Specificity protein 1 (SP1) and 4 (SP4) transcription factors have been related to both brain development and psychiatric disorders. SP4 is highly expressed in the hippocampus, and plays a role in postnatal hippocampal development. By contrast, SP1 is ubiquitously expressed. Both transcription factors are expressed in peripheral blood mononuclear cells (PBMC) and highly intercorrelated. *In vitro* studies demonstrated that SP1 is involved in the regulation of Reelin gene expression, a secretory protein responsible for the lamination of the hippocampus, providing SP1 with a possible role in hippocampal development. In people with chronic schizophrenia, SP1 gene expression has been found to be dysregulated across different brain regions and PBMC, suggesting that SP1 peripheral levels could be related to altered SP1 brain levels. Recent neuroimaging studies have extended previous post-mortem schizophrenia research showing cellular and molecular tissue abnormalities in the medial temporal lobe, demonstrating, at the imaging level, a bilateral hippocampal reduction in first-episode psychosis. Nevertheless, there is still no consensus regarding the molecular pathways underlying such volume reductions in schizophrenia from the early stages of disease. We therefore hypothesised that such neuroanatomical alterations could be related to SP1 and SP4 variations and aimed to investigate the relationship between SP1 and SP4 peripheral levels and bilateral hippocampal volume in participants with first-episode psychosis and healthy controls.

**Method**

We studied 11 patients with first-episode psychosis (FEP group) from the acute psychiatric ward of Parc Sanitari Sant Joan de Deu and 14 age- and gender-matched healthy controls. Inclusion and exclusion criteria, clinical assessments and treatments are detailed in the online data supplement. All participants gave written informed consent after a full description of the study, which was approved by the Institutional Review Board and the Institutional Ethics Committee.

Participants had a blood sample drawn and underwent a structural magnetic resonance imaging (MRI) scan. Blood analyses consisted of PBMC isolation and subsequent total protein or RNA extraction. We then performed protein and gene expression determinations for SP1 and SP4 as previously described (see online data supplement). A high-resolution spoiled gradient recalled echo (SPGR) T1-weighted anatomical scan was acquired for each participant on a 1.5 Tesla MRI scan (Signa Horizon, General Electric Medical Systems, Milwaukee, Wisconsin, USA) (repetition time (TR) = 1234 ms, echo time (TE) = 5.18 ms, 160 sagittal slices, voxel size 0.43 × 0.43 × 1 mm, field of view (FOV) = 512 mm × 512 mm, slice thickness, 1 mm, no gap). Scan processing and analysis were performed as detailed in the online data supplement. Our analyses aimed to (a) examine voxel-wise volumetric differences between the two groups, and (b) investigate the relationship between regional brain volumes and specificity protein and gene expression levels in both groups.

**Results**

Sociodemographic, clinical and cell-related data are provided in online Table DS1. SP4 protein levels were reduced in the FEP group compared with the control group ($t_{(23)} = 2.052, P = 0.0259$), and we also observed a trend for SP1 protein level reduction ($t_{(23)} = 1.659, P = 0.0553$) (online Fig. DS1(a)). Conversely, between-group differences were not observed in SP1 and SP4 gene expression levels (online Fig. DS1(b)). Exploratory imaging whole-brain analyses did not reveal any significant finding. By contrast, in ROI analyses we observed a right hippocampal volume reduction in the FEP group compared with the control group (peak difference at the Montreal Neurological Institute coordinates (26, −15, −12), with a $t$-value of 2.92 and a statistical significance of $P_{\text{FWE-SVC}} = 0.046$). Moreover, right hippocampal volume was associated with SP4 and SP1 protein levels in the FEP group but not in the control group (online Fig. DS2). Specifically, the FEP group showed significant positive associations between right hippocampal volume and SP4 ($t_{(21)} = −4.67, P_{\text{FWE-SVC}} = 0.002$) and SP1 ($t_{(21)} = −4.30, P = 0.0259$) gene expression levels and hippocampal volumes. Moreover, in the FEP group, SP1 and SP4 protein and gene expression levels, as well as regional hippocampal volumes, were not associated with age, daily antipsychotic doses or measurements of disease severity.

**Discussion**

Our findings show that a reduction of SP1 and SP4 protein levels in peripheral cells is significantly associated with a smaller right...
hippocampal volume in individuals with first-episode psychosis. Hippocampal volume reduction in humans could be linked to specific protein molecular mechanisms. Studies with Sp4 null mutant mice have shown a reduction of dentate granule cell density in the hippocampus. Sp4 hypomorphic transgenic mice displayed different morphological and molecular alterations such as dentate gyrus vacuolization and a decrease in NRI N-methyl-D-aspartate (NMDA) receptor subunit levels. In addition, Sp4 hypomorphic mice showed some hippocampal-dependent behavioural deficits that could be related to cognitive impairments described in schizophrenia. Interestingly, cortical and cerebellar studies revealed a dysregulation in Reelin gene expression, which is involved in the reduced hippocampal volume of individuals with first-episode psychosis. The proteasome, suggesting that similar modifications could be occurring in the hippocampus of people with first-episode psychosis. In this regard, it has been shown that hypoxia in rats leads to oxidative-dependent degradation of SP3 by the proteasome, raising the possibility that a hypoxia-degenerative mechanism in the early phases of psychosis could be involved in the reduced hippocampal volume of individuals with first-episode psychosis associated with specificity proteins.

