ORIGINAL ARTICLE

Lower levels of the glial cell marker TSPO in drug-naive first-episode psychosis patients as measured using PET and [11C]PBR28

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Several lines of evidence are indicative of a role for immune activation in the pathophysiology of schizophrenia. Nevertheless, studies using positron emission tomography (PET) and radioligands for the translocator protein (TSPO), a marker for glial activation, have yielded inconsistent results. Whereas early studies using a radioligand with low signal-to-noise in small samples showed increases in patients, more recent studies with improved methodology have shown no differences or trend-level decreases. Importantly, all patients investigated thus far have been on antipsychotic medication, and as these compounds may dampen immune cell activity, this factor limits the conclusions that can be drawn. Here, we examined 16 drug-naive, first-episode psychosis patients and 16 healthy controls using PET and the TSPO radioligand [11C]PBR28. Gray matter (GM) volume of distribution (V_T) derived from a two-tissue compartmental analysis with arterial input function was the main outcome measure. Statistical analyses were performed controlling for both TSPO genotype, which is known to affect [11C]PBR28 binding, and gender. There was a significant reduction of [11C]PBR28 V_T in patients compared with healthy controls in GM as well as in secondary regions of interest. No correlation was observed between GM V_T and clinical or cognitive measures after correction for multiple comparisons. The observed decrease in TSPO binding suggests reduced numbers or altered function of immune cells in brain in early-stage schizophrenia.

INTRODUCTION

Schizophrenia is a severe mental disorder for which currently available treatment is satisfactory only in a minority of cases. Cognitive impairments, such as memory dysfunction and reduced speed of processing, are present already at an early stage of the disease, and are particularly difficult to ameliorate.[1] The development of new, improved treatment approaches is presently hampered by a lack of understanding of the pathophysiology of the disease.

Genetic and epidemiological data are indicative of an involvement of the immune system in the development of schizophrenia.[2,3] In patients, an on-going immune activation is suggested by studies showing an elevation of immune markers in blood and cerebrospinal fluid both during long-term illness, as well as in first-episode psychosis.[4–7] Using positron emission tomography (PET) and radioligands for the translocator protein 18kDA (TSPO), which in the brain is expressed primarily in microglia and astrocytes, it is possible to index brain immune cell activation *in vivo*.[8–10] Initial TSPO PET studies in small samples have shown increased binding in patients with schizophrenia as compared with controls;[11,12] however, a radioligand with low signal-to-noise ratio was used, as well as an outcome measure generally shown to be less reliable.[13] More recent studies using

novel TSPO radioligands have failed to replicate these findings. Although increases in a relative measure of binding was recently reported in schizophrenia patients and ultra-high-risk (UHR) individuals, there was no absolute TSPO elevation using standard methods of quantification.[14] This observation is in line with results from other studies in patients with long-term illness,[15,16] as well as a recent study in patients with recent-onset disease where a trend-level reduction in TSPO was observed.[17]

A major limitation of the studies published thus far is that all patients have been on treatment with antipsychotic medication. Importantly, several of these compounds have shown to attenuate blood cytokine levels in patients[18] as well as decrease binding to TSPO in preclinical studies.[19] Although the high-risk individuals investigated by Bloomfield *et al.*[14] were unexposed to antipsychotic medication, on average less than 30% of this group go on to develop a psychotic disorder,[20] indicating that the group is heterogeneous and cannot be equated with a prodromal phase of schizophrenia.

The aim of the present study was to overcome the critical drawback of previous investigations by measuring TSPO binding in antipsychotic-naive, first-episode psychotic patients, as compared with age-matched control subjects.

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MATERIALS AND METHODS

Patients and control subjects

The study was approved by the Regional Ethics Committee in Stockholm and the Radiation Safety Committee at the Karolinska University Hospital, Stockholm. Subjects were included after providing written informed consent after receiving a complete description of the study.

