Correlation of Specific Amyloid-β Oligomers With Tau in Cerebrospinal Fluid From Cognitively Normal Older Adults

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Importance: To improve the ability to develop treatments that prevent incipient Alzheimer disease (AD) from progressing to overt AD, it is important to understand the molecular basis of the earliest pathophysiological abnormalities and to determine how amyloid-β (Aβ) is involved very early in its pathogenesis.

Objective: To investigate 2 specific Aβ oligomers, Aβ trimers and Aβ*56, in human cerebrospinal fluid (CSF); evaluate the effects of aging and AD; and obtain support for the hypothesis that they may be pathogenic by determining their relationships to CSF tau.

Design: A CSF sampling study.

Settings: The University of Minnesota Medical School in Minneapolis, Minnesota, and the Sahlgrenska University Hospital, Sweden.

Participants: Forty-eight older adults with mild cognitive impairment or AD (impaired group); 49 age-matched cognitively intact control subjects (unimpaired group); and 10 younger, normal control subjects.

Main Outcome Measures: Measurements of CSF Aβ trimers, Aβ*56, the 42-amino acid Aβ isoform (Aβ1-42), total tau (T-tau), and phospho-tau 181 (p-tau181). The hypothesis being tested was formulated after data collection.

Conclusions and Relevance: In cognitively intact older adults, CSF Aβ trimers and Aβ*56 were elevated in individuals at risk for AD, and they showed stronger relationships with tau than did Aβ1-42, a surrogate for Aβ fibril deposition. These findings suggest that prior to overt symptoms, 1 or both of the Aβ oligomers, but not fibrillar Aβ, is coupled to tau; however, this coupling is weakened or broken when AD advances to symptomatic stages. The uncoupling is interesting in light of the failure of experimental Aβ therapies to improve mild cognitive impairment/AD, which has prompted a shift in the timing of Aβ therapies to asymptomatic subjects. Knowing which Aβ species to target in asymptomatic subjects may enhance the success of future treatments for AD.

Results: We observed that Aβ trimers and Aβ*56 levels increased with age; within the unimpaired group, they were elevated in subjects with T-tau/Aβ1-42 ratios greater than a cutoff that distinguished the unimpaired group from subjects with AD. In the unimpaired group, T-tau and p-tau181 were found to correlate strongly with Aβ trimers and Aβ*56 (r > 0.63), but not with Aβ1-42 (−0.10 < r ≤ −0.01). The strong correlations were found to be attenuated in the impaired group.

Alzheimer disease (AD), the most common form of dementia among elderly individuals, threatens to become a major public health hazard as more people live beyond the eighth decade of life. Treatments that are administered after the symptoms of dementia appear do not effectively alter the course of illness, possibly because the pathophysiological processes causing neuronal loss in demented patients have become self-sustaining in ways that are difficult to curb. To improve our ability to detect incipient AD and develop treatments that prevent it from progressing to overt AD, it is important to identify and understand the molecular basis of the earliest pathophysiological abnormalities.

Although the exact cause of AD is unknown, it is widely believed to be triggered by abnormal aggregates of amyloid-β (Aβ), which collaborate with the microtubule-binding protein tau to produce widespread neuronal degeneration and dysfunction (reviewed in an article by Ashe and Zahs1). The disease process begins 1 or 2 decades prior to the onset of neuron loss or overt symptoms.2-4 There
have been many investigations of various Aβ species present in end-stage AD (reviewed in an article by Benilova et al), but our understanding of Aβ species in the initial stages of AD remains limited. One species of Aβ with the potential to be pathogenic in the initial, preclinical stages of AD is Aβ*56, a soluble 56-kDa oligomer that correlates with memory dysfunction independently of neuron loss or plaque deposition in several lines of mice overexpressing Aβ and disrupts cognition when injected into the cerebral ventricles of young, healthy rats. It has been suggested that Aβ*56 consists of 4 Aβ trimers, a conjecture that is based on its pattern of dissociation in a polar solvent. Amyloid-β trimers were the only oligomeric species present in mice prior to the appearance of Aβ*56 in Tg2576 mice modeling preclinical AD, providing additional support for the idea that they are basic compositional units for higher order oligomers. Interestingly, low levels of Aβ trimers were the only Aβ oligomers found in the brains of children and adolescent humans. Amyloid-β trimers were not shown to disrupt cognition when applied to rats, and they did not correspond well to memory deterioration in mice. To our knowledge, there is at least 1 report of Aβ trimers disrupting neural function in vitro, but their presence in children and adolescent humans, who presumably are free from AD, suggests that they are benign at low concentrations.

