Archival Report

Glutamatergic Neurometabolite Levels in Patients With Ultra-Treatment-Resistant Schizophrenia: A Cross-Sectional 3T Proton Magnetic Resonance Spectroscopy Study

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ABSTRACT

BACKGROUND: In terms of antipsychotic treatment response, patients with schizophrenia can be classified into three groups: 1) treatment resistant to both non-clozapine (non-CLZ) antipsychotics and CLZ (ultra-treatment-resistant schizophrenia [URS]), 2) treatment resistant to non-CLZ antipsychotics but CLZ-responsive schizophrenia [non-URS]), and 3) responsive to first-line antipsychotics (non-treatment-resistant schizophrenia). This study aimed to compare glutamatergic neurometabolite levels among these three patient groups and healthy control subjects using proton magnetic resonance spectroscopy.

METHODS: Glutamate and glutamate+glutamine levels were assessed in the caudate, the dorsal anterior cingulate cortex (dACC), and the dorsolateral prefrontal cortex using 3T proton magnetic resonance spectroscopy (point-resolved spectroscopy, echo time = 35 ms). Glutamatergic neurometabolite levels were compared between the groups.

RESULTS: A total of 100 participants were included, consisting of 26 patients with URS, 27 patients with non-URS, 21 patients with non-treatment-resistant schizophrenia, and 26 healthy control subjects. Group differences were detected in ACC glutamate+glutamine levels ($F_{3,96} = 2.93$, $p = .038$); patients with URS showed higher dACC glutamate+glutamine levels than healthy control subjects ($p = .038$). There were no group differences in the caudate or dorsolateral prefrontal cortex.

CONCLUSIONS: Taken together with previous studies that demonstrated higher ACC glutamate levels in patients with treatment-resistant schizophrenia, this study suggests that higher levels of ACC glutamatergic metabolites may be among the shared biological characteristics of treatment resistance to antipsychotics, including CLZ.

Keywords: Antipsychotic, Clozapine, Glutamate, ^{1}H-MRS, Schizophrenia, Treatment-resistant

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Schizophrenia is a debilitating illness that affects approximately 1% of the global population (1). Currently, the primary treatment for schizophrenia involves dopamine receptor antagonism by antipsychotics (2). The clinical effects of antipsychotics have provided the basis for the dopamine hypothesis of schizophrenia (3), which posits that aberrant dopaminergic function is implicated in schizophrenia pathophysiology (4). However, approximately 20% to 35% of patients with schizophrenia do not respond to first-line antipsychotics and are thus considered to have treatment-resistant schizophrenia (5).

Clozapine (CLZ) is the most effective antipsychotic for treatment-resistant schizophrenia. In contrast to other antipsychotics, CLZ has lower affinity for dopamine D_2 receptors (6). That it is effective when other D_2 antagonists are not suggests that the clinical efficacy of CLZ in treatment-resistant schizophrenia may be associated with other mechanisms as well. Moreover, two studies employing [^{18}F]-DOPA positron emission topography have demonstrated lower dopamine synthesis capacity in the striatum of patients with treatment-resistant schizophrenia compared with patients with treatment-responsive schizophrenia (7,8). Taken together, these findings suggest that the pathophysiology of treatment-resistant schizophrenia might not be associated with increased striatal dopamine levels.

The glutamatergic hypothesis is an alternative mechanism by which to explain the pathophysiology of schizophrenia (9,10). A recent meta-analysis was conducted by Merritt *et al.* to examine glutamatergic neurometabolite levels, as measured by proton magnetic resonance spectroscopy (^{1}H-MRS), in patients with schizophrenia (11). The authors reported that there were elevations in levels of glutamate (Glu) and

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glutamate+glutamine (Glx) in the basal ganglia and Glx levels in the medial temporal lobe within this patient population, suggesting that schizophrenia may be associated with elevations in levels of glutamatergic neurometabolites across several brain regions. However, it is noteworthy that these findings considered participants within stages of illness that included the high-risk state, first-episode psychosis (FEP), and chronic schizophrenia. The authors also conducted subgroup analyses and found increased medial frontal Glx in high-risk individuals, elevated basal ganglia Glx levels in patients with FEP, and increased frontal white matter and medial temporal Glx levels in chronic schizophrenia.

Moreover, in patients with treatment-resistant schizophrenia, one group noted elevated Glu levels in the anterior cingulate cortex (ACC) of this population compared with healthy control subjects (HCs) and responders to first-line antipsychotics (12,13). The results put forth by this group suggest a link between elevated levels of glutamatergic neurometabolites and treatment-resistant schizophrenia.

Importantly, it has also been reported that 40% to 70% of patients with treatment-resistant schizophrenia do not respond to CLZ (14,15). This subset of patients is considered to have ultra-treatment-resistant schizophrenia (URS), and this distinction in clinical response implies that pathophysiological mechanisms may be different between URS and CLZ-responsive treatment-resistant schizophrenia (non-URS).