Sixteen first-episode psychotic patients (11 male, 5 female, mean age 28.5 (s.d. 8.4)) were recruited from psychiatric emergency wards and out-patient clinics in Stockholm. At the time of investigation, all patients were naive to antipsychotic treatment and met the diagnostic criteria for schizophrenia ($n = 4$), schizophreniform psychosis ($n = 7$), psychosis NOS ($n = 4$) or brief psychosis ($n = 1$), according to DSM-IV. Exclusion criteria were neurologic or severe somatic illness, current use or history of abuse of illegal drugs (including cannabis) and autism-spectrum disorder. Absence of major brain abnormalities was confirmed using magnetic resonance imaging. Occasional medication with sedatives and anxiolytics, including benzodiazepines, were allowed during the course of the study, as well as medication with antidepressants. In cases where diazepam was used, the daily dose was significantly lower than equivalent concentrations of this drug shown to affect TSPO binding according to in vitro data.[21]

Sixteen control subjects (7 male, 9 female, age 26.4 (8.4)) were recruited by advertisement. They were healthy according to medical history, clinical examination, routine laboratory blood and urine tests as well as a brain magnetic resonance imaging (MRI) examination. The Mini International Neuropsychiatric Interview was used to exclude previous or current psychiatric illness. Further exclusion criteria were previous or current use of illegal drugs and first-degree relatives with psychotic illness. None of the control subjects were on any medication at the time of the study.

The TSPO genotype was assessed in all subjects as described previously[22] using DNA extracted either from whole blood or saliva. There were eight high-affinity binders (HABs) and eight mixed-affinity binders (MABs) among the patients and nine HABs and seven MABs in the control group (Table 1).

Behavioral measures

In patients, psychotic symptoms were assessed using the Positive and Negative Syndrome Scale for Schizophrenia (PANSS) and the Clinical Global Impression (CGI) scale. Cognitive functioning was assessed in all subjects using tests from the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) Consensus Cognitive Battery (MCCB),[23,24] which is designed specifically to measure cognition in research on schizophrenia.[1] The cognitive domains assessed were speed of processing (using the tests Category Fluency: Animal Naming, Trail Making Test: Part A), attention/vigilance (Continuous Performance Test-Identical Pairs), working memory (Wechsler Memory Scale: Spatial Span), verbal learning (Hopkins Verbal Learning Test-Revised) and visual learning (Brief Visuospatial Memory Test-Revised). We also performed the Wisconsin Card Sorting Test and included categories completed and percent errors as outcome measures.

Magnetic resonance imaging

Brain MRI was performed at the MR Centre, Karolinska University Hospital, using a 3-T General Electric Discovery MR750 system (GE, Milwaukee, WI, USA). T2-weighted images were acquired for evaluation regarding pathology by a neuroradiologist, and T1-weighted images were acquired for definition of regions of interest (ROIs).

PET procedures

PET measurements were performed at the PET Centre at the Karolinska University Hospital, Stockholm, Sweden, using a high-resolution research tomograph (HRRT, Siemens Molecular Imaging, Knoxville, TN, USA). Individualized plaster helmets were made for each subject and used together with a head fixation system to minimize movement artifacts. A 6-min transmission scan using a ^{137}Cs source was performed for attenuation correction. [^{11}C]PBR28 was prepared as described previously[25] and injected as a bolus over ~10 s into the cubital vein. Emission data were acquired in list mode for up to 91 min, except for one patient (85 min) and two control subjects (77 min). The inclusion of data from these subjects was supported by analyses of a previous data set[22] where 63 min two-tissue compartment model (2TCM) for gray matter (GM) showed high correlation to that of 91 min ($n = 12$, $r = 0.997$, $P = 8 \times 10^{-13}$). PET images were reconstructed using ordered subset expectation maximization with modeling of the point-spread function, and subsequently corrected for head movement using a frame-by-frame realignment process as described previously.[26,27]

An automated blood-sampling system (ABSS, Allogg, Mariefred, Sweden) was used during the first 5 min of each PET measurement. Discrete arterial blood samples (2–4 ml) were drawn manually at 1, 3, 5, 7, 9, 10.5, 20, 30, 40, 50, 60, 70, 80 and 90 min, except for one patient, for whom the samples were drawn at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10.5, 20, 30, 40, 50, 60, 70, 80 and 90 min, and two control subjects with sampling times of 2, 4, 6, 8, 10, 15, 20, 25, 30, 40, 50, 60 and 72 min. The radioactivity was measured immediately after sampling in a well counter cross-calibrated with the PET system. After centrifugation, 0.7–1.5 ml plasma was pipetted and the radioactivity was measured in the same well counter.