Longitudinal studies in humans indicate that subtle memory deficits, as well as functional and metabolic brain abnormalities, presumably the result of synaptic dysfunction, precede neuron loss at least a decade before overt cognitive symptoms emerge. The 42–amino acid Aβ isoform (Aβ1–42), total tau (T-tau), and tau phosphorylated at threonine 181 (p-tau181) have emerged as cerebrospinal fluid (CSF) biomarkers for the preclinical stages of AD. Not only are CSF T-tau and p-tau181 elevated in cognitively normal elderly individuals who later go on to develop AD, their levels also robustly correlate with glucose hypometabolism, making them presumptive biomarkers for synaptic dysfunction during the preclinical stages of AD.

In this study, we measured Aβ trimers and Aβ*56 in lumbar CSF using a highly sensitive and specific immunoblot assay. Our aims were to evaluate the effects of aging and AD and their relationships to CSF tau. Our goal was to obtain support for the hypothesis that specific Aβ oligomers induce changes in tau in the preclinical stages of AD, thereby advancing our understanding of how Aβ may be involved very early in its pathogenesis.

METHODS

We obtained CSF samples from 107 subjects, including 10 young, normal control subjects and older subjects consisting of 26 with AD, 22 with mild cognitive impairment (MCI), and 49 age-matched healthy control subjects. We made clinical diagnoses of AD according to the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer’s Disease and Related Disorders Association criteria and of MCI according to the Petersen criteria. We combined the subjects with AD and those with MCI to form the impaired group. The unimpaired control group consisted of 49 age-matched individuals without signs of psychiatric or neurologic disease, malignant disease, or systemic disorders (eg, rheumatoid arthritis or infectious disease). Cognition in the unimpaired and impaired groups was assessed using the Mini Mental State Examination.

We obtained CSF samples by lumbar puncture in the L3/L4 or L4/L5 interspaces. The CSF samples were collected in polypropylene tubes, gently mixed to avoid possible gradient effects, centrifuged, aliquoted, and stored at −80°C, pending biochemical analyses, without being thawed or refrozen. All procedures were approved by the institutional review board of the University of Göteborg.

Levels of CSF T-tau, p-tau181, and Aβ1–42 for all but 2 specimens were obtained using an enzyme-linked immunosorbent assay, as described in the eAppendix (http://www.jamaneuro.com).

To detect Aβ trimers and Aβ*56 in the CSF, we optimized the sensitivity of our immunoprecipitation and immunoblot protocols to measure and quantify Aβ trimers and Aβ*56 in 240 μL of CSF (detailed methods in the eAppendix). We received 750 μL of CSF from each subject, permitting us to perform each measurement in triplicate. The percentages coefficient of variance of triplicate measurements were 13% for Aβ trimers and 15% for Aβ*56. All measurements were performed blind to the clinical and demographic characteristics of the subjects. All statistical analyses were performed and graphs plotted using SPSS version 19 (SPSS Inc). Nonnormal data were log-transformed. The χ² and t tests were used to compare demographic and clinical data and CSF measurements. Best-fitting exponential curves were generated and provided for illustration purposes only.

RESULTS

DEMOGRAPHICS OF STUDY PARTICIPANTS

The demographic and clinical characteristics of the subjects are presented in the Table.

INCREASED CSF Aβ TRIMERS AND Aβ*56 WITH AGING

To determine the effects of aging on the levels of CSF Aβ trimers and Aβ*56, we calculated Pearson correlation coefficients between these oligomers and age in the combined young, normal control and unimpaired groups. The correlation coefficients were 0.31 between age and Aβ trimers (P = .02; n = 59) and 0.25 between age and Aβ*56 (P = .07) (Figure 1A). We also compared the levels of the oligomers in unimpaired subjects 65 years of age or older and in younger subjects between 35 and 50 years of age, and we found 9.2% higher levels of Aβ trimers (P < .01) and 6.9% higher levels of Aβ*56 (P = .04) in the older subjects (Figure 1B). These results suggest that CSF Aβ trimers and Aβ*56 increase with aging, but they do not distinguish between an age-dependent phenomenon that is related to AD from one that is unrelated to AD.

