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Altered Patterns of Brain Glucose Metabolism Involve More Extensive and Discrete Cortical Areas in Treatment-resistant Schizophrenia Patients Compared to Responder Patients and Controls: Results From a Head-to-Head 2-[^{18}F]-FDG-PET Study

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Abstract

***Background and Hypothesis*. Treatment resistant schizophrenia (TRS) affects almost 30% of patients with schizophrenia and has been considered a different phenotype of the disease. In vivo characterization of brain metabolic patterns associated with treatment response could contribute to elucidate the neurobiological underpinnings of TRS. Here, we used 2-[^{18}F]-fluorodeoxyglucose (FDG) positron emission tomography (PET) to provide the first head-to-head comparative analysis of cerebral glucose metabolism in TRS patients compared to schizophrenia responder patients (nTRS), and controls. Additionally, we investigated, for the first time, the differences between clozapine responders (Clz-R) and non-responders (Clz-nR). *Study Design*. 53 participants underwent FDG-PET studies (41 patients and 12 controls). Response to conventional antipsychotics and to clozapine was evaluated using a standardized prospective procedure based on PANSS score changes. Maps of relative brain glucose metabolism were processed for voxel-based analysis using Statistical Parametric Mapping software. *Study Results*. Restricted areas of significant bilateral relative hypometabolism in the superior frontal gyrus characterized TRS compared to nTRS. Moreover, reduced parietal and frontal metabolism was associated with high PANSS disorganization factor scores in TRS ($P < .001$ voxel level uncorrected, $P < .05$ cluster level FWE-corrected). Only TRS compared to controls showed significant bilateral prefrontal relative hypometabolism, more extensive in CLZ-nR than in CLZ-R ($P < .05$ voxel level FWE-corrected). Relative significant hypermetabolism was observed in the temporo-occipital regions in TRS compared to nTRS and controls. *Conclusions*. These data indicate that, in TRS patients, altered metabolism involved discrete brain regions not found affected in nTRS, possibly indicating a more severe disrupted functional brain network associated with disorganization symptoms.**

Key words: dopamine/glutamate/antipsychotics/clozapine/superior frontal gyrus/prefrontal cortex

Introduction

According to the treatment response, schizophrenia patients have been phenotypically subtyped as: responders to conventional antipsychotics (thereafter: nTRS), treatment resistant (TRS), and clozapine resistant (a special case of TRS).[1] TRS is regarded as the most severe and disabling clinical phenotype of the disorder.[2] In recent years, accumulating clinical and neuroimaging evidence[3–6] has tentatively conceptualized TRS as a categorically different illness subtype to treatment-responsive schizophrenia.[7] However, the neurobiological underpinnings of TRS have not yet been fully elucidated. While schizophrenia has traditionally been considered a disorder of cortical/subcortical dopamine transmission,[8,9] disturbances of glutamatergic networks within the anterior

cingulate cortex and anterior prefrontal cortex may be prominent in TRS.[5,10–13] One purported neurobiological mechanism of TRS may be the abnormal activity of multiple and discrete cortical areas, resulting in a disorganization of neural networks that cannot be reverted by antipsychotics.[14]

Based on this supposed mechanism, the evaluation of in vivo brain activity by functional neuroimaging in TRS patients compared to nTRS may provide crucial information on the neurobiological basis of the disorder. Positron emission tomography (PET) with 2-[^{18}F]-fluorodeoxyglucose (FDG), which measures the cerebral metabolic rate of glucose (CMRGlc), and mainly reflects neuronal and astrocyte activity, is considered one of the most powerful tools for investigating in vivo brain metabolism.[15]

Currently, there is no easily manageable method available in the regular clinical context to detect a potential or surrogate biomarker that can be used to provide a TRS-specific biological signature. In this scenario, FDG-PET may represent a feasible, reliable, and reproducible methodology.

Despite these premises, brain glucose metabolism in schizophrenia patients has been investigated in a restricted number of previous reports, and no recent investigations have been performed.[16–20] Early quantitative FDG-PET studies showed lower cortical and caudate metabolic rates in unmedicated schizophrenia patients compared to nonaffected controls.[21]

Contrasting evidence has been provided on the effects of antipsychotic agents on brain metabolism and few studies investigated brain metabolic changes in relation to response to antipsychotics and/or in TRS patients. The methodology and design of these studies are highly heterogeneous. Only one study with TRS patients (n = 14) compared regional brain metabolism in responder and nonresponder patients.[22] However, this study used a pre/post-intervention evaluation design and was mostly aimed at evaluating the effects of a haloperidol challenge. A second FDG-PET study compared brain metabolism in nonresponder patients prior to and after clozapine administration[23] and a third one was a post hoc analysis comparing clozapine-treated patients, neuroleptic-naïve recent-onset patients, and nonaffected controls.[24] Therefore, these early studies were not conclusive.

Another area that remains elusive is the putative effect of the clozapine response on glucose metabolism in TRS patients. Currently, three regional cerebral perfusion SPECT studies have analyzed the pattern of brain dysfunction in clozapine responders vs nonresponders,[25–27] providing contrasting results.

Taken together, these earlier studies allow to hypothesize that TRS and nTRS patients may differ in terms of quantitative and possibly regional metabolic changes, due to a putatively different neurobiological basis. However, several questions remain elusive:

1. Do pharmacologically stabilized TRS and nTRS patients exhibit different brain metabolic patterns in terms of topography and extent?
2. Do metabolic patterns correlate with specific domains of psychotic symptoms in TRS and nTRS patients?
3. Does response vs nonresponse to clozapine treatment result in different metabolic patterns in pharmacologically stabilized TRS patients?

The present study was specifically designed to address these questions by providing, to our knowledge, the first head-to-head comparison of FDG-PET-measured relative brain metabolism between TRS, nTRS, and controls. Moreover, the additional subdivision of TRS patients in clozapine responders and nonresponders allowed us to explore possible different metabolic changes between TRS subgroups.