To date, one study compared levels of Glu and Glx among patients with URS, non-URS, responders to first-line antipsychotics (non-treatment-resistant schizophrenia [nTRS]), and HCs (16). This study found that Glx levels in the dorsolateral prefrontal cortex (DLPFC) were lower in patients with URS than in patients with nTRS, while Glx levels in the putamen were higher in patients with URS than in patients with non-URS and patients with nTRS. However, in this study, the authors differentiated URS from non-URS when patients had add-on antipsychotics on top of CLZ. In addition, they included patients with, at most, mild illness severity in both the URS and non-URS to minimize the state effects of symptom severity. However, this study did not consider past treatment failure before CLZ treatment; patients might have started CLZ owing to side effects or intolerance to the previous medications. Thus, it was not clear whether patients with mildly ill URS benefit from CLZ or add-on antipsychotics.

To further elucidate the relationships between glutamatergic neurometabolites and treatment-resistant schizophrenia, the current study included both symptomatic and nonsymptomatic patients with treatment-resistant schizophrenia undergoing CLZ monotherapy. More specifically, we classified patients into three groups regarding their antipsychotic treatment response: 1) URS, 2) non-URS, and 3) nTRS. In addition, we used existing guidelines to establish treatment resistance to non-CLZ antipsychotics (17). The primary aim of this study was to compare glutamatergic neurometabolite levels in the caudate, dorsal ACC (dACC), and DLPFC among the patient groups and HCs. Of note, the caudate voxel was placed on the dorsal part of the caudate to represent the functional striatum subdivision of the associative striatum, where a recent meta-analysis demonstrated most significant increases of presynaptic dopamine function in patients with schizophrenia compared with HCs (18). In addition, previous ^{1}H-MRS studies have demonstrated increased Glu levels in patients with FEP and in subjects at ultra-high risk for psychosis in the associative striatum (19,20). The secondary aim of this study was to explore the relationships between glutamatergic neurometabolite levels and symptom severity.

METHODS AND MATERIALS

Participants

This single-center cross-sectional study was conducted at the Centre for Addiction and Mental Health between 2014 and 2017. All the participants have been treated within the regular clinical practice at the Centre for Addiction and Mental Health. Patients met inclusion criteria if they had a DSM-IV/Structured Clinical Interview for DSM (21) diagnosis of schizophrenia spectrum disorders. Antipsychotic treatment resistance was defined by the modified Treatment Response and Resistance in Psychosis Working Group Consensus criteria (17). Optimal antipsychotic treatment was defined as ≥6 consecutive weeks with ≥400 mg of chlorpromazine (CPZ) equivalent daily dose antipsychotic treatment (22). Treatment response was defined by 1) a Clinical Global Impression Severity (CGI-S) (23) score of ≤3, 2) scores of ≤3 on all positive symptom items of the Positive and Negative Syndrome Scale (PANSS) (24), and 3) no symptomatic relapse during the previous 3 months. Antipsychotic treatment failure was defined by 1) a CGI-S score of ≥4 (moderate) and 2) a score of ≥4 (moderate) on two PANSS positive symptom items after optimal antipsychotic treatment. To establish failure to previous antipsychotic treatments, CGI-S scores were retrospectively collected based on available information provided by the patients, their psychiatrists, the chart, or other sources. The CGI-S scores were independently determined by two investigators (YI and SN). If there were discrepancies between them, we further discussed with another investigator (AG-G) to reach a consensus on group classification.

More specifically, URS criteria included 1) current intake of CLZ, 2) a history of treatment failure to optimal treatment with at least two previous non-CLZ antipsychotics, and 3) subsequent treatment failure with CLZ after patients had taken CLZ for ≥6 weeks at a minimum dose of 300 mg/day. Non-URS criteria included 1) current intake of CLZ, 2) a history of treatment failure to optimal treatment with at least two previous non-CLZ antipsychotics, and 3) subsequent treatment response to CLZ. nTRS criteria included 1) current intake of a non-CLZ single antipsychotic and 2) treatment response. Lastly, HCs met inclusion criteria if they had no history of psychiatric illness as assessed by the Mini-International Neuropsychiatric Interview (25). Exclusion criteria for all groups consisted of 1) substance abuse or dependence during the past 6 months, 2) positive urine drug screen at inclusion or prior to magnetic resonance imaging (MRI) scanning, 3) history of head trauma resulting in loss of consciousness >30 minutes, 4) unstable physical illness or neurological disorder, and 5) current administration of lamotrigine, topiramate, or memantine. The patient groups and HCs were matched as closely as possible for age and sex. The sample size was calculated based on a previous study comparing Glu levels between patients with FEP versus HCs (19). Using that study, it was determined that 20 subjects in each group would provide at least 85% power to detect the expected difference in Glu levels with an alpha = .05.

Magnetic Resonance Imaging

All participants were scanned at the Centre for Addiction and Mental Health in a 3T GE Discovery MR750 scanner equipped with an eight-channel head coil. Participants had a three-dimensional inversion recovery–prepared T1-weighted MRI scan (BRAVO; GE Healthcare, Wauwatosa, WI) (echo time = 3.00 ms, repetition time = 6.74 ms, inversion time = 650 ms, flip angle = 8°, field of view = 230 mm, 256 × 256 matrix, slice thickness = 0.9 mm).

