Neurofibrillary Tangles Mediate the Association of Amyloid Load With Clinical Alzheimer Disease and Level of Cognitive Function

David A. Bennett, MD; Julie A. Schneider, MD; Robert S. Wilson, PhD; Julia L. Bienias, ScD; Steven E. Arnold, MD

Objective: To test the hypothesis that the association of amyloid load with clinical Alzheimer disease (AD) and cognitive impairment is mediated through neurofibrillary tangles.

Design: Longitudinal clinicopathologic cohort study.

Participants and Setting: Forty-four individuals with clinically diagnosed AD and 53 without dementia who participated in the Religious Orders Study underwent a uniform structured clinical evaluation for AD and cognitive testing about 8 months prior to death, and brain autopsy at death.

Methods: The percent area occupied by amyloid-β/H9252 and the density of neurofibrillary tangles were quantified from 6 brain regions and averaged to yield summary measures of amyloid load and neurofibrillary tangles. Multivariate regression analyses were used to simultaneously examine the effects of amyloid load and neurofibrillary tangles on clinically diagnosed AD and level of cognition.

Main Outcome Measures: Clinically diagnosed AD and level of global cognitive function proximate to death.

Results: In separate logistic regression analyses, each 1% increase in amyloid load was associated with about a 50% increase in the odds of clinical AD (P=.002), and each neurofibrillary tangle was associated with a greater than 20% increase in the odds of clinical AD (P<.001). When a term for tangles was added to the regression model with amyloid, the association of amyloid load with clinical disease was reduced by more than 60% and was no longer significant, whereas the association of tangles with clinical disease was essentially unchanged. Similar results were found in analyses of global cognitive function.

Conclusion: These findings are consistent with a sequence of pathologic events whereby the effect of amyloid deposition on clinical disease is mediated by neurofibrillary tangles.

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MYLOID-β PEPTIDE PLAQUES and hyperphosphorylated paired helical filament tau protein–rich neurofibrillary tangles are the principal pathologic lesions of Alzheimer disease (AD).1 Determining how these 2 pathologic indices are related to each other and to the clinical manifestations of AD has important implications for strategies to treat and ultimately to prevent AD. Several lines of evidence suggest that deposition of amyloid-β peptide is the first step in a sequence of events that ultimately leads to clinical disease.2 By contrast, some have argued that these lesions are not directly related but are a consequence of a third underlying variable.3 It had been hoped that clinicopathologic studies would further elucidate this important controversy. However, few clinicopathologic studies have quantified amyloid load and tangles in persons with and without dementia,4,5 and only 3 small studies used multivariate statistical techniques to simultaneously examine the relationship of amyloid load and tangles to clinical status.5-8 In general, these studies suggest that neurofibrillary tangles correlate better with the presence and severity of dementia than do plaques. However, the results are also consistent with a sequence of events in which tangles are an intermediate step linking amyloid deposition to clinical disease. We used clinical data and postmortem tissue from older persons participating in the Religious Orders Study to test the hypothesis that the association of amyloid load with clinical AD and cognitive impairment is mediated through neurofibrillary tangles.

METHODS

SUBJECTS

Subjects were older Catholic nuns, priests, and brothers participating in the Religious Orders Study (see the “Acknowledgment” section).9,10

From the Rush Alzheimer’s Disease Center and the Departments of Neurological Sciences (Drs Bennett, Schneider, and Wilson) and Psychology (Dr Wilson), and the Rush Institute for Healthy Aging and Department of Internal Medicine (Dr Bienias), Rush University Medical Center, Chicago, Ill; and the Center for Neurobiology and Behavior, University of Pennsylvania, Philadelphia (Dr Arnold).
Each participant agreed to an annual clinical evaluation and signed an informed consent form and an Anatomical Gift Act, donating his or her brain to Rush investigators at the time of death. The study was approved by the institutional review board of Rush University Medical Center (Chicago, Ill.). Since January 1994, more than 900 persons have enrolled in the study and completed the baseline evaluation. Participation in the annual follow-up evaluations exceeds 95% of survivors, and the autopsy rate exceeds 90%.10

CLINICAL EVALUATION

Subjects underwent a uniform structured clinical evaluation, including a medical history, neurologic examination, cognitive performance testing, and review of a brain scan when available. Twenty-one cognitive performance tests were administered that assessed a broad range of cognitive abilities commonly affected by aging and AD, as previously described.9,10 Cognitive test results were reviewed by a board-certified neuropsychologist, and participants were evaluated in person by a physician with expertise in the evaluation of older persons with and without dementia. On the basis of this evaluation, participants were classified with respect to AD and other common conditions affecting the elderly as described previously.11-13 Follow-up evaluations, identical in all essential details, were performed annually by examiners blinded to previously collected data. At the time of death, all available clinical data were reviewed, and a summary diagnostic opinion was rendered regarding the most likely clinical diagnosis at the time of death. The reviewers were blinded to all postmortem data.

A global cognitive summary measure was used to minimize floor and ceiling effects and other sources of measurement error. It was constructed by converting the raw scores from 19 individual tests to Z scores, using the mean and standard deviation from the baseline evaluation of all participants and averaging the Z scores. To have a valid summary score, at least half of the component scores must be present. The derivation of the summary measure has been previously reported in detail.9-11

TISSUE PREPARATION

The brains of deceased subjects were removed in a standard fashion, its length (up to 6 blocks per case).