Methods

Subjects

All patients were from the Unit for Treatment Resistant Psychosis, "Federico II" University Hospital of Naples. All diagnoses were made by trained psychiatrists (FI and AdB). The study was approved and registered (registration number: 195/2019) through the Ethical Committee for Clinical Studies of the University of Naples Federico II, and it began after the release of registration procedure of the participating institutions, including the Department of Neuroscience, Reproductive Science and Odontostomatology and the Department of Advanced Biomedical Sciences. All procedures, including patients' recruitment, clinical evaluations, MR/PET scans, took place from March 2019 to July 2020.

All consecutive patients who met the eligibility criteria and conferred consent were recruited. Inclusion criteria were: age within the 18–55 years range; diagnosis of schizophrenia according to DSM-5[28]; being on antipsychotics without medication switch or dose changes (ie, >10% baseline dose)[29,30] within the last six months; stabilized symptoms, including persistent psychotic symptoms without evidence of current or recent worsening (ie, acute psychotic breakthrough with significant deterioration of function and/or need for hospitalization), within the last three months prior to evaluations.

Exclusion criteria were: disease duration less than at least 2 years; nonschizophrenia psychotic disorders; psychotic symptoms due to another medical condition or to substances/medications; substance use disorder or frequent substance use within 6 months before recruitment; moderate or severe neurological disorders, whose severity may impact clinical presentation or affect antipsychotic response; intellectual disability (according to DSM-5 diagnostic criteria) or nonschizophrenia-related neurocognitive impairment; severe medical diseases (as certified by clinical records, medical examination and/or

by ad hoc adjunctive analyses); structural brain anomalies; pregnancy or lactation; nonstabilized diabetes mellitus or glucose intolerance with fasting glucose >160 mg/dl; lack of the capacity or refusal to provide consent; enrollment in any sort of experimental clinical trial within 3 months from recruitment.

Fiftythree participants underwent FDG-PET studies (41 patients and 12 controls). All patients performed preliminary diagnostic morphological MRI or CT studies and clinical assessments within 6 months from FDG-PET studies. Brain structural images were analyzed and reviewed by trained neuroradiologists (SC and GP) to rule out, with a consensus process, the presence of detectable brain abnormalities, including cortical atrophy, and two additional subjects were excluded from further analyses due to morphological anomalies (one had left sylvian fissure atrophy; one had partial dysgenesis of the corpus callosum). Twelve controls (18–60 years, mean: 44 ± 13; 7 females/5 males), free from centrally acting medications, without history of neurologic or psychiatric diseases, with no computerized tomography (CT) or magnetic resonance imaging (MRI) evidence of brain lesions were retrospectively selected from our normative FDG-PET GE Discovery ST[31] and Philips Ingenuity TF 64 PET-CT scanners database. They were either healthy subjects who participated as control subjects in previous prospective studies or patients referred for non-CNS pathology. Controls did not differ significantly in mean age from TRS and nTRS patients (one-way ANOVA: $P > .05$).

Overall, the final sample included 12 controls (CTRL), 20 nTRS, and 21 TRS patients. In a separate analysis, the TRS patients were divided into 11 clozapine non-responders (CLZ-nR) and 10 clozapine responders (CLZ-R).

All participants signed a written informed consent form, approved by our local Ethical Committee. All procedures carried out in the present study complied with the principles laid down by the Declaration of Helsinki, revised Hong Kong, 1989.

Response to Antipsychotics Evaluation

According to the operational criteria for the definition of TRS,[32] potential TRS patients should have a history of nonresponse to at least two different antipsychotics in the last five years, given for an adequate period (ie, six weeks) and at appropriate doses (ie, 600 mg/day chlorpromazine equivalents). Clinical information and previous medical records were used to assess the history of nonresponse. Only those cases whose medication history could be reliably reconstructed were included in subsequent evaluations.

Severity of psychotic symptoms was measured using the Positive and Negative Syndrome Scale (PANSS).[33] The cut-off to consider a patient not actively symptomatic was set at a score of 70, as described in previous reports.[34,35]

If the historical and current clinical severity criteria were met, patients underwent a prospective trial which may follow two routes, based on clinical advice: starting titration with a new antipsychotic agent until reaching the target dose; or continuing the ongoing antipsychotic until reaching the target dose (if already not reached). Target dose was defined as the recommended dose interval by regulatory agencies (eg, U.S. Food and Drug Administration and European Medical Agency).

PANSS ratings were collected at baseline (ie, before starting the new antipsychotic or before a dose increase of an already ongoing antipsychotic) and at fixed timepoints during the follow-up. Since the standard PANSS ratings could underestimate the response, we adopted rescaling, as described elsewhere.[36] Although antipsychotics are predicted to ameliorate positive symptoms mainly, we chose the change in PANSS total score for response detection, in agreement with the most recent guidelines.[32]

In the prospective trial, full response was stringently defined as the reduction of at least 50% baseline PANSS score after four weeks of antipsychotic treatment at therapeutic dose.[37] Nonresponse was defined as a reduction of <25% baseline PANSS (or even worsening). Nonresponder patients were ultimately considered as TRS and were then prescribed clozapine unless clinical conditions or previous severe adverse reactions contraindicated it. Partial response was indicated by a PANSS reduction <50% but >25% from baseline. Partial responder patients underwent an additional 4-week trial.

The prospective trial was carried out on outpatients. However, admission to the inpatient unit was considered when nonadherence was reliably detected. Additionally, at least one-third of patients underwent a trial with long-acting injectable antipsychotics to test for putative pseudo-resistance factors.

