Correlation of Cerebrospinal Fluid Levels of Tau Protein Phosphorylated at Threonine 231 With Rates of Hippocampal Atrophy in Alzheimer Disease

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Background: The microtubule-associated tau protein abnormally phosphorylated at threonine 231 (p-tau231) has been investigated as a potential marker of Alzheimer disease. Levels of cerebrospinal fluid (CSF) p-tau231 vary across patients with Alzheimer disease. We hypothesized that these variations partially reflect differences in the degree of neuronal damage and therefore may be used to predict structural disease progression.

Objective: To investigate whether CSF p-tau231 levels correlate with rates of hippocampal atrophy as an in vivo marker of regional neuronal loss.

Design and Patients: We measured hippocampal volumes on the basis of serial magnetic resonance image examinations in 22 patients with Alzheimer disease. In addition, we determined CSF p-tau231 levels at baseline.

Results: Levels of CSF p-tau231 were significantly correlated with baseline hippocampal volumes (P<.001) and rates of hippocampal atrophy (left hippocampus, P<.001; right hippocampus, P=.02), independent of disease duration and severity.

Conclusion: These findings suggest that variations in p-tau231 levels may be used to predict progression of brain atrophy in patients with Alzheimer disease.

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Methods

We studied 22 patients (13 women and 9 men) with the clinical diagnosis of probable AD according to the criteria of National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association. After describing the study to each subject, the holder of a durable power of attorney, or a legal guardian, written informed consent was obtained. Mean age was 67.8 years (SD, 7.9 years). Global cognitive functioning was assessed with the Mini-Mental State Examination (MMSE). The mean MMSE score was 23.1 (SD, 4.0). Twenty AD patients underwent MRI twice, and 2 underwent MRI 3 times. Length of observation time ranged from 11.3 to 41.0 months (mean, 18.4 months [SD, 9.4 months]). Originally, 25 patients had mild AD; for 2 patients the second and for 1 patient the first MRI from the series could not be used.

The MRIs were acquired on the same 1.5-T scanner (Magnetom Vision; Siemens AG, Ernsthof, Germany).
Mean levels of p-tau231 were 729.6 pg/mL (SD, 404.3 pg/mL). Hippocampal volumes were 2267 mm³ (SD, 640 mm³) for the left and 2263 mm³ (SD, 651 mm³) for the right side. As shown in the Table, the mixed general linear model yielded a significant effect of p-tau231 on rates of hippocampal atrophy and left and right hippocampal volumes. Higher p-tau231 levels corresponded to higher rates of atrophy and higher baseline volumes (Figure 1).

There was a significant effect of t-tau levels on baseline volumes of the left hippocampus (β = 0.50 [P < .01]) and right hippocampus (β = 0.46 [P = .03]), with higher t-tau levels corresponding to higher baseline hippocampal volumes. There was no significant effect of t-tau levels on rates of left and right hippocampal atrophy (β = -0.27 [P = .14] and β = -0.10 [P = .51], respectively).

The statistical significance of these findings remained unchanged when main and interaction effect terms for MMSE and disease duration were added to the model.

There was no significant correlation between p-tau231 and t-tau levels and CSF volume (Pearson r² = 0.10 [P = .68] and r² = 0.06 [P = .78], respectively), gray matter volume (r² = -0.13 [P = .58] and r² = -0.26 [P = .26], respectively), or white matter volume (r² = 0.27 [P = .24] and r² = 0.16 [P = .48], respectively).

Higher rates of MMSE score decline correlated significantly with higher rates of atrophy of left and right hippocampal volumes (p = 0.52 [P = .02] and p = 0.47 [P = .03], respectively) (Figure 2). There was, however, no effect of p-tau231 levels on MMSE scores at baseline (F₁,20 = 1.57 [P = .22]) or on rate of point loss in MMSE scores (F₁,22 = 1.81 [P = .19]).

### RESULTS

In AD patients, higher CSF p-tau231 levels were significantly correlated with higher rates of hippocampal atrophy progression, even after controlling for disease duration and severity. This finding is consistent with the hypothesis that increased levels of CSF p-tau231 reflect more extensive neuronal damage, leading to higher rates of hippocampal atrophy.