^{1}H-MRS Acquisition and Data Processing

^{1}H-MRS was collected using point-resolved spectroscopy (echo time = 35 ms, repetition time = 2000 ms, spectral width = 5000 Hz, 4096 datapoints, 128 water-suppressed and 16 water-unsuppressed averages, 8 number of excitations). Shimming was performed to achieve a full width at half maximum ≤ 12 Hz measured on the unsuppressed water signal from the voxel. ^{1}H-MRS voxels were placed in the left dorsal caudate (associative striatum) (voxel size = 7.5 mL), bilateral dACC (voxel size = 9.0 mL), and left DLPFC (voxel size = 13.5 mL). The detailed voxel placement procedures, locations of the ^{1}H-MRS voxels, and representative spectra are provided in Figures 1 and 2. Water-suppressed spectra were analyzed using LCModel version 6.3-0E (26). Metabolite levels were estimated with a field appropriate basis set with matching echo time (= 35 ms) provided by LCModel. Then, metabolite levels were expressed in institutional units by normalizing each metabolite's peak area to the peak area of the unsuppressed water signal. The metabolite levels were expressed as institutional units. In the current work, neurometabolites of interest included Glu and Glx. As supplemental neurometabolites, myo-inositol, glycerophosphocholine+phosphocholine, *N*-acetylaspartate+*N*-acetylaspartylglutamate, and creatine+phosphocreatine were also collected. All the LCModel spectrum outputs were

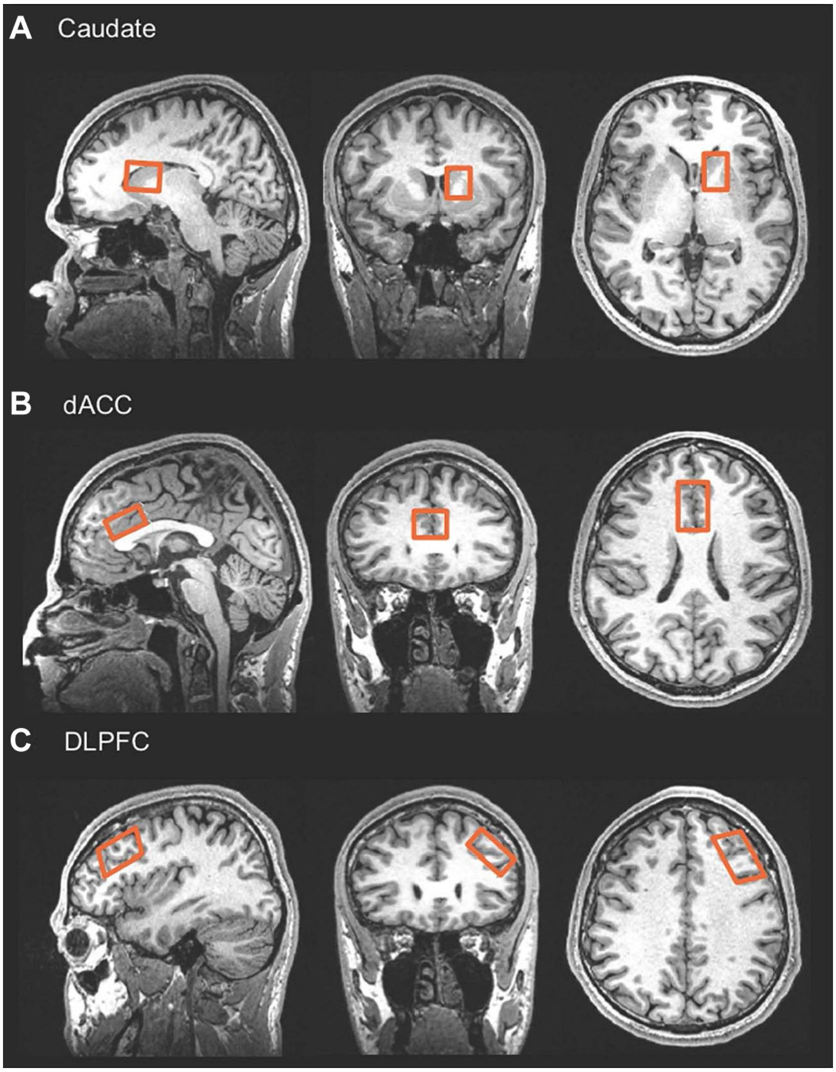

Figure 1. Voxel locations and placement procedures of the proton magnetic resonance spectroscopy voxels. **(A)** The dorsal caudate voxel was positioned on an oblique axial image acquired parallel to the anterior commissure–posterior commissure (AC-PC) line; the voxel was 7.5 mL (2.5 × 1.5 × 2.0 cm^3), and its center was 14 mm superior to the AC-PC line. **(B)** The dorsal anterior cingulate cortex (dACC) voxel (voxel size: 9.0 mL [3.0 × 2.0 × 1.5 cm^3]) was positioned on an oblique axial image acquired parallel to the AC-PC line and an oblique sagittal image acquired to parallel to head midline. The tip of the voxel was placed on top of the most anterior part of genu with paralleling to the cingulate cortex. **(C)** For the dorsolateral prefrontal cortex (DLPFC) voxel (voxel size: 13.5 mL [3.0 × 3.0 × 1.5 cm^3]), in AC-PC space markers were placed on the most anterior point of the frontal pole and the tip of the temporal pole. Using these markers, the posterior and anterior boundaries of the DLPFC were determined as follows: the average distance from the tip of the temporal lobe to the posterior vertical boundary was 20% of ~50 mm, or ~10 mm; the average distance from the tip of the frontal pole and the anterior vertical boundary was 40% of ~50 mm, or ~20 mm.