TRS patients prescribed clozapine were again evaluated in a time range of 8 weeks from reaching the target dose. In agreement with previous studies including as responsive those patients who had a diagnosis of TRS and showed at least a >25% reduction in PANSS scores following clozapine, ECT, or other augmentation strategies,[38–40] we considered patients on clozapine as responders (CLZ-R) if they had a PANSS reduction of at least >25% from baseline, and as non-responders (CLZ-nR) those who did not reach this cut-off improvement. The CLZ-R category also included four patients who discontinued clozapine due to untoward side effects, although clozapine was efficacious.

Assessments

Clinical and demographic data from the sample were recorded. Antipsychotic doses (CPZ) were transformed into chlorpromazine-equivalent doses.[41] Clinical Global Impression-Severity (CGI-S) was rated. The 5-factor

subdivision of the PANSS score was used,[42] in addition to the classical subdivision.

FDG-PET Studies

PET images of brain FDG uptake (FDG-PET) were performed according to the European Association of Nuclear Medicines (EANM) guidelines.[43,44] All scans took place after the patient was considered TRS or non-TRS, and after the antipsychotic treatment had been stabilized, that is: without therapeutic changes in the last six months before assessment. The authors who analyzed the neuroimaging scan ad related data set were blind to the response status of the patients. At the time of the scan, patients were off antidepressants from at least six months and off benzodiazepines from at least five half-lives, PET acquisition began between 45 and 60 min after the injection of 200–250 MBq of ^{18}F-FDG and lasted 15 min. Brain images were acquired in 3D mode using time-of-flight PET/TC system (Philips Ingenuity TF 64, Philips Medical Systems, Best, The Netherlands) with an axial field of view of 18 cm yielding 90 slices of 2 mm thickness and an axial and transaxial resolution (full width at half maximum [FWHM]) of 4.7 and 4.8 mm respectively. Images were reconstructed with the iterative time-of-flight reconstruction algorithm (BLOB-OS-TF) and corrected for attenuation using CT scans.

FDG-PET Image Analysis

FDG-PET images were processed for voxel-based analysis using the Statistical Parametric Mapping (SPM) software (Wellcome Department of Cognitive Neurology, London, UK, https://www.fil.ion.ucl.ac.uk/spm/) version 12 (SPM12) running in Matlab 2016b (Mathworks Inc.). Images were spatially normalized in the Montreal Neurological Institute (MNI) space using the PET template and the default parameters (affine transformation with nonlinear components, voxel size of 2 × 2 × 2 mm) of the old normalization in SPM12. For statistical analysis, spatially normalized FDG-PET images smoothed with an isotropic 3D Gaussian kernel filter of 8 mm. Intensity normalization to the global mean values, obtained with a threshold masking of 0.9, was performed using proportional scaling.

Comparison of TRS Patients, nTRS, and Controls. TRS, nTRS, and controls were compared using the voxel-based SPM one-way ANOVA statistical model with age and sex as nuisance variables. The F-contrast was performed to test the overall effect of group on relative brain glucose metabolism and the following post hoc comparisons were then performed using *t*-test to assess differences between groups as follows: TRS < nTRS, TRS > nTRS, TRS < CTRL, and nTRS < CTRL. Increased relative glucose metabolism in TRS and nTRS patients compared to controls was also tested. Significance thresholds for ANOVA F-contrast were set at uncorrected $P < .001$ at voxel-level and at $P < .05$ family-wise-error (FWE)-corrected for multiple comparisons at cluster level. To capture regions showing the most significant differences between groups, we set significance thresholds of post hoc *t*-test comparisons at both voxel and cluster level as follows: $P < .05$ FWE-corrected for multiple comparisons at voxel-level, and of $P < .008$ FWE-corrected at cluster level (Bonferroni correction for 6 comparisons = .05/6). Only clusters containing more than 50 voxels were deemed significant. To explore the relative glucose metabolic changes at an individual level in the different groups, we extracted all the significant clusters resulting from each post hoc *t*-test comparison as volume of interest (VOI), using a binary mask. Mean clusters' counts for each contrast were calculated in each subject and normalized to the global mean values.

Voxel-based SPM Correlation Analysis. Voxel-based multiple correlation analysis was performed in SPM to assess the relationship between relative glucose metabolism, chlorpromazine equivalent doses, PANSS total score, and the 5-factor domains scores, in TRS and nTRS patients, setting age, sex, and disease duration as covariates. Interaction analysis was performed to assess differences between TRS and nTRS patients in the correlations between relative brain glucose metabolism and the covariates of interest (ie, PANSS total score and domains scores). A less conservative statistical threshold of $P < .001$ uncorrected at voxel-level and $P < .05$ corrected at cluster level was set for correlation analysis.

Comparison of Clozapine Responder Patients, Clozapine Nonresponders, and Controls. To assess possible different effects on regional glucose metabolism related to the different response to clozapine, CLZ-nR, CLZ-R, and controls were compared using the voxel-based SPM one-way ANOVA statistical model with age and sex as covariates. As reported above, F-contrast was carried out to assess the overall significant group effect and post hoc comparisons were then performed using *t*-test to assess differences between groups. Significance thresholds for ANOVA F-contrasts were set at uncorrected $P < .001$ at voxel-level and at $P < .05$ FWE-corrected for multiple comparisons at cluster level. Significance thresholds for post hoc *t*-test comparisons were set at both voxel and cluster level as follows: $P < .05$ FWE-corrected for multiple comparisons at voxel-level and $P < .008$ FWE-corrected for multiple comparisons at cluster level (Bonferroni correction for 6 comparisons = .05/6). Only clusters containing more than 50 voxels were deemed significant. We also explored the relative glucose metabolic changes at an individual level in the different groups in VOIs extracted from each post hoc *t*-test comparison as reported above.

Statistical Analyses of Demographic and Clinical Data

All statistical procedures were run using the SPSS 24.0 software. Descriptive statistics were used to report

clinical and socio-demographic data. Independent-sample Student *t*, one-way ANOVA, and Chi-square tests were used to compare quantitative and categorical data.

