No correlation between CSF tau protein phosphorylated at threonine 181 with neocortical neurofibrillary pathology in Alzheimer’s disease

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Sir, We have shown recently that tau protein hyperphosphorylated at threonine 231 (P-tau231P) in cerebrospinal fluid (CSF), a major candidate marker of Alzheimer’s disease (AD), correlates with neurofibrillary pathology in the brain of mostly severely demented AD patients (Buerger et al., 2006). In a clinical study, however, it had been reported earlier, that P-tau subtypes perform differently in the discrimination between mild to moderate AD and comparison groups (Hampel et al., 2004). Recently, Engelberghs and colleagues investigated correlations between CSF levels of P-tau phosphorylated at threonine 181 (P-tau181P), another widely used P-tau candidate marker, and Braak NFT and NP stages (Braak and Braak, 1991) and found no associations (Engelberghs et al., in press).

Therefore, we tested whether CSF P-tau181P correlates with neurofibrillary pathology in our previously studied population. Post-mortem assessed parameters were amount of neurofibrillary tangles (NFT), neuritic plaques (NP), neuropil threads (HP-tau load) and P-tau181P concentration in tissue homogenates measured within the brain of 26 AD patients. The study population, materials and methods as well as statistical analyses were identical to our recent report (Buerger et al., 2006). Levels of P-tau181P in CSF and brain homogenates were determined using a commercially available ELISA (Innotest-Phospho-Tau181P, Innogenetics, Belgium). To compare tau deposits stained by antibodies against phosphorylated tau at threonine 231 and threonine 181 in the brain of AD patients, additional immunohistochemical staining on tissue samples obtained from temporal cortex of all patients included in the study was carried out. Three different tau antibodies were used: monoclonal PHF-TAU clone AT8 (Innogenetics BR-03) that recognizes PHF-tau and the epitope has been shown to contain serine 202; PHF-TAU clone AT180 (Innogenetics BR-07) that recognizes PHF-tau and the epitope has been determined as being threonine 231; and PHF-TAU clone AT270 (Innogenetics BR-08) that recognizes PHF-tau and the epitope has been determined as being threonine 181. All antibodies were used in dilution of 1/500 and the staining was carried out without pretreatments with incubation overnight. Immunoreactivity was estimated semiquantitatively as mild, moderate or severe.

Mean level of CSF P-tau181P was 64.84 pg/ml (SD 32.34). CSF P-tau181P and CSF P-tau231P were highly correlated ($\rho = 0.71, P < 0.001$). Results of the bootstrapped regression models for each neuropathological marker and brain region are displayed in Table 1.

CSF-levels of P-tau181P were not associated with P-tau181P concentrations as measured in homogenates of frontal cortex. CSF P-tau181P was not correlated to scores of NFTs and NPs as well as HPtau load in the regions studied here. Findings were independent of MMSE score, age at lumbar puncture, time interval between lumbar puncture and death, disease duration and ApoE genotype.

Comparing the staining results using three different tau antibodies, the AT270 antibody labelled diffusely the neuropil whereas antibodies AT8 and AT180 lacked such
staining. All neuronal inclusions, i.e. neurofibrillary tangles, neurites and dystrophic neurites of neuritic plaques were labelled with all three antibodies, but in various extents. AT270 seemed to label as many immunoreactive (IR) structures as AT8 whereas there seemed to be fewer IR structures when using AT180 antibody. This difference was most notable in cases with only few IR structures i.e. when some or occasional AT8 and AT270 structures were seen hardly any AT180 IR were noted.

We found no association between CSF P-tau181P and neurofibrillary pathology. Recently, we found positive correlations for CSF P-tau231P in the same study group, on the same material, applying the same statistical analyses (Buerger et al., 2006). Our results suggest that various phosphorylated tau proteins might behave differently with regard to neurofibrillary pathology.

