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Short report

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

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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.

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,[1] and plays a role in postnatal hippocampal development.[2] 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,[3] providing SP1 with a possible role in hippocampal development. SP1 and SP4 are reduced in PBMC in first-episode psychosis[4] and in the brain in bipolar disorder.[5] In people with chronic schizophrenia, SP1 gene expression has been found to be dysregulated across different brain regions and PBMC,[6] 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,[7] demonstrating, at the imaging level, a bilateral hippocampal reduction in first-episode psychosis.[8] 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[4] (see online data supplement). A high-resolution spoiled gradient recalled echo (SPGR) T_1-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) = 512mm × 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. Significance was set at $P<0.05$ after family-wise error (FWE) correction for multiple comparisons (across the whole-brain in exploratory analyses, or applying small volume correction (SVC) procedures when analyses were restricted to our ROI).

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 $P_{FWE-SVC}=0.046$). 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 (21, −16, −14, $t=4.67$, $P_{FWE-SVC}=0.002$) and SP1 (21, −16, −14, $t=4.30$; $P_{FWE-SVC}=0.004$) 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

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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.[2] *Sp4* hypomorphic transgenic mice displayed different morphological and molecular alterations such as dentate gyrus vacuolisation and a decrease in NR1 *N*-methyl-D-aspartate (NMDA) receptor subunit levels.[9] In addition, *Sp4* hypomorphic mice showed some hippocampal-dependent behavioural deficits that could be related to cognitive impairments described in schizophrenia.[9–11] Interestingly, cortical and cerebellar granule neuron studies reported that SP4 is modulated by NMDA receptor activity, suggesting that SP4 could contribute to altered NMDA-dependent glutamatergic signalling.[12] Similar regulation could occur in hippocampal neurons. Furthermore, post-mortem studies revealed a dysregulation in Reelin gene expression, which is regulated by SP1, in hippocampal neurons of people with bipolar disorder and schizophrenia.[13] We propose that SP1 and SP4 dysfunction could be a plausible pathway that leads to abnormal postnatal hippocampal formation through complex intermediate mechanisms that include glutamate circuitry and the regulation of key neurodevelopmental genes relevant 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,[5,14,15] 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 in the hippocampus,[16] 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[17] 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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