Results

Included Sample and Demographics

Among the 41 patients who underwent FDG-PET, 20 were classified as nTRS (21–53 years, mean: 38 ± 10; 8 females/12 males) and 21 as TRS (21–54 years, mean: 37 ± 10 years; 3 females/18 males). All TRS patients were prescribed clozapine after being considered nonresponders in the prospective trial. However, four subjects experienced intolerable side effects with clozapine and were then switched to another antipsychotic drug. TRS and nTRS patients did not differ for mean age and education years. TRS patients were more frequently male compared to both nTRS (table 1) and controls (chi-square, $P = .002$), in agreement with the observation that male sex is a predictor of poor treatment outcome.[45] No significant differences in sex rates were found between nTRS and controls (chi-square, $P > .05$). No patient was on antidepressants or benzodiazepines. Two TRS patients and two nTRS patients were on mood stabilizers (chi-square, $P > .05$).

Clinical Features

As described in previous studies,[46,47] TRS patients had an earlier age at onset of psychotic symptoms, a higher number of overall hospitalizations, and were prescribed higher mean antipsychotic doses compared to nTRS patients. TRS patients had significantly higher mean total PANSS score, higher mean scores in virtually all PANSS factors, and higher mean CGI-S score than nTRS patients (table 1).

FDG-PET Image Analysis

Comparison of TRS, nTRS, and Controls. One-way ANOVA F-contrast revealed significant group differences in large areas including frontal, fusiform, and occipital gyri bilaterally (Supplementary Figure 1, Supplementary Table 1).

Post hoc *t*-test comparisons revealed a significant bilateral relative glucose hypometabolism in restricted areas of the superior frontal gyrus in TRS compared to nTRS. Compared to CTRL, only TRS showed a widespread prefrontal relative glucose hypometabolism, more marked in the left hemisphere, involving the left superior (SFG), middle (MFG), medial (mFG), and inferior frontal gyri (IFG), and the right superior and middle frontal gyri. An increase in relative glucose metabolism was found only in TRS compared to nTRS and CTRL, located in the posterior cortical regions, including the right lingual/fusiform gyrus, right middle occipital gyrus (MOG), and the left fusiform gyrus and cuneus. The results are reported in figure 1 and table 2. The plots of individual values of relative glucose metabolism showing differences among groups for each post hoc *t*-test comparison are reported in Supplementary Figure 2.

Voxel-based SPM Correlation Analysis

We investigated whether brain glucose metabolism may be associated with relevant clinical presentations of schizophrenia, including the PANSS 5-factor positive and disorganization domains. We chose to include the

Table 1. Patients' Demographic and Clinical Data

	TRS	nTRS	*P*
Age	36.9 ± 9.8	37.1 ± 11.1	ns
Sex (m/f%)	90/10	60/40	.023
Education years	12.1 ± 2.7	12.4 ± 3.6	ns
Age at onset	19.2 ± 6.5	23.9 ± 6.3	.025
Duration of disease	16.9 ± 8.7	14.1 ± 8.5	ns
Hospitalizations	2.04 ± 1.7	0.5 ± 0.8	.003
Antipsychotic dose	654.7 ± 416.2	276.5 ± 175.1	.001
PANSS total	98.8 ± 15.4	81.1 ± 9.3	<.0005
Positive factor	26.8 ± 5.3	18.6 ± 5.9	<.0005
Negative factor	27.3 ± 5.1	23.8 ± 6.3	.06
Disorganization factor	34.4 ± 6.5	27.7 ± 4.1	.001
Excitement factor	22.5 ± 4.9	17.6 ± 3.9	.001
Emotional distress factor	27.8 ± 5.7	22.5 ± 4.6	.004
CGI-S	4.4 ± 0.7	3.7 ± 0.5	.002

Note: TRS, Treatment Resistant Schizophrenia; nTRS, Non-treatment Resistant Schizophrenia; PANSS, Positive and Negative Syndrome Score; CGI-S, Clinical Global Impression – Severity; ns, not significant.
All analyses were carried out as independent sample Student *t* tests, with the exception of gender rates that were compared by chi square test.