The reason for the lack of a correlation between P-tau181P in brain tissue and all the other parameters studied here is unclear and can currently only be speculated upon. For determination of P-tau181P, brain homogenates underwent one more freeze-thaw-cycle than for measurement of P-tau231P which may have caused alterations in the phosphorylation state of the protein. Even if the lack of a correlation between P-tau181P in the CSF and in brain homogenates was due to methodological problems, there is still the lack of an association between CSF P-tau181P and measures of neurofibrillary pathology as determined here which currently cannot be explained.

In additional immunohistochemical analyses, we found differences in the extent of staining which might explain for the fact that we do see a correlation between P-tau231P in CSF and brain, but not for P-tau181P. When sections from temporal cortex were stained at the same time using the same detection system one presumes that we found real differences, i.e. AT8-IR equals AT270-IR and both are more pronounced than AT180-IR. Interestingly, when using AT270 staining, there was in addition to a strong IR of neuronal inclusions and threads a strong neuropil labelling. Moreover, we do not know whether there are additional variables contributing to the release of various P-tau subtypes to the CSF and these unknown factors might exert different effects on PHF tau subtypes. One particular phosphorylation might entrap the protein making it less movable from tissue and vice versa.

In the light of our findings it could be concluded that CSF phosphorylated tau proteins differ with regard to correlations with AD neuropathological markers. From a clinical study we know that P-tau subtypes perform differently in the discrimination between AD and comparison groups (Hampel et al., 2004). The association between CSF candidate markers and AD neuropathology awaits further study.

References

Table 1: Regression coefficient, standard error, and 95% CI for CSF levels of P-Tau181P as a predictor of neuropathological markers specified for each brain region.

<table>
<thead>
<tr>
<th>Lesion assessed</th>
<th>Brain region</th>
<th>( \beta )</th>
<th>SE</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFT count</td>
<td>Frontal</td>
<td>0.046</td>
<td>0.033</td>
<td>−0.018 to 0.11</td>
</tr>
<tr>
<td></td>
<td>Temporal</td>
<td>0.033</td>
<td>0.028</td>
<td>−0.021 to 0.089</td>
</tr>
<tr>
<td></td>
<td>Parietal</td>
<td>0.051</td>
<td>0.026</td>
<td>−0.003 to 0.10</td>
</tr>
<tr>
<td></td>
<td>Hippocampus CA1</td>
<td>−0.030</td>
<td>0.040</td>
<td>0.11 to 0.048</td>
</tr>
<tr>
<td>NP count</td>
<td>Frontal</td>
<td>0.033</td>
<td>0.025</td>
<td>−0.016 to 0.082</td>
</tr>
<tr>
<td></td>
<td>Temporal</td>
<td>0.011</td>
<td>0.019</td>
<td>−0.026 to 0.047</td>
</tr>
<tr>
<td></td>
<td>Parietal</td>
<td>0.0016</td>
<td>0.027</td>
<td>−0.052 to 0.055</td>
</tr>
<tr>
<td></td>
<td>Hippocampus CA1</td>
<td>−0.018</td>
<td>0.041</td>
<td>−0.098 to 0.062</td>
</tr>
<tr>
<td>P-tau181P_brain</td>
<td>Frontal</td>
<td>0.012</td>
<td>0.0082</td>
<td>−0.0039 to 0.028</td>
</tr>
<tr>
<td></td>
<td>Temporal</td>
<td>0.0084</td>
<td>0.0071</td>
<td>−0.0056 to 0.022</td>
</tr>
<tr>
<td></td>
<td>Parietal</td>
<td>0.012</td>
<td>0.010</td>
<td>−0.0078 to 0.033</td>
</tr>
<tr>
<td></td>
<td>Hippocampus CA1</td>
<td>0.0023</td>
<td>0.0048</td>
<td>−0.0070 to 0.012</td>
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<tr>
<td></td>
<td>Frontal</td>
<td>0.0088</td>
<td>0.12</td>
<td>−0.23 to 0.25</td>
</tr>
</tbody>
</table>

\( \beta \) = regression coefficient, SE = standard error and 95% CI = 95% confidence interval of the regression coefficient.

*Statistically significant at \( \alpha = 0.05 \).