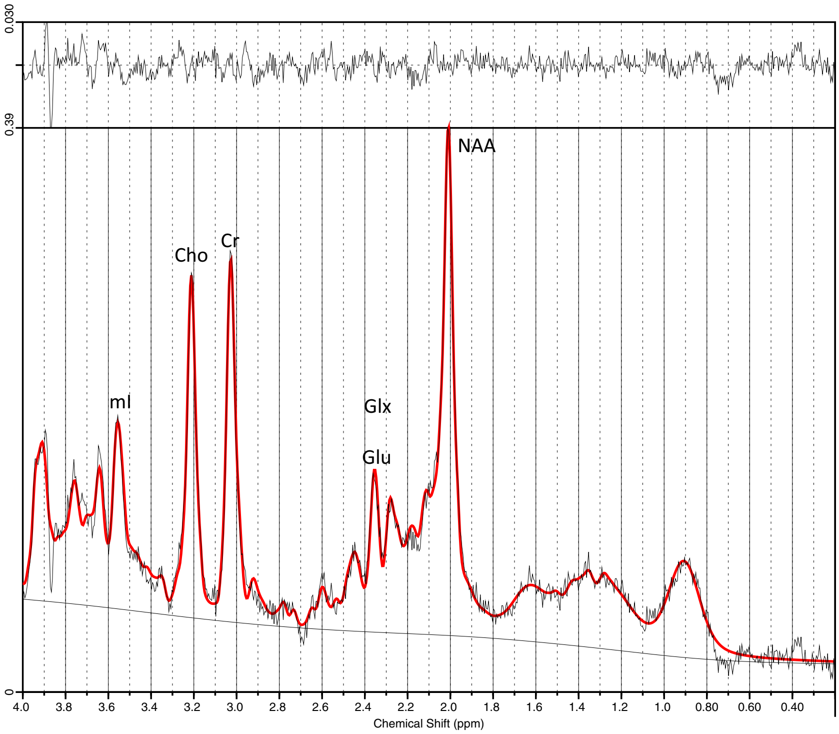

Figure 2. Representative spectra. Cho, glycerophosphocholine+phosphocholine; Cr, creatine+phosphocreatine; Glu, glutamate; Glx, glutamate+glutamine; mI, myo-inositol; NAA, *N*-acetylaspartate+*N*-acetylaspartylglutamate; ppm, parts per million.

visually checked. Percentage SD values ≥ 20% were deemed poor quality and excluded from subsequent analyses.

T1-weighted MRI scans were segmented into gray matter (GM), white matter, and cerebrospinal fluid using the FIRST tool (27) (version 5.0; FMRIB Software Library, Oxford, UK) (28). A MATLAB-based software package (The MathWorks, Inc., Natick, MA) named Gannet (http://www.gabamrs.com) was used to create a mask of the voxel size and location on the segmented T1-weighted image while using the spatial coordinates in the scanner, permitting correction of neurometabolite levels for fraction of cerebrospinal fluid in the region of interest (ROI) (19).

Statistical Analysis

Statistical analyses were performed using SPSS Statistics version 21 (IBM Corp., Armonk, NY). Clinicodemographic characteristics and spectrum quality indices (Cramer–Rao lower bound values, full width at half maximum values, and signal-to-noise ratios) and GM ratios within ^{1}H-MRS voxel brain tissue (GM/[GM+white matter]) were compared between groups using analyses of variance for continuous variables and chi-square tests for categorical variables. The Shapiro-Wilk test was performed to test normality of neurometabolite data. The relationships between glutamatergic neurometabolite levels, clinicodemographic characteristics, and GM ratios were examined within each group. A significance level of $p < .05$/number of comparisons was used.

For primary analyses, group differences in glutamatergic neurometabolite levels were examined. First, glutamatergic neurometabolite levels were compared between groups using an analysis of variance. Then, analyses of covariance were performed while controlling for age, GM ratio, and spectrum quality values that were significantly different between groups. Additional analyses for the patient groups were also performed using an analysis of variance while controlling for smoking status and CPZ daily dose individually. To further examine the effects of symptom severity on glutamatergic neurometabolite levels, we compared Glu and Glx levels between symptomatic patients (URS) and nonsymptomatic patients (combination of non-URS and nTRS). On the basis of previous literature reporting higher glutamatergic neurometabolite levels in the ACC of patients with TRS (12,29), dACC Glu- and Glx-level group comparisons used a significance level of $p < .05$. For comparisons involving the caudate and DLPFC, we used an adjusted significance level ($p < .025$ [= .05/2 ROIs]). Planned post hoc comparisons were performed using the Bonferroni method to correct for multiple comparisons. We hypothesized that the URS group would have higher levels of glutamatergic neurometabolite in the dACC, representing a neurobiological trait of treatment resistance.

For secondary analyses, correlations were explored to examine the association between symptom severity scales and glutamatergic neurometabolite levels using a significance threshold of $p < .01$ (.05/5) owing to the number of comparisons (PANSS total and subscale scores and CGI-S scores) in the whole patient sample. The inclusion of age, sex, tobacco use, and CPZ daily dose as covariates was also tested.