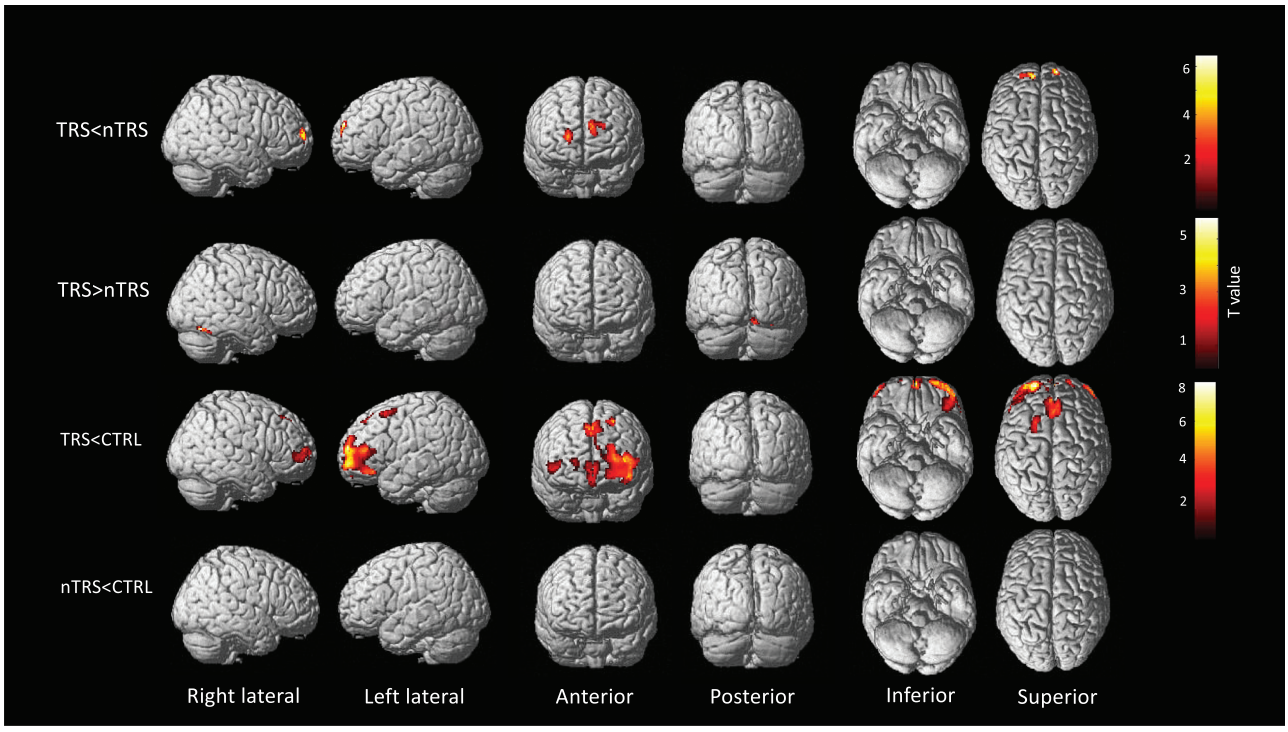

Fig. 1. Results of comparison between treatment resistant (TRS), non-treatment resistant (nTRS) patients and controls (CTRL). Age and sex were included as covariates. Significant clusters of reduced or increased relative glucose metabolism in TRS compared to controls and nTRS are superimposed on a volume rendered of the normal brain for anatomical localization and reported for the left and right brain hemispheres (statistical threshold: $P < .05$ FWE, corrected for both voxel height and cluster extent). Color bar illustrates the magnitude of the effects. .

disorganization domain since the productive psychotic symptoms of schizophrenia have been framed into two independent dimensions: reality distortion (such as delusion and hallucinations), and disorganization (ie, conceptual disorganization or bizarre behavior),[48] which has been associated with impaired cognitive performances.[48] Therefore, the disorganization factor could represent a predictor of TRS, as well as positive symptoms.

A significant negative correlation was found between relative brain glucose metabolism, total PANSS, and disorganization factor score in TRS but not in nTRS patients. Increased total PANSS scores were correlated with a reduced relative glucose metabolism in the parietal cortex bilaterally, including the right inferior parietal lobule and the left angular gyrus. A higher disorganization factor score was correlated with a lower relative glucose metabolism in the left angular gyrus, and in the left middle frontal gyrus (figure 2; Supplementary Table 2). The results of the interaction analysis revealed, however, that these correlations were not significantly different between TRS and nTRS patients. There was no significant correlation between relative brain glucose metabolism and chlorpromazine equivalent doses in either TRS or nTRS patients, as well as with other PANSS 5-factor domains (data not shown).

Comparison of CLZ-Rs, CLZ-nRs, and Controls. One-way ANOVA F-contrasts revealed significant group differences in the frontal, occipital cortices fusiform gyrus, bilaterally (Supplementary Figure 1, Supplementary Table 3). Post hoc analysis showed a significant widespread reduction of relative glucose metabolism in the prefrontal cortex located in the bilateral superior, middle, and inferior frontal gyri, in the left orbitofrontal cortex (gyrus rectus, BA11), left medial frontal gyrus, and left anterior cingulate in CLZ-nR patients compared to controls. Conversely, CLZ-R patients showed only one cluster of relative glucose hypometabolism located in the left superior frontal gyrus (figure 3, table 2). No significant differences were found in the direct comparison of CLZ-nR and CLZ-R, however, the possibility to find significant differences may be prevented by the relatively small sample size. The main significant cluster of relative hypermetabolism was found in CLZ-R and CLZ-nR compared to CTRL in right fusiform gyrus (18 −62 −10 *x*,*y*,*z* MNI coordinates; *Z*: 5.61; and 16 −64 −10 *x*,*y*,*z* MNI coordinates; *Z* values: 5.35, respectively). The plots of individual values of relative glucose metabolism showing differences among groups for each post hoc *t*-test comparison are reported in Supplementary Figure 3.

Discussion

The present study mapped brain glucose metabolism with the aim of providing preliminary support to the view that TRS and nTRS subjects have distinct patterns of brain

Table 2. Results of SPM Post hoc *t*-test Analysis of Relative Brain Glucose Metabolism Comparison Between TRS, nTRS, and Controls (CTRL; top) and Between Clozapine Responders (CLZ-R), Clozapine Non Responders (CLZ-nR), and Controls (Bottom)

				MNI Coordinates (mm)				
	Cluster Extent (Voxels)	pFWE Corrected Cluster Level	Z Score	*x*	*y*	*z*	Region	BA
TRS < nTRS	134	<.0001	6.52	−10	58	22	L SFG	10
	94	<.0002	6.44	18	62	12	R SFG	10
TRS < CTRL	1481	<.0001	6.32	−24	60	18	L SFG	10
			5.95	−36	56	−4	L MFG	10
			5.93	−40	42	14	L MFG	46
			5.64	−38	30	−14	L IFG	47
			4.81	−6	54	26	L mFG	9
	149	<.0001	5.74	48	52	2	R MFG	10
	162	<.0001	5.66	−20	16	56	L SFG	6
			5.30	−22	4	60	L MFG	6
	475	<.0001	5.36	6	34	46	R mFG	8
			5.24	−2	26	50	L mFG	8
	306	<.0001	5.30	−4	64	2	L mFG	10
TRS > nTRS	60	<.001	5.04	24	−66	−16	R FusiG	37
TRS > CTRL	594	<.0001	6.01	20	−64	−12	R LG/FusiG	19/37
	558	<.0001	5.76	−20	−58	−12	L FusiG	19/37
	143	<.0001	5.59	−10	−90	26	LCuneus	18
	73	<.0003	6.32	30	−80	8	R MOG	19
CLZ-nR < CTRL	1274	<.0001	6.56	−24	62	20	L SFG	10
			5.93	−38	56	0	L MFG	10
			5.85	−36	52	10	L MFG	46
	158	<.0001	6.04	64	10	24	R IFG	44
	146	<.0001	5.92	−38	32	−14	L IFG	47
	488	<.0001	5.85	18	66	2	R SFG	10
			5.17	44	56	0	R MFG	10
	55	<.0002	5.80	−8	40	−26	L GR	11
	67	<.0001	5.45	−8	38	46	L mFG	8
	76	<.0001	5.24	−22	2	60	L MFG	6
CLZ-R < CTRL	250	<.0001	6.62	−24	62	20	L SFG	10

