente-Sandoval et al,[18] 2011	0.97
de la Fuente-Sandoval et al,[17] 2013	0.80
Tayoshi et al,[55] 2009	0.00
Summary: $g = 0.63$ (95% CI, 0.15-1.11); $I^2 = 57\%$	



Source	Hedges *g* (95% CI)
Medial temporal lobe: Glx	
Capizzano et al,[75] 2011	-0.42
Capizzano et al,[75] 2011	0.22
Stone et al,[62] 2009	0.13
Wood et al,[49] 2010	1.52
Wood et al,[49] 2010	0.63
Galińska et al,[30] 2009	0.14
Hasan et al,[33] 2014	-0.11
Szulc et al,[24] 2004	0.82
Wood et al,[48] 2008	0.16
Wood et al,[48] 2008	0.38
Chang et al,[14] 2007	0.92
da Silva Alves et al,[16] 2011	1.23
Hutcheson et al,[34] 2012	0.00
Kegeles et al,[36] 2000	0.17
Kraguljac et al,[38] 2012	-0.10
Kraguljac et al,[21] 2013	0.61
Szulc et al,[24] 2004	0.43
Szulc et al,[43] 2011	0.37
Summary: $g = 0.32$ (95% CI, 0.12-0.52); $I^2 = 42\%$	



Source	Hedges *g* (95% CI)
Thalamus: glutamine	
Bustillo et al,[76] 2010	-0.08
Théberge et al,[9] 2002	0.70
Théberge et al,[54] 2003	0.89
Summary: $g = 0.56$ (95% CI, 0.02-1.09); $I^2 = 41\%$	



Each data marker represents a study, and the size of the data marker is proportional to the total number of individuals in that study. The summary effect size for each brain region is denoted by a blue diamond. FEP indicates first-episode psychosis; Glx, combined glutamate and glutamine signal; and HR, high risk.

Heterogeneity

Significant heterogeneity was found in numerous groups for all regions (Table), which justifies the use of a random-effects model to combine the effect sizes. Heterogeneity may result from methodologic differences (eTable 3 in the Supplement). Meta-regression analysis investigated possible sources of heterogeneity.

Meta-regression

The clinical and demographic measures that were available from each study for meta-regression are presented in eTable 1 in the Supplement. In all brain regions, there were no significant correlations between the study effect sizes for glutamate, glutamine, or Glx and the mean PANSS subscale scores, chlorpromazine-equivalent dose, and duration of psychotic illness of patients with FEP or schizophrenia.

In all brain regions, there was no association between the mean age of the patients and the effect size for glutamate, glutamine, or Glx.

Small-Study Bias

Small-study bias, which could reflect publication bias, was evident for reports of Glx in the medial frontal region (Egger test, $P = .01$) and Glx in the basal ganglia ($P = .04$). The year of publication was not significantly associated with metabolite reports in any brain region.

Sensitivity Analysis

Leave-1-out sensitivity analysis showed that significant results were generally robust. Significant differences did not remain in 2 of 3 tests for cases of glutamine in the thalamus, indicating an unreliable result.

Discussion

The number of publications reporting proton MRS measures of brain glutamate, glutamine, or Glx in schizophrenia has more than doubled since the last meta-analysis.[65] In addition to analyzing data from a large number of new studies, we were able to include findings from brain regions precluded from previous meta-analysis in the present study.

We found significant differences in glutamatergic metabolites across several cortical and subcortical regions in cases compared with controls. Although the nature of the findings varied depending on the patient subgroup and brain region, all of the significant findings reflected elevations in glutamatergic metabolites in patients and HR individuals. This finding is consistent with data from animal models of schizophrenia that propose an increase in glutamatergic activity resulting from NMDAR hypofunction.[66] The finding is also consistent with human studies of NMDAR hypofunction that show increases in both glutamate and glutamine concentrations in the cortex following ketamine administration to healthy volunteers.

The HR, FEP, and schizophrenia groups had higher glutamate and Glx levels in the basal ganglia, higher glutamine levels in the thalamus, and higher Glx levels in the MTL. In contrast, there were generally no significant findings in the DLPFC or cerebellum, and significant findings in the medial frontal cortex and frontal white matter were observed only in specific patient subgroups. Preclinical models propose that glutamatergic overactivity in hippocampal areas drive excessive subcortical dopamine release via polysynaptic glutamatergic projections to the striatum. Likewise, abnormalities in striatal glutamate may influence striatal dopaminergic signaling since glutamate in the basal ganglia modulates tonic dopamine release presynaptically via NMDARs. Finally, the thalamus receives efferent input from the striatum,[78] and NMDAR antagonism in the thalamus causes cortical neurotoxic injury via corticothalamic loops.