The plasma radioactivity curve for the first 5 min was generated by combining the ABSS data with an interpolated curve from the manual samples, and then multiplied with the blood-to-plasma ratio obtained from the plasma samples to create an arterial plasma curve. The time curve for parent fraction of the radioligand was determined using high-performance liquid chromatograhpy (HPLC), interpolated and multiplied with the plasma curve to generate the arterial input function. The area under the curve (AUC) of the standardized uptake value (SUV) for the metabolite-corrected plasma curves was calculated for all subjects from 0 to 72 min. To assess the impact of protein binding, the free fraction of radioligand was analyzed using an ultrafiltration method. For additional details on these procedures see Collste et al.[25] and Kanegawa et al.[28]

Quantification of [^{11}C]PBR28 binding

MR images were realigned, segmented and co-registered to PET images using SPM5 in MATLAB R2007b (Wellcome Trust Centre for Neuroimaging, London, UK; The Mathworks, Natick, MA, USA).[25] ROIs were defined using an automated procedure based on the FreeSurfer software (version 5.0.0, http://surfer.nmr.mgh.harvard.edu/), previously validated for PET data analysis.[29] GM was selected as the primary ROI. In addition, we examined white matter, frontal cortex, temporal cortex and hippocampus on account of their central interest for research in schizophrenia as well as to enable comparison with previous TSPO PET studies.[12,14] Furthermore, a FreeSurfer whole-brain (WB) ROI was created for calculation of distribution volume ratios (DVRs), as described previously.[14] Volumetric data for all ROIs used in the PET analysis were extracted from FreeSurfer, as well as for the total intracranial volume.

The main outcome measure of TSPO binding was calculated using the 2TCM with a metabolite-corrected arterial plasma curve as input function. Binding was expressed as the total volume of distribution (V_T), which corresponds to the ratio between the sum of specific and non-displaceable concentrations of radioligand in the target region to the concentration in plasma at equilibrium. As a secondary analysis to allow for comparison with Bloomfield et al.,[14] data were analyzed using the 2TCM-1K model that includes an additional compartment with irreversible uptake hypothesized to account for endothelial binding in blood vessels.[30] All kinetic analyses were performed using MATLAB R2007b. Finally, DVRs were calculated as the ratio between GM V_T and WB V_T for both 2TCM and 2TCM-1K to enable additional comparisons with previously published data.[14,17]

Statistics

Normality of all demographic data, clinical and cognitive variables, radiochemical data and uptake outcome measures were evaluated using Kolmogorov–Smirnov tests as well as ocular inspection of histograms. Independent sample two-tailed t-tests were performed to examine potential group differences in demographic data, measurements of cognitive function, radiochemical data, ROI volumes and free fraction of radioligand in plasma as well as differences in plasma SUV AUC values. For variables that were not normally distributed, a Mann–Whitney U-test was performed to examine group differences. Group differences in categorical variables were assessed using a X^2-test.

Effects of genotype, sex and age on GM V_T were examined using independent sample t-tests (two-tailed) and Pearson's correlation. Multivariate analysis of covariance with all ROIs as dependent variables was deemed invalid because of high multicollinearity between the outcome measures. For the main statistical analysis, the difference between patients and controls in [^{11}C]PBR28 binding in GM was, therefore, examined using a univariate analysis of covariance (ANCOVA). GM V_T derived from the standard 2TCM model was used as dependent variable, group (patients versus control subjects) as independent variable

Table 1. Demographic, radiochemical and clinical data for first-episode psychosis patients and control subjects