Note: MNI, Montreal Neurological Institute; L, left; R, right; SFG, superior frontal gyrus; MFG, middle frontal gyrus; IFG, inferior frontal gyrus; mFG, medial frontal gyrus; FusiG, Fusiform gyrus; MOG, middle occipital gyrus; LG, lingual gyrus; BA, Brodmann's areas; FWE, Family-Wise Error rate; GR, gyrus rectus.
Significant differences were set at both voxel and cluster level with thresholds set at $P < .05$ FWE-corrected at voxel level and $P < .008$ FWE-corrected at cluster level. Only clusters with extent higher than 50 voxels were considered significant and reported in the table. Age and sex were included as covariates.

metabolic changes. To the best of our knowledge, based also on the systematic evaluation of the literature,[49,50] our study is the first to provide a comparison of brain metabolic patterns in stabilized TRS patients compared to nTRS and controls, whereas several structural neuroimaging studies have been published,[49–51] reporting heterogenous and often nonconsistent findings. The results of this study may be relevant to provide at least a surrogate biological signature of TRS.

The main findings of the study were: (1) TRS patients had significantly lower relative metabolism in discrete areas within the superior frontal gyrus compared to nTRS; (2) Reduced brain metabolism in parietal and frontal cortices correlated with worse clinical outcomes in TRS but not in nTRS patients; (3) Compared to controls, only TRS patients displayed extensive areas of significant relative hypometabolism at our stringent statistical thresholds, more widespread in TRS patients who were nonresponders to clozapine than in TRS who responded to clozapine; (4) Relative posterior cortical hypermetabolism characterizes TRS compared to nTRS and controls.

The results of our study are partially consistent with previous reports on brain metabolic activity in TRS patients or in clozapine-treated patients. A condition of frontal hypometabolism in schizophrenia patients with various antipsychotics, including clozapine, and increased metabolism in occipital areas by clozapine has already been described in several reports,[17–20] although other reports questioned these findings.[52,53]

Cortical hypometabolism/hypoperfusion was also found in studies that included treatment-resistant patients and/or explored metabolic rates or cerebral perfusion after 6 months of clozapine treatment,[22,23,54] although

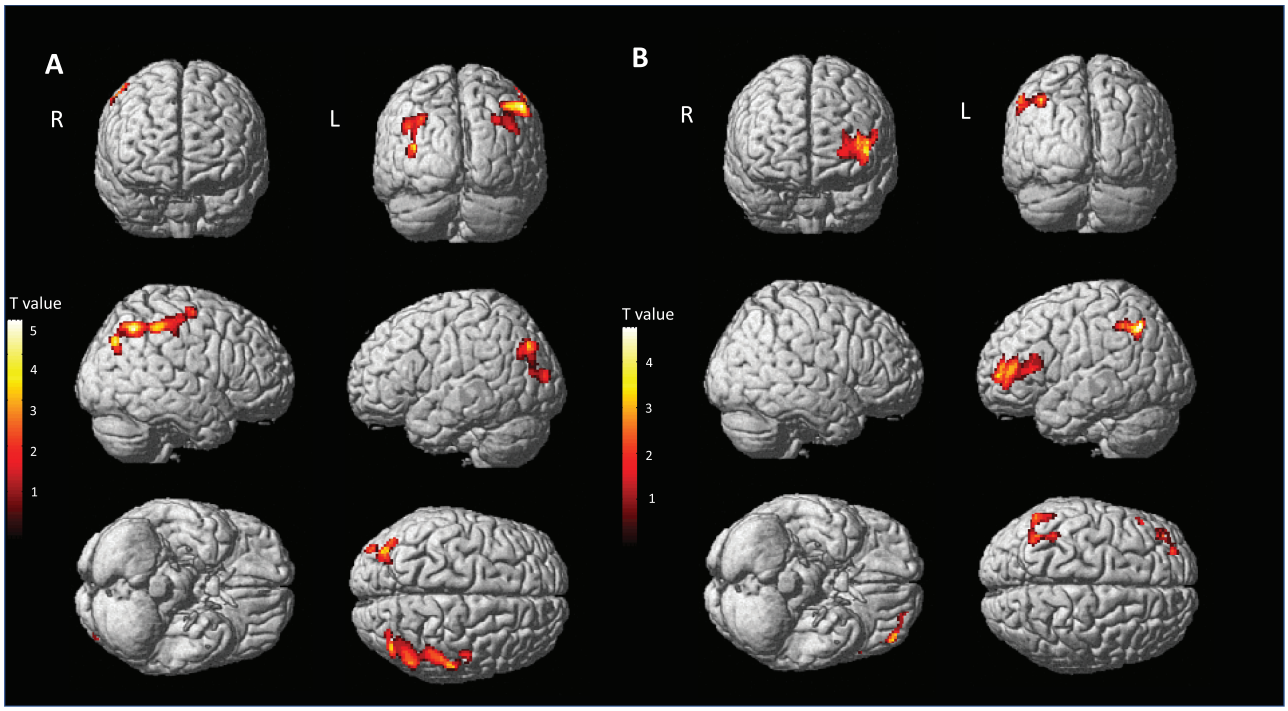

Fig. 2. Clusters of significant negative correlations between FDG uptake and total PANSS score (A) or PANSS disorganization score (B) in TRS patients are superimposed on a volume rendered of the normal brain for anatomical localization and reported for the left (L) and right (R) hemispheres (statistical threshold: $P < .001$, uncorrected for voxel height and $P < .05$ FWE, corrected for cluster extent). Age and sex were included as covariates. Color bar illustrates the magnitude of the effects. .