In all regions except the basal ganglia, the HR, FEP, and schizophrenia groups had significant elevations in the Glx or glutamine signal rather than the glutamate signal, which may partially reflect the greater number of studies reporting on Glx rather than glutamate or glutamine separately. Although the glutamate signal accounts for most (80%-90%) of the Glx signal at field strengths of 1.5 T to 3 T,[6] it is possible that Glx level elevations could be driven by increases in glutamine rather than glutamate levels. Following neurotransmission, glutamate is converted to glutamine in astrocytes for recycling. Elevations in glutamine levels may thus reflect increases in glutamatergic synaptic activity. The previous meta-analysis[27] reported reduced medial frontal glutamate but elevated glutamine levels in schizophrenia that were not detected in the present analysis. This discrepancy may reflect improved methods in more recent studies since more studies acquired data at higher field strengths, correct for voxel cerebrospinal fluid, and specify more conservative thresholds for the acceptability of metabolite fitting (eTable 3 in the Supplement).

There was some evidence that the regional degree of glutamatergic elevation may be sensitive to illness stage. The Glx level elevations in the medial frontal cortex were apparent in HR individuals but not in those with schizophrenia. Similarly, medial frontal glutamine levels were elevated in 2 studies[9,76] of patients with FEP, but no differences were seen in patients with schizophrenia. Conversely, MTL Glx levels were elevated in individuals with schizophrenia but not in the FEP or HR groups. One interpretation of these findings is that the regional pattern of glutamatergic abnormalities progress with the clinical course of the disorder or show differential responses to antipsychotic treatment. Most HR individuals will not develop psychosis and were not receiving antipsychotic medication; exclusion of this group generally resulted in similar effect sizes. This observation suggests that inclusion of HR groups did not dilute the findings in patients and that the same pattern of glutamatergic-level elevation is apparent in individuals at HR for psychosis. Few studies have directly compared different patient groups[23,24,57-59] or repeatedly assessed glutamatergic metabolites over long periods.[79] Interpretation was limited because there were insufficient data to analyze all glutamatergic measures for each patient group separately in every region.

The meta-regression did not find support for the hypothesis that glutamatergic metabolite concentrations in patients vary in association with age, antipsychotic treatment, or symp-

tom severity. The latter may be relevant to the interpretation of cross-sectional studies comparing regional glutamatergic measures in association with antipsychotic treatment response[8] because this finding suggests that such differences may not simply involve group differences in symptom severity. Detailed information on antipsychotic treatment was available in few data sets, and mean chlorpromazine-equivalent doses may not account for medication adherence and do not discriminate between the effects of different antipsychotic medications. Longitudinal MRS studies have not found effects of antipsychotic treatment on medial frontal glutamatergic measures,[76,79] although medication effects in the striatum have been reported.[17] One previous study[20] reported higher medial frontal Glx in patients not receiving vs receiving medications. The meta-analysis found higher medial frontal glutamine (but not glutamate or Glx) levels in patients with FEP, all of whom were not receiving medications.

One limitation of the present meta-analysis is that, when clinical groups were analyzed separately, the number of studies per group for some regions was small. We conducted the meta-analysis when at least 3 independent data sets were available; however, findings based on a low number of data sets should be considered preliminary and are presented to stimulate further research. Increases in Glx levels in the medial frontal cortex in HR individuals and increases in MTL Glx levels in patients with schizophrenia were reported by relatively large numbers of studies; thus, these investigations represent the most robust of the findings in patient subgroups.

Our HR category included studies of people at increased familial risk for schizophrenia as well as those showing subclinical signs of psychosis since there were too few studies to permit separate meta-analyses of each group. The risk of psychosis differs between these groups, and this heterogeneity may explain why we did not find elevated Glx levels in the basal ganglia and lower glutamate levels in the thalamus that have been reported[52] in clinical HR individuals. In addition, glutamate may be increased only in HR persons who will later develop psychosis or show poorer outcomes.[52]

The resonance frequencies of glutamate and glutamine significantly overlap at 1.5 T, whereas glutamate can be largely resolved at 3 T, and field strengths of 4 T or more are needed to measure glutamine accurately.[6] For glutamine reports, a sufficient number of studies were performed at 4 T only in the thalamus. Given that glutamine may provide a measure of glutamate turnover and our general finding of increased glutamine or Glx rather than glutamate in patients, additional studies at higher field strengths optimized for glutamine resolution should be a priority. Another limitation of this study is that there was much variability in data acquisition and analysis methods (eTable 3 in the Supplement), which will affect data quality. Full reporting of such information in future studies will help to address sources of heterogeneity in subsequent meta-analyses.

Use of proton MRS provides a measure of total concentrations within the voxel studied and thus does not infer the functional significance of the metabolites measured. However, glutamine can act as an indirect measure of neurotransmitter glutamate turnover since 80% of glutamine is used for glutamate neurotransmitter cycling.[7]

Conclusions

This meta-analysis indicates that schizophrenia is associated with glutamatergic-level elevations in several brain regions. These findings further support the idea that pharmacologic compounds that can reduce glutamatergic neurotransmission may have therapeutic potential.

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