	Controls (n = 16)	Patients (n = 16)	X^2	t-value[a]	df	Mann–Whitney U-value	P-value
Age	26.4 (8.4)	28.5 (8.4)				106.5	0.415
Gender							
Male/female	7/9	11/5	2.032		1		0.154
Genotype							
HAB/MAB	9/7	8/8	0.125		1		0.723
Descriptive statistics							
BMI	21.9 (1.9)	22.9 (4.8)		− 0.722*	19.8		0.479
Education years	14.9 (2.2)	13.6 (3.3)		1.278	25		0.213
Nicotine-user							
Yes/no	0/16	2/14	NA[b]				
PET measures							
Injected mass tracer (µg)	0.68 (0.57)	0.66 (0.32)		0.166	30		0.870
Specific radioactivity (GBq µmol^{-1})	313.8 (213.4)	276.4 (170.8)				120.5	0.777
Injected radioactivity (MBq)	403.9 (52.5)	402.1 (69.5)		0.083	30		0.934
Duration of illness (months)[c]	7.9 (9.6)						
PANSS[c]							
Positive		20.3 (4.9)					
Negative		18.1 (7.0)					
General		39.1 (10.8)					
Total		77.4 (18.3)					
Level of functioning[c]							
CGI		4.6 (1.2)					
	n = 16	n = 15					
Cognitive measurements							
MATRICS							
TMT	22.6 (4.6)	35.5 (14.4)				36	8.65×10^{-4}
HVLT-R	28.4 (2.3)	21.2 (5.8)				26	1.65×10^{-4}
WMS-III-SS	19.0 (2.2)	14.8 (3.4)				32.5	4.90×10^{-4}
BVMT-R	29.0 (5.4)	19.1 (6.6)		4.581	29		8.10×10^{-5}
Fluency	25.6 (6.7)	18.8 (4.2)		3.368	29		0.002
		n = 14					
CPT-IP	2.9 (0.4)	2.0 (0.4)		5.316	28		1.20×10^{-5}
	n = 15	n = 13					
WCST							
PctErrors	14.6 (7.5)	33.3 (18.2)				27.5	0.00123
CatCompl	5.9 (0.3)	4.5 (2.1)				64	0.0315

Abbreviations: BMI, body mass index; BVMT-R, Brief Visuospatial Memory Test Revised; CatCompl, Categories completed; CGI, Clinical Global Impression (1-6); CPT-IP, Contiuous Performance Test-Identical Pairs; df, degree of freedom; HAB, high-affinity binder; HVLT-R, Hopkins Verbal Learning Test - Revised; MAB, mixed-affinity binder; MATRICS, Measurement and Treatment Research to Improve Cognition in Schizophrenia; NA, not applicable; PANSS, Positive and Negative Symptom Scale in Schizophrenia; PctErrors, Percent errors; PET, positron emission tomography; TMT, Trail Making Test: Part A; WCST, Wisconsin Card Sorting Test; WMS-III-SS, Wechsler Memory Scale III: Spatial Span. Averages and s.d.'s are reported as the mean (s.d.). *Equality of variance not assumed, Welch's Independent sample t-test was performed. [a]In the case of equality of variance Welch's t-test was performed. [b]Expected cell counts too low to perform a X^2-test. [c]Ratings only exist for the patient group.

and gender and genotype as covariates. An analysis was also performed excluding the five patients on benzodiazepine treatment. Furthermore, effect sizes for GM binding was estimated for MABs and HABs separately, using partial eta-squared values obtained from ANCOVAs with gender as covariate.

Secondary, to examine group differences in binding in frontal cortex, temporal cortex, hippocampus and white matter, we performed four additional univariate ANCOVAs, each having sex and genotype as covariates. To allow for comparison with previously published results, the same set of univariate ANCOVAs was performed using regional V_T values derived from 2TCM-1K, as well as DVR values based on both 2TCM and 2TCM-1K methods as dependent variables.[14] For the main analysis of GM V_T differences, alpha was set to 0.05.

The relationships between GM V_T and symptom levels (PANSS), global functioning (CGI) and duration of illness were assessed using partial correlation controlling for genotype and gender. For correlations between GM V_T and cognitive measures, age was added as a covariate and the threshold for significance was Bonferroni-corrected for multiple comparisons (alpha = 0.05/8 = 0.00625). All statistical analyses were performed in SPSS 23 (IBM, Armonk, NY, USA) or R (version 3.2.4 'Very Secure Dishes').

RESULTS

Patient and control groups did not differ significantly in age, sex distribution, body mass index or genotype distribution (Table 1).

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Duration of illness was 7.9 (9.6) months and total PANSS score 77.4 (18.3). None of the patients had been exposed to antipsychotic medication at the time of investigation. Nine patients received anxiolytics or sedatives, of which five patients received benzodiazepines (diazepam (5 mg per day) in one case and oxazepam (10–30 mg per day) in four cases; Supplementary Table 1). One patient was prescribed paroxetin 20 mg per day. Two of the patients and none of the controls were cigarette smokers. Regional brain volumes did not differ significantly between patients and controls (Table 2) also after normalizing with intracranial volume (ST2); therefore, correction for partial volume effects was not performed for analyses of [^{11}C]PBR28 binding.