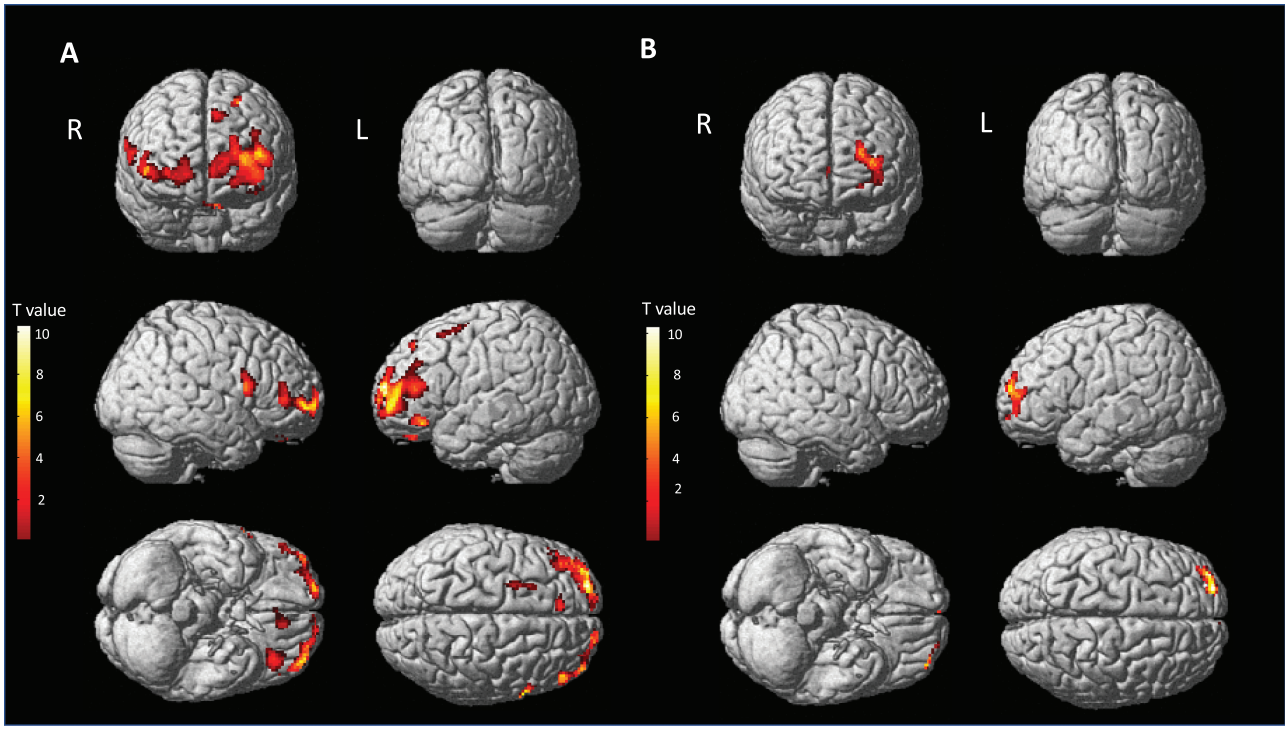

Fig. 3. Results of comparison between clozapine nonresponders and controls (A) and clozapine responders and controls (B). Age and sex were included as covariates. Significant clusters of reduced relative glucose metabolism are superimposed on a volume rendered of the normal brain for anatomical localization and reported for the left (L) and right (R) hemispheres (statistical threshold: $P < .05$ FWE, corrected for both voxel height and cluster extent). Color bar illustrates the magnitude of the effects. .

their designs were different from ours and did not allow to conclude whether the hypometabolism was specific to TRS or common to nTRS. Furthermore, previous studies on clozapine effects investigated relative cerebral blood flow, rather than brain metabolism, and did not separate between responders and nonresponders,[25–27] preventing the possibility of comparing their results with our data.

The clusters of relative hypometabolism found in this study were largely mapped in the rostral portions of the prefrontal cortex,[55] that have been already implicated in multiple disturbances of executive functions in schizophrenia patients. In multiple studies, the R-MFG was found hypoactivated in tasks exploring working memory (WM) of schizophrenia patients compared with healthy controls[56] and in at-risk mental state patients.[57–59] L-SFG has been described as one of the main areas of altered regional homogeneity (ReHO) in schizophrenia patients.[60] Notably, a larger activation in the SFG has been reported in chronic schizophrenia patients during a WM task compared to first episode ones.[61] SFG activation also correlated with antipsychotic dose.[61] Therefore, reduced glucose metabolism in R-MFG, R-SFG, and L-SFG may contribute to severe executive dysfunctions described in TRS and may represent one neurobiological mechanism of treatment resistance.

In particular, we found a significant negative correlation between the 5-factor PANSS disorganization factor score and glucose metabolism in the left medial frontal gyrus (BA46) in TRS but not in nTRS patients. There is evidence that the scores on the disorganization factor, also termed as the PANSS cognitive factor, may be inversely associated with performance on tools assessing executive functions.[62] Future studies should explore the relationship between cerebral glucose metabolism and performance in specific cognitive functions in TRS and nTRS patients.