RESULTS

Characteristics of Participants

A total of 100 participants were included in this study, consisting of 26 patients with URS, 27 patients with non-URS, 21 patients with nTRS, and 26 HCs. Characteristics of the participants are presented in Table 1. HCs showed a lower ratio of tobacco use and more years of education compared with the patient groups. CLZ doses were higher in the URS group compared with the non-URS group. The URS group showed higher symptom severity scores compared with the non-URS and nTRS groups. In the CLZ-treated patient groups, duration of illness before CLZ initiation was longer in the URS group than in the non-URS group. One caudate Glu datapoint in a patient with URS was excluded owing to high percentage SD (>20%). Three participants (1 URS and 2 non-URS) and 10 participants (5 URS and 5 non-URS) did not complete scans for the caudate and DLPFC, respectively. Full width at half maximum values in the DLPFC were higher in HCs than in the URS and non-URS groups. GM ratios were higher in the nTRS group compared with the URS and non-URS groups in the caudate and were higher in the DLPFC compared with the URS group. Spectrum qualities and GM ratios are displayed in Supplemental Table S1.

Glutamatergic Neurometabolite Levels Between the Groups

The relationships between participants' clinicodemographic characteristics and glutamatergic neurometabolite levels are displayed in Supplemental Table S2. Male subjects had higher levels of Glu and Glx in the dACC than female subjects in the URS group. GM ratios were associated with glutamatergic neurometabolite levels in the dACC of the URS group and in the DLPFC of the URS, non-URS, and HC groups. In the patients with non-URS, years of CLZ treatment were negatively correlated with DLPFC Glu levels.

Figure 3 and Table 2 show comparisons of neurometabolite levels between groups. A significant group difference was found in dACC Glx levels between groups. Post hoc analyses revealed higher dACC Glx levels in patients with URS compared with HCs, while no other differences were found. The results remained significant after controlling for age and GM ratio. There was a trend toward significant differences in dACC Glu levels between groups, and the comparison became significant after controlling for GM ratio. In the caudate and DLPFC, there were no group differences in Glu or Glx levels.

Regarding the comparison among three patient groups, there were no significant differences between groups regardless of whether tobacco use or CLZ daily dose was included as a covariate for all ROIs (Supplemental Table S3). Supplemental Table S4 depicts the comparisons of other neurometabolites and Cramer–Rao lower bound values between groups. Lastly, no differences were observed in Glu and Glx levels between

Table 1. Characteristics of Participants

	URS (*n* = 26)[a]	Non-URS (*n* = 27)[a]	nTRS (*n* = 21)[a]	HCs (*n* = 26)	ANOVA or Chi-Square	
					F Value or *df*	*p* Value
Age, Years	45.1 (±12.9)	40.5 (±11.2)	46.3 (±12.7)	40.8 (±13.2)	$F_{3,96}$ = 1.35	.26
Sex, Female	5 (19.2)	8 (29.6)	5 (23.8)	7 (26.9)	3	.84
Tobacco Use	11 (42.3)	12 (44.4)	13 (61.9)	1 (3.8)	3	< .001[b]
Education, Years	12.3 (±2.4)	12.7 (±2.9)	13.1 (±2.5)	15.6 (±2.0)	$F_{3,96}$ = 9.21	< .001[c]
Schizophrenia	19 (73.1)	17 (63.0)	15 (71.4)		2	.70
Schizoaffective	7 (26.9)	10 (37.0)	6 (28.6)			
Age of Onset, Years	21.4 (±5.1)	24.0 (±6.9)	25.9 (±7.7)		$F_{2,71}$ = 2.78	.07
DUI, Years	23.6 (±12.7)	16.4 (±9.7)	20.0 (±12.2)		$F_{2,71}$ = 2.57	.08
CPZ Dose, mg/day	643.2 (±179.2)	527.1 (±201.7)	443.1 (±188.1)		$F_{2,71}$ = 6.60	.002[d]
CLZ Dose, mg/day	428.8 (±119.5)	351.4 (±134.5)				
PANSS Total Score	82.8 (±11.7)	56.1 (±10.9)	57.2 (±9.5)		$F_{2,71}$ = 49.72	< .001[e]
Positive subscale	22.5 (±3.9)	11.5 (±2.0)	10.9 (±2.3)		$F_{2,71}$ = 127.60	< .001[e]
Negative subscale	20.9 (±4.2)	16.1 (±4.8)	16.0 (±3.6)		$F_{2,71}$ = 10.61	< .001[f]
General subscale	39.5 (±7.0)	28.5 (±5.6)	30.3 (±4.7)		$F_{2,71}$ = 26.03	< .001[e]
CGI-S	4.6 (±0.8)	2.8 (±0.4)	2.7 (±0.5)		$F_{2,71}$ = 90.91	< .001[e]
Duration of CLZ, Years	8.6 (±5.6)	7.7 (±6.1)[g]			$F_{1,50}$ = 0.27	.60
DUI before CLZ, Years	15.0 (±11.7)	9.0 (±8.8)[g]			$F_{1,50}$ = 4.34	.04[h]

Values are mean (±SD) or *n* (%).

ANOVA, analysis of variance; CGI-S, Clinical Global Impression Severity scale; CLZ, clozapine; CPZ, chlorpromazine; DUI, duration of illness; HCs, healthy control subjects; LAI, long-acting injection; nTRS, non-treatment-resistant schizophrenia; PANSS, Positive and Negative Syndrome Scale; URS, ultra-treatment-resistant schizophrenia.