Group effects on regional [^{11}C]PBR28 binding
There was a significant effect of both genotype and sex on GM V_T ($t = 3.5$, degree of freedom (df) = 30, $P = 0.001$ and $t = 2.1$, df = 30, $P = 0.043$, respectively), whereas no correlation was observed between age and GM V_T ($r = -0.09$, $P = 0.62$). Hence, only sex and genotype were included as covariates in the ANCOVA. A decrease in [^{11}C]PBR28 GM V_T was observed in patients as compared with controls, with a significant effect of diagnostic group obtained in the ANCOVA (F = 6.19, df = 1.28, $P = 0.019$). The decrease was more prominent in HAB patients, with an effect size of 0.38 (partial eta square) compared with 0.02 for the MAB subgroup (Figure 1). The effect remained when excluding the five patients on benzodiazepine treatment (F = 6.71, df = 1.23, $P = 0.016$). In the regional analysis, significant group effects were observed for frontal cortex (F = 6.08, df = 1.28, $P = 0.020$), temporal cortex (F = 6.51, df = 1.28, $P = 0.016$) and hippocampus (F = 5.49, df = 1.28, $P = 0.026$), but not for white matter F = 1.54, df = 1.28, $P = 0.23$; ST3. There was also a significant effect of group for GM V_T derived using 2TCM-1k (F = 6.66, df = 1.28, $P = 0.015$), whereas no difference was found when analyzing DVR values calculated either using 2TCM or 2TCM-1K V_T (ST3). No statistically significant difference between patients and control subjects was observed for plasma SUV AUC (mean = 2.53×10^3 (s.d. = 1.0×10^3) versus 2.20×10^3 (0.66×10^3); $t = -1.1$, df = 30, $P = 0.28$) or free fraction (8.2 (3.1) versus 9.0 (2.8); $t = -0.74$, df = 30, $P = 0.46$).

Correlations between [^{11}C]PBR28 binding and behavioral measures
In patients, there was no significant association between GM V_T and either positive, negative, general or total PANSS scores, CGI or duration of psychosis. The patients performed more poorly than controls in all the cognitive outcome measures (Table 1). However, there were no significant correlations between GM V_T and cognitive performance in patients after correction for multiple comparisons (Table 3).

Table 2. Comparison of measured region of interest volumes between first-episode patients with psychosis and healthy control subjects

Region	Controls (n = 16)		Patients (n = 16)		t-value	P-value
	Mean	s.d.	Mean	s.d.		
Whole brain	1230.7	130.8	1256.0	126.2	− 0.557	0.582
Gray matter	738.7	61.7	739.1	71.8	− 0.015	0.988
Frontal cortex	153.7	12.3	156.1	18.5	− 0.431	0.670
Temporal cortex	110.5	7.5	111.8	13.1	− 0.357	0.723
Hippocampus	8.8	0.8	8.7	0.7	0.255	0.801

All volumes expressed in cm^3. P-value as determined using an independent sample t-test.

DISCUSSION
The results of the present study are novel in two important respects. First, this is, to our knowledge, the only study thus far to investigate TSPO binding in drug-naive patients with psychosis. The finding of reduced TSPO levels suggests that the lack of increases, or even trend-level decreases in V_T reported in recent studies employing novel TSPO radioligands[14,15,17] is not explained by the effects of antipsychotic medication. Second, patients had a mean duration of illness of less than 8 months, which is significantly shorter than in previous studies, including the average of 2.2 years reported in a recently published report in recent-onset schizophrenia.[17] Hence, the results imply that there is no TSPO elevation also in very early stages of the disease. Instead, first-episode psychosis patients may even be characterized by having decreased glial cell activation.

TSPO is present in myeloid and astrocytic cells throughout the whole brain, also at physiological conditions.[31,32] Therefore, quantification of radioligand binding to TSPO requires a metabolite-corrected plasma input function as reference. To reduce the variability induced by plasma measurements, relative outcome measures of binding such as SUV ratio or DVR have been suggested instead of the widely used V_T values.[33] In the study by Bloomfield et al.,[14] a nonsignificant reduction of ~ 10% in regional V_T was found in patients and UHR individuals, whereas V_T values normalized to WB binding showed an apparent increase in both groups. However, the normalization approach has been criticized.[34] Importantly, the WB values used for the normalization procedure were ~ 20% lower both for the UHR and patient groups as compared with controls, a difference that in patients appeared to be driven by significant decreases in white matter. Moreover, DVR values were obtained using WB as a covariate in the statistical analysis rather than dividing the ROI by WB. As a high degree of correlation is expected between WB and GM regions, this method may reduce variability significantly, resulting in increased effect sizes. In the present study, the observed reduction of GM TSPO in patients did not remain when using WB-normalized V_T (DVR, ratio method) as outcome measure. The effect of using DVR instead of V_T was thus in the same direction as for Bloomfield et al.[35] The lack of significant increases in our case may be explained by the statistical DVR procedure as well as the use of different WB templates, which could influence WB V_T values. Finally, we observed no group difference in the metabolite-corrected input function or free plasma fraction of radioligand in our data, providing support for the validity of our primary outcome measures.