CSF Aβ TRIMERS AND Aβ*56 HIGHER IN SUBJECTS AT RISK FOR AD

To determine whether elevated levels of Aβ trimers or Aβ*56 in cognitively intact subjects were related to AD,
we identified subjects at high risk for AD based on CSF T-tau/Aβ1-42 ratios, which have been shown to be elevated in individuals with AD and cognitively intact individuals who go on to develop AD. Using receiver operating characteristic analysis, we demonstrated that a T-tau/Aβ1-42 cutoff of 1.005 provided sensitivity of 92.3% and specificity of 89.6% for distinguishing the unimpaired group from subjects with AD. We found that subjects in the unimpaired group with a T-tau/Aβ1-42 ratio of 1.005 or greater had 10.7% higher levels of Aβ trimers (P < .01) and 10.9% higher levels of Aβ*56 (P < .01) (Figure 1C). These results support the hypothesis that age-dependent elevations in Aβ trimers and Aβ*56 are related to AD.

**CSF Aβ AND TAU IN COGNITIVELY NORMAL OLDER ADULTS**

To examine relationships between CSF Aβ oligomers and tau in cognitively normal older adults, we calculated age-adjusted partial correlation coefficients and P values in the unimpaired group. Correlation coefficients were 0.64

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**Table. Subject Demographic Characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Young, Normal Control Subjects</th>
<th>Unimpaired</th>
<th>Mild Cognitive Impairment</th>
<th>Alzheimer Disease</th>
<th>P Valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, No.</td>
<td>10</td>
<td>49</td>
<td>22</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Age at lumbar puncture, mean (SD), y</td>
<td>41.7 (4.9)</td>
<td>64.8 (7.9)b</td>
<td>65.3 (8.7)</td>
<td>66.9 (6.5)</td>
<td>.09</td>
</tr>
<tr>
<td>Sex, female/male, No. (% female)</td>
<td>7/3 (70)</td>
<td>32/16 (65)c</td>
<td>14/8 (64)</td>
<td>17/9 (65)</td>
<td>.90</td>
</tr>
<tr>
<td>Mini Mental State Examination score, mean (SD) [range, 0-30]</td>
<td>NA</td>
<td>29.3 (0.97)d</td>
<td>28.2 (1.66)</td>
<td>21.6 (4.59)e</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Abbreviation: NA, not applicable.

a Comparison of unimpaired, mild cognitive impairment, and Alzheimer disease groups.

b N = 46.

c N = 48.

d N = 47.

e N = 25.

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**Figure 1.** Alzheimer disease–related, age-dependent increase in cerebrospinal fluid amyloid-β (Aβ) oligomers, Aβ*56 and Aβ trimers. In cognitively normal subjects, cerebrospinal fluid Aβ*56 and Aβ trimers increased with aging (A and B), and they were higher in subjects with total tau (T-tau)/Aβ1-42 ratios greater than 1.005 (C), which provided sensitivity of 92.3% and specificity of 89.6% for distinguishing cognitively normal older adults from subjects with Alzheimer disease. DLU indicates densitometry light units. The asterisks indicate P < .05.
between A\(\beta\)\(^{\text{400}}\) and T-tau (\(P < .01\)) (Figure 2A) and 0.70 between A\(\beta\)\(^{\text{56}}\) and p-tau\(_{\text{181}}\) (\(P < .01\)) (Figure 2D). The correlation coefficients were 0.65 between A\(\beta\) trimers and T-tau (\(P < .01\)) (Figure 2B) and 0.71 between A\(\beta\) trimers and p-tau\(_{\text{181}}\) (\(P < .01\)) (Figure 2D).

The relationships between CSF A\(\beta\)1-42 and tau were also examined by calculating age-adjusted partial correlation coefficients and \(P\) values. Correlation coefficients were \(-0.10\) between A\(\beta\)1-42 and T-tau (\(P = .50\)) (Figure 2C) and \(-0.01\) between A\(\beta\)1-42 and p-tau\(_{\text{181}}\) (\(P = .95\)) (Figure 2F).