[a]Antipsychotics: All URS and non-URS participants were currently taking CLZ. nTRS participants were currently taking flupenthixol (*n* = 1), haloperidol (*n* = 2), loxapine (*n* = 1), olanzapine (*n* = 8), paliperidone (*n* = 1), risperidone (*n* = 1), ziprasidone (*n* = 1), flupenthixol LAI (*n* = 2), fluphenazine LAI (*n* = 1), paliperidone LAI (*n* = 1), or risperidone LAI (*n* = 2).

[b]Bonferroni-corrected *p* value < .05: URS > HCs (*p* = .006), non-URS > HCs (*p* = .004), nTRS > HCs (*p* < .001).

[c]Bonferroni-corrected *p* value < .05: HCs > URS (*p* < .001), HCs > non-URS (*p* < .001), HCs > nTRS (*p* = .006).

[d]Bonferroni-corrected *p* value < .05: URS > nTRS (*p* = .002).

[e]Bonferroni-corrected *p* value < .05: URS > non-URS (*p* < .001), URS > nTRS (*p* < .001).

[f]Bonferroni-corrected *p* value < .05: URS > non-URS (*p* < .001), URS > nTRS (*p* = .001).

[g]Unclear data for 1 participant.

[h]The result changed after excluding the participants who did not complete the caudate scans (*p* = .09) and the dorsolateral prefrontal cortex scans (*p* = .13).

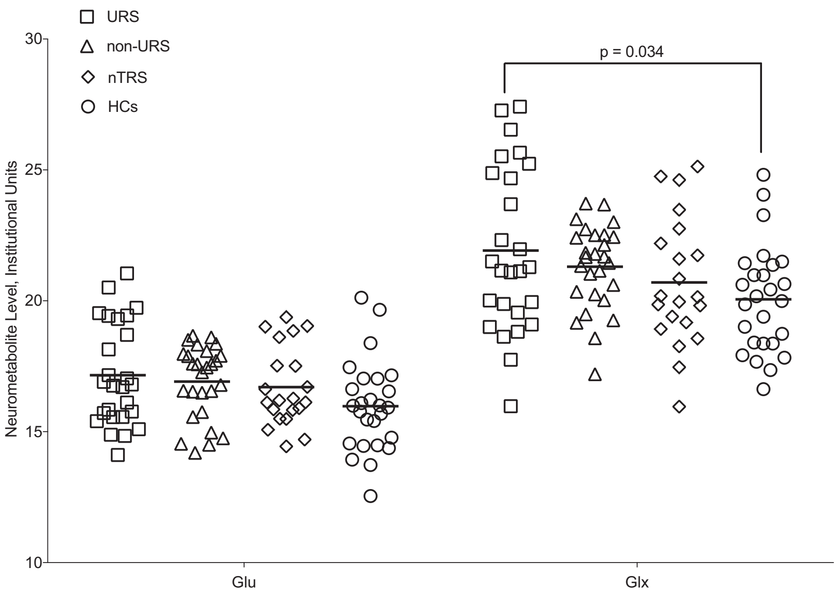

Figure 3. Glutamatergic neurometabolite levels in the dorsal anterior cingulate cortex. Glu, glutamate; Glx, glutamate+glutamine; HCs, healthy control subjects; nTRS, non-treatment-resistant schizophrenia; URS, ultra-treatment-resistant schizophrenia.

the symptomatic patients with URS and nonsymptomatic patients (combination of non-URS and nTRS) in all ROIs (Supplemental Table S5).

Psychopathological Scale and Its Relationships to Glutamatergic Neurometabolite Levels

Glu and Glx levels were not related to PANSS total or subscale scores and CGI-S scores in the whole patient sample (Supplemental Table S6). Results remained unchanged after controlling for age, sex, tobacco use, and CPZ daily dose.

DISCUSSION

This is the first ^{1}H-MRS study comparing glutamatergic neurometabolite levels in the caudate, dACC, and DLPFC among patients with symptomatic URS, non-URS, and nTRS as well as HCs. This study included a larger number of participants compared with the study by Goldstein *et al.* (100 vs. 58) (16). Our findings are as follows. First, dACC Glx levels were higher in patients with URS than in HCs (Cohen's d = 0.64). Second, no differences were observed in glutamatergic neurometabolite levels in the caudate, dACC, and DLPFC among patients with URS, non-URS, and nTRS. Third, there were no differences between symptomatic patients (URS) and nonsymptomatic patients (combination of non-URS and nTRS) in terms of glutamatergic neurometabolite levels in any of the ROIs. Fourth, glutamatergic neurometabolite levels were not related to symptom severity scores in the patient groups. Finally, negative associations were found between Glu levels in the DLPFC and years of CLZ treatment in patients with non-URS.