Evidence for a heightened immune response in schizophrenia patients comes primarily from biomolecular studies, showing increases in pro-inflammatory markers both in long-term illness and in early psychosis.[4–7] With regard to the role of brain immune cells, some post-mortem studies have shown increased levels of microglia in patients; however, no differences or even decreases have also been reported.[36] For instance, two autoradiography studies assessing TSPO have yielded contrasting results, reporting reductions in [^{3}H]PK11195 and increases in [^{3}H]PBR28 binding, respectively.[37,38] Whereas initial in vivo PET studies using [^{11}C] PK11195 in small samples have shown increased TSPO binding in antipsychotic drug-treated schizophrenia patients, this finding has not been replicated using more recently developed TSPO radioligands with higher sensitivity. Importantly, preclinical studies combining PET with post-mortem immunohistochemistry have shown a close correspondence between TSPO levels and glial cell markers.[8,9] Consequently, although the high variability observed in human TSPO PET studies may limit the sensitivity,[15,25,37] a plausible interpretation of both in vitro and in vivo TSPO studies across different disease stages is that glial upregulation is not a robust feature of schizophrenia.

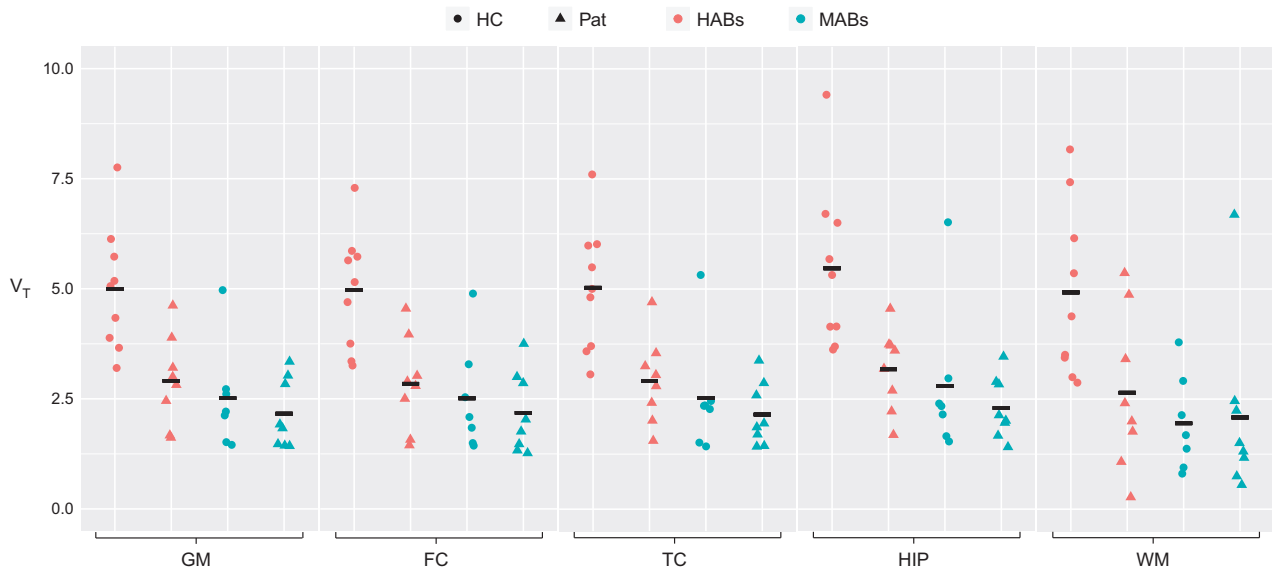**Figure 1.** Regional V_T values in first-episode psychosis patients (Pat) and healthy control subjects (HC) estimated using 2TCM. FC, frontal cortex; GM, gray matter; HAB, high-affinity binder; HIP, hippocampus; MAB, mixed-affinity binder; TC, temporal cortex; WM, white matter; V_T, volume of distribution.