PANSS Disorganization score and PANSS total score were also significantly and negatively correlated with relative glucose metabolism in the posterior cortical regions, mostly in the parietal lobe regions, including the inferior parietal lobule bilaterally (BA 40), the left angular gyrus (BA39). In particular, the inferior parietal lobule, which includes the supramarginal and angular gyri, has been considered one of the most severely disrupted brain regions in schizophrenia and participates in key pathophysiological processes, including impairment of sensory integration and executive functions.[63] The reduction of gray matter volume in IPL has been associated with more severe disease in schizophrenia.[64] Structural and functional alterations of the precuneus, including reduced regional homogeneity and functional connectivity, have been repeatedly described in schizophrenia patients.[65–67] The significant correlations with symptom severity suggest that parietal lobe dysfunction might be involved in TRS neurobiology. However, the lack of significant differences in the correlation between TRS and nTRS patients, possibly due to the sample size, could in part limit the interpretations of our results. Future studies are required to assess whether these findings are specific to the neurobiology of TRS.

The findings of the present study suggest that TRS and nTRS patients share regional metabolic frontal involvement of different degrees; however, relative hypometabolism involved more extensive brain areas in TRS than in nTRS, including discrete brain regions within the frontal cortex possibly less or not affected in nTRS. Interestingly, alterations in frontal metabolism appeared to be more profound in clozapine nonresponder TRS than in clozapine responders. The mechanisms of action of clozapine on FDG uptake are not yet fully understood. Recent interesting in vivo ^{18}FDG-microPET and ex-vivo/in vitro data in adult rats suggest that clozapine might induce widespread cortical glucose hypometabolism and network alteration by reducing the Glut1 transporter expression in the astrocytes.[68] Our results may suggest that hypofrontality, one of the most replicated findings in functional neuroimaging studies on schizophrenia,[69] may be more pronounced or fails to be reverted in those patients who do not respond to conventional antipsychotic agents and even less in those who do not respond to clozapine. Indirect support to this hypothesis comes from the findings that frontal perfusion was more largely enhanced in those patients who responded to clozapine[27] and from the observation that a challenge with haloperidol was associated with a wide reduction in brain metabolism in nonresponders but not responder patients.[22]

An alternative explanation may be that the more severe hypofrontality is one of the neurobiological underpinnings of TRS, whose unique relevance to this subgroup of patients has not been previously noticed since the populations included in previous studies were mixed TRS and nTRS.

Nonetheless, to assess whether hypofrontality precedes or not antipsychotic therapy, a rigorous longitudinally design with larger cohorts of patients and controls is required.

We have also found a higher glucose metabolism in the R-FG of TRS compared to nTRS. R-FG is part of the ventral visual system and is involved in object recognition (mostly face).[70] Hyperfunctional connectivity between R-FG and visual cortex at resting state in schizophrenia patients has been associated with poorer performance on the face detection task.[71] Disturbed connectivity in this area has also been associated with worse executive functions in schizophrenia patients.[72] Relative hypermetabolism was also found in TRS compared to controls in the left fusiform gyrus and occipital cortex, a finding previously observed after chronic clozapine treatment,[23] suggesting possible effects due to clozapine that deserve further investigation. However, we cannot exclude that normalization of FDG images

to global mean values lowered by the widespread frontal hypometabolism present in our patients might contribute to this apparent hypermetabolism that may only reflect relatively preserved metabolism in these regions, as also suggested in previous studies.[23] It should be acknowledged that this is a common bias in most part of nonquantitative FDG studies in schizophrenia and neurological patients. Only the absolute quantification of CMRGlu might clarify this point. However, the invasiveness of this method limits its use.

This study has limitations that should be taken into account in the interpretation of findings. The first limitation of the study is the relatively small sample size, although our study included one of the highest numbers of participants among studies evaluating glucose brain metabolism in schizophrenia. Furthermore, the present investigation was sufficiently powered to detect voxel-wise differences in brain metabolic activity and a very stringent and conservative threshold for statistical significance was adopted, to avoid false positives and detect differences between TRS and nTRS. Another limitation is that data acquisition of our control group has been performed with two different scanners. However, it should be stressed that the main objective of our study was the comparison of TRS and nTRS patients and that no significant differences were found in direct comparisons of the two groups of controls acquired with different scanners (data not shown).

Additionally, the limited availability of research-level structural brain imaging in our sample prevented us from applying a proper partial volume correction strategy. This makes our analyses less robust towards the potential spurious effects caused by structural brain changes, which have been reported in patients with schizophrenia,[73,74] and may be related to clinical and neurobiological heterogeneity of TRS patients.[75] However, the spatial distribution and the direction of the observed results make them unlikely to be definitely driven by putative structural changes, especially when looking at the comparison between TRS and nTRS patients.

We were not able to provide blood antipsychotic levels due to technical restrictions at our site. This step is requested by current procedure to assess whether nonresponse to antipsychotics may be due to pharmacokinetics issues.[76] As a proxy to infer dose levels in target sites, patients were evaluated for the occurrence of dose-related side effects of antipsychotics. We considered sufficiently reliable this alternative procedure to broadly separate patients who had presumably reached the adequate dose level from those who had not and who may suffer from pseudo-resistance.

Another relevant caveat is the lack of correction for some potential confounders, including the effect of cumulative antipsychotic dose, which has been proposed to cause changes in brain metabolic homeostasis, including a purported normalization of brain glucose metabolism.[77] Nonetheless, we found that current antipsychotic dose was not correlated with brain metabolic activity in TRS or in nTRS patients, partially excluding this variable as a possible confounder.

Despite these limitations, our results show coherent patterns of altered glucose metabolism in multiple brain regions of TRS patients, putatively originating from a generalized disconnection of neural circuits that is not counterbalanced by antipsychotics.

Supplementary Material

Supplementary material is available at https://academic.oup.com/schizophreniabulletin/.

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