Specificity proteins 1 and 4, hippocampal volume and first-episode psychosis

Montserrat Fusté,* Iria Meléndez-Pérez,* Victoria Villalta-Gil, Raquel Pinacho, Núria Villalmanzo, Narcís Cardoner, José M. Menchón, Josep Maria Haro, Carles Soriano-Mas** and Belén Ramos**

Summary
We assessed specificity protein 1 (SP1) and 4 (SP4) transcription factor levels in peripheral blood mononuclear cells and conducted a voxel-based morphometry analysis on brain structural magnetic resonance images from 11 patients with first-episode psychosis and 14 healthy controls. We found lower SP1 and SP4 levels in patients, which correlated positively with right hippocampal volume. These results extend previous evidence showing that such transcription factors may constitute a molecular pathway to the development of psychosis.

Declaration of interest
None.

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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 PTWE–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 (21, −16, −14, t = 4.67, PTWE–SVC = 0.002) and SP1 (21, −16, −14, t = 4.30; PTWE–SVC = 0.004) levels (Fig. DS2). By contrast, we did not find any relationship between the SP1 and SP4 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 specificity 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 vacuolisation 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 behavioural deficits that could be related to cognitive impairments hypomorphic mice showed some hippocampal-dependent disorder and schizophrenia. We propose that SP1 and SP4 are regulated by SP1, in hippocampal neurons of people with bipolar disorder. Ubiquitination of SP1 and/or SP4 and subsequent degradation that we found associations between SP1 and SP4 and right hippocampal volume in people with first-episode psychosis, suggesting that these associations may ultimately be of relevance to psychosis. The fact that we found associations between SP1 and SP4 and right hippocampal volume exclusively at the protein, but not at the gene, expression level suggests that these factors might be regulated by post-translational events, leading to protein degradation. Indeed, several studies have shown that different insults lead to the ubiquitination of SP1 and/or SP4 and subsequent degradation by 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 SP1 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>Feature</th>
<th>Control group (n = 14)</th>
<th>First-episode psychosis group (n = 11)</th>
<th>t (d.f.)</th>
<th>w^2</th>
<th>U</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sociodemographic features</strong></td>
<td></td>
<td></td>
<td></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>
<td></td>
<td></td>
<td>0.751</td>
</tr>
<tr>
<td></td>
<td>Men</td>
<td>23.99 (5.04)</td>
<td>1.055 (11)</td>
<td></td>
<td></td>
<td>0.314</td>
</tr>
<tr>
<td></td>
<td>Women</td>
<td>26.95 (5.44)</td>
<td>0.034 (10)</td>
<td></td>
<td></td>
<td>0.973</td>
</tr>
<tr>
<td></td>
<td>Women, n (%)</td>
<td>6 (42.9)</td>
<td>0.34 (1)</td>
<td></td>
<td></td>
<td>0.561</td>
</tr>
<tr>
<td></td>
<td>College education, n (%)</td>
<td>7 (50.0)</td>
<td>4.57 (1)</td>
<td></td>
<td></td>
<td>0.032</td>
</tr>
<tr>
<td><strong>Clinical features, mean (s.d.)</strong></td>
<td></td>
<td></td>
<td></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>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily dosage antipsychotics, mg</td>
<td>N/A</td>
<td>324.2 (172)</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
<td></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>
<td>N/A</td>
<td></td>
<td></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>
<td>0.0016</td>
<td></td>
<td></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>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cell-related feature, mean (s.d.)</strong></td>
<td></td>
<td></td>
<td></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>
<td>0.657</td>
<td></td>
<td></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 and w^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).

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 (RIN) = 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 IPO8 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.
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.
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Supplementary Material
Supplementary material can be found at:
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