Table 3. Partial correlations between GM V_T and clinical and cognitive variables in patients with first-episode psychosis

	r	df	P-value
Duration of illness (months)[a]	0.035	12	0.91
PANSS[a]			
Positive	0.45	12	0.11
Negative	0.10	12	0.73
General	0.29	12	0.31
Total	0.35	12	0.22
Level of functioning[a]			
CGI	0.41	12	0.14
Cognitive measures[b,c]			
MATRICS (n = 15)			
TMT	− 0.41	10	0.19
HVLT-R	0.70	10	0.012
WMS-III-SS	− 0.082	10	0.80
BVMT-R	0.21	10	0.52
Fluency	0.016	10	0.96
CPT-IP (n = 14)	− 0.15	9	0.66
WCST (n = 13)			
PctErrors	−0.049	8	0.89
CatCompl	0.016	8	0.97

Abbreviations: BVMT-R, Brief Visuospatial Memory Test-Revised; CatCompl, Categories completed; CGI, Clinical Global Impression (1–6); CPT-IP, Contiuous Performance Test-Identical Pairs; df, degree of freedom; GM, gray matter; HVLT-R, Hopkins Verbal Learning Test-Revised; MATRICS, Measurement and Treatment Research to Improve Cognition in Schizophrenia; PANSS, Positive and Negative Symptom Scale in Schizophrenia; PctErrors, percent errors; r, partial correlation coefficient; TMT, Trail Making Test: Part A; TPSO, translocator protein; WCST, Wisconsin Card Sorting Test; WMS-III-SS, Wechsler Memory Scale III: Spatial Span. [a]Controlling for TSPO genotype and gender. [b]Controlling for TSPO genotype, gender and age. [c]Bonferroni-corrected alpha-value = 0.00625.

Our findings of reduced TSPO levels in drug-naive patients with psychosis are congruent with the numerically lower V_T values reported in antipsychotic drug-treated patients with long-term schizophrenia,[14,15] as well as the trend-level reductions shown in recent-onset patients.[17] It may be hypothesized that reduced glial activation in early-stage psychosis reflects a failure of the immune system to adapt to other pathological processes associated with being in a psychotic state, thus promoting the development of the disorder. Importantly, both microglia and astrocytes also have neuroprotective and pro-inflammatory roles in the brain,[39] and the immune hypothesis in schizophrenia may thus be thought of as an imbalance rather than a one-dimensional activation. A limitation is that TSPO radioligands cannot differentiate between pro- and anti-inflammatory cell phenotypes, and there is thus a need for immune cell markers with improved functional specificity. This view is corroborated by the lack of significant correlations between TSPO V_T values and symptom levels or cognitive functioning observed in our study as well as in previous reports.[15,17]

In the present study the decrease in TSPO binding was more prominent in the group of HAB individuals. Although the lack of statistical power limits the conclusions that can be drawn when examining the genetic groups separately, this effect may partly be explained by the higher ratio of specific to non-displaceable binding in HABs compared with MABs.[31,40] This pattern needs confirmation in larger samples or as part of a meta-analysis.

An alternative hypothesis that allows for reconciliation of the lack of TSPO elevation at least with genetic and epidemiological data[2,3] is that pro-inflammatory microglial activation exerts its major influence preceding the onset of manifest disease. For instance, both human and animal research has suggested a link between immune activation and excessive synaptic pruning,[41,42] which may correspond to the cortical thinning observed already in early stages of schizophrenia.[43] Consequently, although all patients in the present study were examined during their first psychotic episode, it may be speculated that this was still too late to detect a hypothesized increase in immune cell activation.

CONCLUSION

In this study we found a decrease in TSPO binding in antipsychotic-naive first-episode psychosis patients compared with control subjects, indicating lower number or altered function of immune cells in brain in early-stage schizophrenia.

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Further studies combining molecular and structural brain imaging with detailed characterization of pro- and anti-inflammatory immune markers over time are needed to clarify the role of the immune system during the different disease stages of the disorder.

CONFLICT OF INTEREST

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MEMBERS OF THE KAROLINSKA SCHIZOPHRENIA PROJECT (KaSP)

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp).