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

Maureen Handoko, PhD; Marianne Grant, BA; Michael Kuskowski, PhD; Kathleen R. Zahs, PhD; Anders Wallin, MD, PhD; Kaj Blennow, MD, PhD; Karen H. Ashe, MD, PhD

**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 Aβ56, 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, Aβ56, 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.

**Results:** We observed that Aβ trimers and Aβ56 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 Aβ56 (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.

**Conclusions and Relevance:** In cognitively intact older adults, CSF Aβ trimers and Aβ56 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.

**Author Affiliations:** N. Bud Grossman Center for Memory Research and Care (Drs Handoko, Zahs, and Ashe, and Ms Grant), Department of Neurology (Drs Handoko, Zahs, and Ashe, and Ms Grant), University of Minnesota; Geriatric Research Education Clinical Center, VA Medical Center (Drs Kuskowski and Ashe), Minneapolis, Minnesota; and Department of Psychiatry and Neurochemistry, University of Göteborg, Hisings Backa, Sweden (Drs Wallin and Blennow).

**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.24 There

©2013 American Medical Association. All rights reserved.
have been many investigations of various Ab species present in end-stage AD (reviewed in an article by Benilova et al 10), but our understanding of Ab species in the initial stages of AD remains limited. One species of Ab with the potential to be pathogenic in the initial, preclinical stages of AD is Ab56, a soluble 56-kDa oligomer that correlates with memory dysfunction independently of neuron loss or plaque deposition in several lines of mice overexpressing Ab-42 and disrupts cognition when injected into the cerebral ventricles of young, healthy rats.7,9

It has been suggested that Ab56 consists of 4 Ab trimers, a conjecture that is based on its pattern of dissociation in a polar solvent.7 Amyloid-β trimers were the only oligomeric species present in mice prior to the appearance of Ab56 in Tg2576 mice modeling preclinical AD,7 providing additional support for the idea that they are basic compositional units for higher order oligomers. Interestingly, low levels of Ab trimers were the only Ab oligomers found in the brains of children and adolescent humans.10 Amyloid-β trimers were not shown to disrupt cognition when applied to rats,7 and they did not correlate well to memory deterioration in mice.7 To our knowledge, there is at least 1 report of Ab trimers disrupting neural function in vitro,11 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.24 The 42–amino acid Ab isoform (Ab1-42), 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,13 their levels also robustly correlate with glucose hypometabolism,15 making them presumptive biomarkers for synaptic dysfunction during the preclinical stages of AD.

In this study, we measured Ab trimers and Ab56 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 Ab oligomers induce changes in tau in the preclinical stages of AD, thereby advancing our understanding of how Ab may be involved very early in its pathogenesis.

RESULTS

DEMOGRAPHICS OF STUDY PARTICIPANTS

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

INCREASED CSF Ab TRIMERS AND Ab56 WITH AGING

To determine the effects of aging on the levels of CSF Ab trimers and Ab56, 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 Ab trimers (P = .02; n = 59) and 0.25 between age and Ab56 (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 Ab trimers (P < .01) and 6.9% higher levels of Ab56 (P = .04) in the older subjects (Figure 1B). These results suggest that CSF Ab trimers and Ab56 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 Ab TRIMERS AND Ab56 HIGHER IN SUBJECTS AT RISK FOR AD

To determine whether elevated levels of Ab trimers or Ab56 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.17-19 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

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>68.9 (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>
</tbody>
</table>
| Mini Mental State Examination score, mean (SD) [range, 0-30] | NA | 29.3 (0.97)d | 28.2 (1.66) | 21.6 (4.59)e | <.001    

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.
between Aβ*56 and T-tau (P < .01) (Figure 2A) and 0.70 between Aβ*56 and p-tau 181 (P < .01) (Figure 2D). The correlation coefficients were 0.65 between Aβ trimers and T-tau (P < .01) (Figure 2B) and 0.71 between Aβ trimers and p-tau 181 (P < .01) (Figure 2D).

The relationships between CSF Aβ1-42 and tau were also examined by calculating age-adjusted partial correlation coefficients and P values. Correlation coefficients were −0.10 between Aβ1-42 and T-tau (P = .50) (Figure 2C) and −0.01 between Aβ1-42 and p-tau 181 (P = .95) (Figure 2F).

These results suggest that in cognitively normal older adults, there are strong relationships between CSF tau and both Aβ trimers and Aβ*56, which contrasts with the absence of relationships between Aβ1-42 and tau.

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

The relationships between CSF Aβ 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 Aβ*56 and T-tau (P = .35) (Figure 3A), 0.19 between Aβ*56 and p-tau 181 (P = .21) (Figure 3B), 0.37 between Aβ trimers and T-tau (P = .01) (Figure 3B), and 0.44 between Aβ trimers and p-tau 181 (P < .01) (Figure 3E). The relationships between CSF Aβ1-42 and tau were similarly assessed. Correlation coefficients were −0.40 between Aβ1-42 and T-tau (P < .01) (Figure 3C) and −0.33 between Aβ1-42 and p-tau 181 (P = .03) (Figure 3F).

These results suggest an attenuation of the relationships between CSF tau and both Aβ 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 Aβ1-42 in the symptomatic group.

**COMMENT**

We measured 2 Aβ oligomers, Aβ*56 and Aβ trimers, in CSF and found age-dependent increases in Aβ 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 Aβ oligomers, but not Aβ1-42, and tau in cognitively normal older adults, and attenuations of these relationships in MCI/AD. Because Aβ1-42 is a surrogate for Aβ fibril deposition, these findings suggest that in the years prior to the onset of overt symptoms, 1 or both of the Aβ oligomers, but not fibrillar Aβ, 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 Aβ therapies to alter the clinical course of patients with MCI or 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 drug development.

To our knowledge, this is the first report in which the levels of specific Aβ 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 Aβ1-42 ($-0.10 < r < -0.01$) 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 Aβ1-42 may not be useful as measures of how 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, 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 if Aβ trimers cluster to form Aβ 56, as is suspected. 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.

Accepted for Publication: January 4, 2013.
Published Online: March 11, 2013. doi:10.1001/jamaneurol.2013.48

Correspondence: Karen H. Ashe, MD, PhD, University of Minnesota, Departments of Neurology and Neuroscience, N. Bud Grossman Center for Memory Research and Care, 2101 6th St SE, Rm 5-190, Minneapolis, MN 55455-3008 (hsiao005@umn.edu).

Author Contributions: Study concept and design: Handoko, Wallin, Blennow, and Ashe. Acquisition of data: Handoko, Grant, Wallin, and Blennow. Analysis and interpretation of data: All authors. Drafting of the manuscript: Handoko and Ashe. Critical revision of the manuscript for important intellectual content: All authors. Statistical analysis: Handoko and Kuskowski. Obtained funding: Wallin, Blennow, and Ashe. Study supervision: Ashe.

Conflict of Interest Disclosures: None reported.

Funding/Support: Dr Ashe’s work is supported by grants RC1-AG35870 and R01-NS33249 from the National Institutes of Health. Dr Blennow and Dr Wallin’s work is supported by grants K2010-61X-1481-07-3 from the Research Council, Sweden.


Additional Contributions: We thank Peng Liu, PhD, and Melanie Kiihn, MA, for helpful discussions.

REFERENCES