Table 2. Glutamatergic Neurometabolite Levels Between the Groups

					ANOVA		ANCOVA, Age Covariate		ANCOVA, GM/(GM + WM) Covariate		ANCOVA, FWHM Covariate	
	URS (n = 26)	non-URS (n = 27)	nTRS (n = 21)	HCs (n = 26)	F Value	p Value	F Value	p Value	F Value	p Value	F Value	p Value
Caudate	n = 25	n = 25	n = 21	n = 26								
Glu	11.36 (1.71)[a]	11.95 (1.74)	12.13 (1.78)	11.50 (2.20)	$F_{3,92}$ = 0.87	.46	$F_{3,91}$ = 0.97	.41	$F_{3,91}$ = 0.86	.47		
Glx	16.34 (2.85)	16.21 (1.87)	16.90 (2.32)	15.76 (2.20)	$F_{3,93}$ = 0.94	.43	$F_{3,92}$ = 0.99	.40	$F_{3,92}$ = 0.79	.50		
dACC	n = 26	n = 27	n = 21	n = 26								
Glu	17.16 (1.97)	16.92 (1.39)	16.70 (1.51)	15.98 (1.74)	$F_{3,96}$ = 2.42	.07	$F_{3,95}$ = 2.40	.07	$F_{3,95}$ = 2.75	.047		
Glx	21.92 (3.14)	21.30 (1.61)	20.71 (2.46)	20.06 (2.06)	$F_{3,96}$ = 2.93	.038[b]	$F_{3,95}$ = 2.79	.045	$F_{3,95}$ = 3.52	.018		
DLPFC	n = 21	n = 22	n = 21	n = 26								
Glu	13.55 (1.60)	13.87 (1.62)	14.65 (1.26)	13.94 (1.32)	$F_{3,86}$ = 1.81	.15	$F_{3,85}$ = 1.85	.14	$F_{3,85}$ = 0.13	.94	$F_{3,85}$ = 1.51	.22
Glx	16.96 (2.50)	17.12 (2.13)	18.05 (1.80)	17.00 (1.68)	$F_{3,86}$ = 1.40	.25	$F_{3,85}$ = 1.31	.28	$F_{3,85}$ = 0.44	.72	$F_{3,85}$ = 1.31	.28

Values are mean (SD).

ANCOVA, analysis of covariance; ANOVA, analysis of variance; dACC, dorsal anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; FWHM, full width at half maximum; Glu, glutamate; Glx glutamate + glutamine; GM, gray matter; HCs, healthy control subjects; nTRS, non-treatment-resistant schizophrenia; URS, ultra-treatment-resistant schizophrenia; WM, white matter.

[a]One datapoint was excluded because of high percentage SD (>20).

[b]Bonferroni-corrected p value < .05: URS > HCs (p = .034).

We conducted a meta-analysis on unmedicated patients exclusively and found no difference in Glu or Glx levels of the medial frontal cortex between unmedicated patients with schizophrenia and HCs (30). In addition, a recent meta-analysis noted that there were no differences in the level of Glu or Glx of the medial frontal cortex between patients with schizophrenia and HCs (11). However, it should be noted that the former study did not consider detailed voxel locations within the ACC (i.e., pregenual ACC or dACC), about which we later address in the limitation paragraph, while the latter study did not consider the voxel locations within the ACC as well as antipsychotic exposure. On the other hand, a systematic review by Egerton *et al.* summarized the prospective studies examining the effects of antipsychotic treatment on brain glutamatergic metabolite levels in patients with schizophrenia (31). According to the authors, there were only two studies that examined the effect of antipsychotic treatment on glutamatergic metabolite levels in the ACC of patients with unmedicated FEP or early stage of psychosis (<6 months). One study reported a significant decrease in glutamatergic metabolite levels (32), while another study found a nonsignificant increase in glutamatergic metabolite levels (33). Since this review article was published, two studies have examined the effects of 4 weeks of antipsychotic treatment on ACC glutamatergic neurometabolite levels in medication-naïve patients with FEP (34,35). De la Fuente-Sandoval *et al.* reported elevations in ACC Glx levels in patients prior to antipsychotic treatment, and normalization of ACC Glx levels, to levels not dissimilar to HCs after 4 weeks of treatment with risperidone (34). On the other hand, Egerton *et al.* demonstrated that there were no differences in Glu levels between patients and HCs at baseline (35), which is consistent with the aforementioned meta-analyses. However, the authors also noted reductions in ACC Glu levels after treatment with amisulpride. In addition, Kegeles *et al.* reported higher ACC Glx levels in patients with unmedicated chronic schizophrenia compared with HCs, while no differences were founded in medicated patients compared with HCs (36). Collectively, this body of work suggests that antipsychotic treatment may lower ACC glutamatergic levels in patients with schizophrenia.

Furthermore, in terms of treatment resistance, Egerton *et al.* noted that higher Glu levels in the ACC at baseline were associated with a lower likelihood of being in remission at 4 weeks, while the reduction in the ACC Glu levels following antipsychotic treatment was not related to treatment response (35). Similarly, previous cross-sectional ^{1}H-MRS studies have found higher glutamatergic metabolite levels in the ACC in patients with treatment-resistant schizophrenia in comparison with HCs (12) and compared with patients who responded to treatment (13). These findings were in line with our result that dACC Glx levels were higher in patients with URS than in HCs. Taken together, the findings within the current work suggest that higher levels of glutamatergic metabolites may be among the shared biological characteristics of treatment resistance to antipsychotics, including CLZ.