These results suggest that in cognitively normal older adults, there are strong relationships between CSF tau and both A\(\beta\) trimers and A\(\beta\)\(^{\text{56}}\), which contrasts with the absence of relationships between A\(\beta\)1-42 and tau.

**CSF A\(\beta\) AND TAU IN COGNITIVELY IMPAIRED OLDER ADULTS**

The relationships between CSF A\(\beta\) oligomers and tau in subjects with MCI/AD were assessed by calculating age-adjusted partial correlation coefficients and \(P\) values in the impaired group. Correlation coefficients were 0.14 between A\(\beta\)\(^{\text{56}}\) and T-tau (\(P = .35\)) (Figure 3A), 0.19 between A\(\beta\)\(^{\text{56}}\) and p-tau\(_{\text{181}}\) (\(P = .21\)) (Figure 3B), 0.37 between A\(\beta\) trimers and T-tau (\(P = .01\)) (Figure 3B), and 0.44 between A\(\beta\) trimers and p-tau\(_{\text{181}}\) (\(P < .01\)) (Figure 3E). The relationships between CSF A\(\beta\)1-42 and tau were similarly assessed. Correlation coefficients were \(-0.40\) between A\(\beta\)1-42 and T-tau (\(P < .01\)) (Figure 3C) and \(-0.33\) between A\(\beta\)1-42 and p-tau\(_{\text{181}}\) (\(P = .03\)) (Figure 3F).

These results suggest an attenuation of the relationships between CSF tau and both A\(\beta\) oligomers in symptomatic individuals with MCI/AD compared with their respective relationships in cognitively normal individuals, while a moderate relationship appeared between CSF tau and A\(\beta\)1-42 in the symptomatic group.

**COMMENT**

We measured 2 A\(\beta\) oligomers, A\(\beta\)\(^{\text{56}}\) and A\(\beta\) trimers, in CSF and found age-dependent increases in A\(\beta\) oligomers in cognitively normal adults, as well as elevated levels of both oligomers in subjects who were at greater risk for AD. We found strong positive relationships between the A\(\beta\) oligomers, but not A\(\beta\)1-42, and tau in cognitively normal older adults, and attenuations of these relationships in MCI/AD. Because A\(\beta\)1-42 is a surrogate for A\(\beta\) fibril deposition, these findings suggest that in the years prior to the onset of overt symptoms, 1 or both of the A\(\beta\) oligomers, but not fibrillar A\(\beta\), is coupled to tau, but that this coupling is weakened or broken when AD advances to symptomatic stages. The uncoupling in MCI/AD is interesting in light of the consistent failure of experimental A\(\beta\) therapies to alter the clinical course of patients with MCI or AD, which has prompted a shift in the timing of A\(\beta\) therapies to asymptomatic subjects. Knowing which A\(\beta\) species to target in asymptomatic subjects may enhance the success of future drug development.

To our knowledge, this is the first report in which the levels of specific A\(\beta\) oligomers were measured in the CSF...
in cognitively normal older adults and shown to correlate with tau. It is interesting to speculate that the strengths of the correlations between tau and the Aβ oligomers (r > 0.63) or Aβ-42 (−0.10 < r < −0.01) reflect the relative participation of the respective Aβ species in the molecular events causing abnormalities in tau or synaptic dysfunction in preclinical AD. If the speculation is true, it suggests that targeting Aβ fibrils alone will not prevent preclinical AD from progressing to symptomatic AD. In addition, it indicates that tracking amyloid deposition or CSF Aβ-42 may not be useful as measures of how well Aβ therapies are blocking the ability of Aβ to engage tau or disrupt synaptic function.

Although animal studies show more evidence supporting a pathogenic role for Aβ*56 than for Aβ trimers,1,7,9 we do not know whether Aβ trimers or Aβ*56 are more likely to play a pathogenic role in humans. The data in this study do not resolve this question because the relationships between tau and each oligomer were equivalently robust. The strong relationships between Aβ trimers and tau may indicate a pathogenic role for trimers or reflect a molecular equilibrium between Aβ*56 and Aβ trimers, which would be expected to exist if Aβ trimers cluster to form Aβ*56, as is suspected.7 Additional molecular studies in animals and cells, as well as longitudinal clinical studies in humans, may better define the pathogenic roles of these oligomers and elucidate their molecular interactions with tau.

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