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### COMMENT

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### Table. Effects of CSF p-tau231 Levels on Hippocampal Volumes*

<table>
<thead>
<tr>
<th>Region</th>
<th>p Value†</th>
<th>F²</th>
<th>P Value</th>
<th>β Value†</th>
<th>F₁,2²</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left hippocampus</td>
<td>0.65</td>
<td>19.8</td>
<td>&lt;.001</td>
<td>-0.36</td>
<td>15.3</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Right hippocampus</td>
<td>0.63</td>
<td>15.1</td>
<td>.001</td>
<td>-0.31</td>
<td>7.1</td>
<td>.02</td>
</tr>
</tbody>
</table>

*Analysis of variance table of results from the mixed-effects general models for left and right hippocampus, investigating the effect of p-tau231 levels on hippocampal volumes at baseline (main effect) and on rates of hippocampal atrophy (interaction effect).
†Indicates the standardized regression weight for the main and interaction effects, respectively.

## Abbreviations
- CSF: cerebrospinal fluid
- p-tau231: tau protein abnormally phosphorylated at threonine 231
- t-tau: tau protein abnormally phosphorylated at threonine 181
- Innotest hTau: diagnostic method to detect tau protein in cerebrospinal fluid
- Innogenetics: company for molecular diagnostics
- SPM2: statistical parametric mapping software
- MMSE: Mini-Mental State Examination
- AD: Alzheimer's disease

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tau$_{231}$ levels and rates of hippocampal atrophy suggest that CSF levels of p-tau$_{231}$ can serve as a marker for the degree of neuronal destruction and may be used to predict structural disease progression. The results were independent from disease duration and severity, indicating that the correlations do not reflect the effects of disease stage on rates of atrophy and p-tau$_{231}$ levels.

Levels of p-tau$_{231}$ and t-tau were positively correlated with baseline hippocampal volumes. There is no conclusive interpretation of this correlation at present. In a study of 8 patients with mild cognitive impairment, CSF p-tau$_{231}$ levels were decreased with larger ventricle size, suggesting a dilution of p-tau$_{231}$. In our patients, however, p-tau$_{231}$ levels were not correlated with CSF volume, indicating that tau proteins are not simply diluted in CSF with larger ventricle size (which is inversely correlated with hippocampal volume). The baseline effect, however, was modeled in the mixed-effect regression analysis to ensure that the correlations between p-tau$_{231}$ and rates of hippocampal atrophy were independent of the p-tau$_{231}$ effects on baseline volumes. Our study did not examine the rate of delivery of p-tau$_{231}$ into the CSF and did not address any changes in the rate of CSF turnover. It is expected that these factors may contribute to the interpretation of the results, and further studies are warranted.

Rates of hippocampal atrophy were correlated with rates of change in MMSE scores, suggesting a functional consequence of progressive hippocampal atrophy. Levels of p-tau$_{231}$ were not correlated with rates of change in MMSE scores, suggesting that the significant correlations between p-tau$_{231}$ levels and hippocampal volume...
do not reflect an effect of p-tau231 levels on rates of clinical deterioration, but may reflect a more specific pathophysiological link between regional brain atrophy and expression of p-tau231 levels in CSF.

There was no significant effect of t-tau levels on rates of hippocampal atrophy. This suggests that the observed correlations between p-tau231 levels and rates of hippocampal atrophy are not merely a global effect of t-tau levels. It remains to be investigated whether these correlations are specific for the threonine 231 epitope or may be found with other phosphoepitopes also. There is evidence of a stage-specific sequence of tau phosphorylation in AD.18 It may be possible that correlations between rates of brain atrophy and levels of subtypes of phosphorylated tau differ across the clinical stages of AD.

CONCLUSIONS

Our data agree with the notion that variations in p-tau231 levels reflect differences in the degree of neuronal damage across AD patients. Although the strength of the correlations presently suggests no sufficient clinical utility to individual patients, p-tau231 levels may be used to predict progression of brain atrophy in AD. To draw more definite conclusions, replication of our findings is needed in larger studies.

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REFERENCES