On the other hand, animal studies have reported that CLZ could increase extracellular Glu levels by increasing Glu release from glial cells or by reducing Glu transporter 1 expression in the astrocyte (37,38). Thus, it may be possible that glutamatergic levels in CLZ-treated patients may have been affected by CLZ. Given that it remains unclear whether glutamatergic metabolite levels may be changed with antipsychotics, including CLZ, further research is clearly warranted to longitudinally examine the effect of CLZ on glutamatergic neurometabolite levels in the brain of patients with treatment-resistant schizophrenia in order to elucidate the mechanisms of action of CLZ.

Comparing similar groups as our study, Goldstein *et al.* examined glutamatergic neurometabolite levels in the putamen and DLPFC, while our ROIs included the caudate, ACC, and DLPFC (16). Goldstein *et al.* reported that patients with URS had lower Glx levels in the DLPFC than patients with nTRS. In line with these findings, our data showed numerically lower Glu levels in the DLPFC in patients with URS than in patients with nTRS (uncorrected $p = .027$). On the other hand, we did not observe group differences in glutamatergic neurometabolite levels in the caudate, while Goldstein *et al.* demonstrated that patients with URS and nTRS had lower Glx levels in the putamen than patients with non-URS (16). This discrepancy may at least partially be attributable to 1) the placement of our ROI in the dorsal caudate, whereas Goldstein *et al.* placed the voxel on the putamen, and 2) our inclusion of symptomatic URS, whereas Goldstein *et al.* enrolled patients with URS who had, at most, mild illness severity.

Despite substantial symptom severity differences, we observed no differences in glutamatergic neurometabolite levels in any of the ROIs among the URS, non-URS, and nTRS groups. This also held true with comparisons between the symptomatic and nonsymptomatic patient groups. Moreover, we did not observe any association between symptom severity and glutamatergic neurometabolites. Overall, glutamatergic neurometabolite levels measured with ^{1}H-MRS in the caudate, dACC, and DLPFC did not appear to be associated with symptom severity. In line with these findings, a recent meta-analysis noted no significant correlations between glutamatergic markers and symptom severity in patients with schizophrenia (39). However, it should be noted that the current study lacked patients with moderate symptom severity as a result of our inclusion criteria. It is possible that patients with a wider range of symptom severity and more sensitive clinical tools may be needed to elucidate the association between glutamatergic neurometabolite levels and symptom severity in this patient population.

Interestingly, we observed negative associations between Glu levels in the DLPFC and years of CLZ treatment in patients with non-URS, although similar relationships were not found in patients with URS. While the effects of CLZ on glutamatergic neurometabolite levels remain unclear, our results indicate potential long-term effects of CLZ on glutamatergic neurometabolite levels in the DLPFC. Notably, we observed such a relationship in the non-URS group exclusively, raising the possibility that Glu level changes in the DLPFC after CLZ administration are specific to patients responsive to CLZ.

There are several limitations to our study. First, ^{1}H-MRS is unable to differentiate neurotransmitter or vesicular and metabolic pools of glutamatergic neurometabolites. Second, although we included the expected number of total participants ($N = 100$), the sample size within each group might still be small. Third, the ratio of tobacco use was significantly lower in HCs than in the patient groups, making it difficult to match the ratio of tobacco use between patient groups and HCs (40). Fourth, although we enrolled patients taking a single antipsychotic, the patients with nTRS were on different antipsychotics, which may have differentially affected glutamatergic neurometabolite levels in the brain. Fifth, although neurochemical levels were corrected for cerebrospinal fluid fraction, relaxation effects were not considered in this study (41). Sixth, it should be noted that this study examined glutamatergic neurometabolite levels in the dACC. Most studies that reported elevations in glutamatergic neurometabolite levels have examined the rostral area of the ACC (12,36,42–50), while most of the studies on the dACC have observed decreased glutamatergic neurometabolites (51–57) (these studies are summarized in Supplemental Table S7). Thus, the negative findings between the patient groups could be attributed to the voxel location of the dACC. Seventh, treatment response and resistance were determined based on symptom severities at the point of assessment owing to the cross-sectional design; we did not examine the effects of antipsychotics on symptom severity in a prospective fashion. Thus, it was possible that even in the URS group, which had residual positive symptoms, there might be participants who had more severe symptoms before CLZ treatment that subsequently improved after CLZ initiation. On the other hand, in the nTRS group, we could not fully rule out participants whose symptoms were not severe even before starting antipsychotic treatment. In such cases, symptom changes might be even less evident. Finally, owing to the nature of the cross-sectional design of this study, we were not able to determine the causal relationships between Glu and Glx levels and CLZ intake. This question may be answered through studies employing a prospective design that permits the measurement of Glu and Glx levels in patients with treatment-resistant schizophrenia before and after CLZ administration. Other limitations are detailed in the Supplement.

In conclusion, our main findings from this cross-sectional ^{1}H-MRS study were 1) higher dACC Glx levels in patients with URS than in HCs and 2) no differences in glutamatergic neurometabolite levels in the caudate, dACC, and DLPFC among patients with nTRS, non-URS, and URS. Few studies have examined the pathophysiology underpinning URS, and to date no robust neuroimaging correlates of URS have been found (58). Given our null finding from the comparisons between CLZ responders and nonresponders, future studies are warranted to elucidate neuroimaging correlates of URS and the mechanisms of action for CLZ.

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ARTICLE INFORMATION

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