Limitations of this study include the small sample size and participants taking antipsychotics. Negative findings should be interpreted with caution because of the limited power of our analyses. Replication with a larger sample of unmedicated patients with first-episode psychosis is warranted. Furthermore, it remains to be established whether SP4 and SP4 changes in peripheral cells in the early stages of the disease are paralleled by specific transcriptional alterations in hippocampal neurons that result in hippocampal volume reduction. However, our findings describe for the first time a direct association between SP1 and SP4 and hippocampal volume in people with first-episode psychosis, suggesting that these associations may ultimately be of relevance for the development of psychosis.

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Table D51  Sociodemographic, clinical and cell-related features of the first-episode psychosis and control groups

<table>
<thead>
<tr>
<th>Features</th>
<th>Control group (n = 14)</th>
<th>First-episode psychosis group (n = 11)</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sociodemographic features</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years: mean (s.d.)</td>
<td>25.26 (5.23)</td>
<td>24.53 (6.02)</td>
<td>0.321 (23)</td>
</tr>
<tr>
<td>Men</td>
<td>23.99 (5.04)</td>
<td>21.48 (1.80)</td>
<td>1.055 (11)</td>
</tr>
<tr>
<td>Women</td>
<td>26.95 (5.44)</td>
<td>27.08 (7.27)</td>
<td>0.034 (10)</td>
</tr>
<tr>
<td>Women, n (%)</td>
<td>6 (42.9)</td>
<td>6 (54.5)</td>
<td>0.34 (1)</td>
</tr>
<tr>
<td>College education, n (%)</td>
<td>7 (50.0)</td>
<td>3 (27.3)</td>
<td>4.57 (1)</td>
</tr>
<tr>
<td>Clinical features, mean (s.d.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of illness, days</td>
<td>N/A</td>
<td>84.27 (56)</td>
<td>N/A</td>
</tr>
<tr>
<td>Daily dosage antipsychotics, mg</td>
<td>N/A</td>
<td>324.2 (172)</td>
<td>N/A</td>
</tr>
<tr>
<td>Positive and Negative Syndrome Scale, total score</td>
<td>N/A</td>
<td>76.5 (10.9)</td>
<td>N/A</td>
</tr>
<tr>
<td>Calgary Depression Scale for Schizophrenia (CDSS), total score</td>
<td>0.4 (1.3)</td>
<td>4.6 (5.2)</td>
<td>28</td>
</tr>
<tr>
<td>Young Mania Rating Scale (YMRS), total score</td>
<td>11.5 (1.2)</td>
<td>14.7 (2.5)</td>
<td>16.5</td>
</tr>
<tr>
<td>Cell-related feature, mean (s.d.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RNA integrity number (RIN)</td>
<td>9.07 (0.46)</td>
<td>9.05 (0.39)</td>
<td>57.5</td>
</tr>
</tbody>
</table>

N/A, not applicable.

We found significant differences regarding the educational level where the first-episode psychosis group had received less formal education. This group also showed higher scores on the YMRS and CDSS. Between-group comparisons were performed with Student's t-test and \( \chi^2 \) test for quantitative and qualitative variables, respectively. Nevertheless, in quantitative comparisons, when normality distribution was not confirmed (according to the Kolmogorov–Smirnov test), we used the non-parametric Mann–Whitney's U-test.

b. Chlorpromazine equivalents.

c. One patient sample was excluded from the analysis because of the low quality of the RNA as detected by RIN (n = 10).

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Fig. DS1  Protein levels of specificity protein 4 (SP4) protein levels are reduced in peripheral blood mononuclear cells (PBMC) of the first-episode psychosis group and SP1 shows a downward trend (#).

(a) Protein levels for SP4 and SP1 normalised to actin levels in extracts from isolated PBMC were analysed by immunoblotting. The resultant bands were quantified by densitometry using Quantity One software version 4 for Windows. The graph represents the differences in protein levels between the control (CTL) and first-episode psychosis (FEP) groups. Values of SP1 and SP4 were normalised to actin and referred to a standard sample. Values represent the mean and the standard error of the mean for each group. Statistical analysis was performed using one-tail unpaired t-test (*P < 0.05). (b) SP4 and SP1 messenger RNA (mRNA) levels in the PBMC of the control (n = 14) and first-episode psychosis (n = 10) groups. One sample was excluded in the first-episode psychosis group in gene expression analysis (RNA integrity number = 3.3). Gene expression levels were determined by retrotranscriptase inverse reaction followed by real-time polymerase chain reaction and normalised to the geometric mean of ITP8 and TBP expression levels, and a control reference sample. Values represent the mean and the standard error of the mean for each group. Statistical analysis was performed using unpaired t-test that was not significant. To plot the graph we used GraphPad Prism version 5.00 for Windows.
Fig. DS2 Reduced specificity protein (SP1) and SP4 protein levels are correlated with smaller right hippocampal volume (RHV).

(a) and (c) Voxel-based morphometry analysis depicting voxel-wise correlations in participants with first-episode psychosis between regional right hippocampal grey matter volumes and SP1 (a) and SP4 (c) protein levels in peripheral blood mononuclear cells (PBMC). At a more liberal significance threshold, such correlations were also observed in the left hippocampus (not shown). (b) and (d) Graph of partial correlations between SP1 (b) and SP4 (d) protein levels and RHV for each group (control and first-episode psychosis) controlled for by gender, total global brain volume and age. As described in the text, correlations were only significant in the first-episode psychosis group, with \( r = 0.79 \) (SP1) and \( r = 0.83 \) (SP4). The values in (b) and (d) are the residual values of the hippocampal volume estimated after taking into account the effect of all the confounding variables. All variables were normally distributed according to a Kolmogorov-Smirnov test. To run the analysis we used IBM SPSS package version 21 for Windows. To plot the graph we used GraphPad Prism version 5.00 for Windows.
Specificity proteins 1 and 4, hippocampal volume and first-episode psychosis
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