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STATISTICAL ANALYSIS

Linear regression was used to examine the relationship between amyloid load and tangles, controlling for age, sex, and education. To examine whether neurofibrillary tangles account for the association of amyloid load with clinical AD, we constructed multiple logistic regression models that examined the relationship of the amyloid load and tangles to clinical diagnosis, first separately and then simultaneously. We examined these models to see if the association of amyloid load with clinical diagnosis was reduced when a term for tangles was added to the model. A similar set of analyses used multiple linear regression to examine the relationship of amyloid load and tangles to level of global cognitive function proximate to death. We have used this analytic approach previously to examine potential events in a causal chain.12,13 All analyses controlled for...
age, sex, and education and were carried out using SAS/STAT software version 8 (SAS Institute Inc, Cary, NC) on a Sun-UltraSparc (SUN Microsystems Inc, Santa Clara, Calif) workstation. Models were validated graphically and analytically.

RESULTS

Ninety-seven deceased participants who underwent brain autopsy between August 28, 1996, and May 5, 2002, were included in these analyses; 44 of these 97 had met clinical criteria for probable AD and 53 did not have dementia. The median interval from the last clinical evaluation to brain autopsy was 8.2 months. Persons with AD were older, had slightly fewer years of education, and had lower scores on the Mini-Mental State Examination and the global measure of cognition (Table 1).

RELATIONSHIP OF AMYLOID LOAD TO TANGLES

The crude association between amyloid load and tangle density is shown in Figure 2. In a regression analysis adjusted for age, sex, and education, each 1% increase in amyloid load was associated with about 2.30 (SE=0.40) additional tangles (P<.001) and contributed 24.7% to the variance of tangles.

RELATIONSHIP OF AMYLOID LOAD AND TANGLES TO CLINICAL AD

Persons with AD had about twice as much amyloid deposition as persons without dementia and had about 4 times as many tangles (Table 1, Figure 3A and B). A series of regression analyses was performed to see if tangles accounted for the relationship of amyloid load to the clinical diagnosis of AD. In separate analyses controlling for

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Table 1. Selected Characteristics of Persons With Alzheimer Disease and Persons Without Dementia

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No Dementia (n = 53)</th>
<th>Alzheimer Disease (n = 44)</th>
<th>Total (N = 97)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, No. (%)</td>
<td>24 (45.3)</td>
<td>17 (38.6)</td>
<td>41 (42.3)</td>
</tr>
<tr>
<td>Age at death, y</td>
<td>82.4 ± 6.5</td>
<td>88.1 ± 5.3</td>
<td>85.0 ± 6.6</td>
</tr>
<tr>
<td>Education, y</td>
<td>18.7 ± 3.3</td>
<td>17.3 ± 3.0</td>
<td>18.1 ± 3.2</td>
</tr>
<tr>
<td>MMSE score</td>
<td>27.66 ± 1.89</td>
<td>16.07 ± 7.38</td>
<td>22.4 ± 7.75</td>
</tr>
<tr>
<td>Global cognition</td>
<td>−0.07 ± 0.53</td>
<td>−1.82 ± 0.83</td>
<td>−0.87 ± 1.11</td>
</tr>
<tr>
<td>Median interval, mo</td>
<td>8.0</td>
<td>8.6</td>
<td>8.2</td>
</tr>
<tr>
<td>Amyloid load, %</td>
<td>1.83 ± 1.84</td>
<td>3.81 ± 2.47</td>
<td>2.73 ± 2.35</td>
</tr>
<tr>
<td>Tangles/mm²</td>
<td>3.51 ± 3.64</td>
<td>14.22 ± 12.38</td>
<td>8.37 ± 10.23</td>
</tr>
</tbody>
</table>

Abbreviation: MMSE, Mini-Mental State Examination.

*Data are given as mean ± SD unless otherwise indicated.

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Figure 1. Inferior temporal cortex immunolabeled for amyloid scattered plaque deposits (A), abundant plaque deposits (B), paired helical filament tau rare neurofibrillary tangles (C), and abundant neurofibrillary tangles (D) (bar=10 µm)
age, sex, and education, each 1% increase in amyloid load was associated with about a 50% increase in the odds of clinical AD (Table 2, model 1), and the odds of clinical AD increased more than 20% for each additional tangle (Table 2, model 2). When tangles were added to the regression analysis with amyloid, the association of amyloid load with clinical disease was reduced by more than 60% and was no longer significant, whereas the association of tangles with clinical disease was essentially unchanged (odds for amyloid, 1.18; 95% confidence interval [CI], 0.87-1.60; odds for tangles, 1.21; 95% CI, 1.08-1.36) (Table 2, model 3). Figure 4A shows how the relationship between amyloid load and the probability of having AD is modified by the inclusion of a term

Table 2. Multiple Logistic Regression Models* Examining the Odds of Clinical AD

<table>
<thead>
<tr>
<th>Terms</th>
<th>Model 1</th>
<th></th>
<th></th>
<th>Model 2</th>
<th></th>
<th></th>
<th>Model 3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P Value</td>
<td>OR (95% CI)</td>
<td>P Value</td>
<td>OR (95% CI)</td>
<td>P Value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amyloid</td>
<td>1.49 (1.15-1.92)</td>
<td>.002</td>
<td>NA</td>
<td>NA</td>
<td>1.18 (0.87-1.60)</td>
<td>.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurofibrillary tangles</td>
<td>NA</td>
<td>NA</td>
<td>1.23 (1.10-1.38)</td>
<td>&lt;.001</td>
<td>1.21 (1.08-1.36)</td>
<td>.006</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AD, Alzheimer disease; CI, confidence interval; NA, not applicable; OR, odds ratio.

*Model 1 = amyloid; model 2 = neurofibrillary tangles; and model 3 = amyloid and neurofibrillary tangles. All models controlled for age, sex, and education.
for tangles in the model. The solid line shows the relationship between amyloid load and the probability of having AD from a model adjusted for age, sex, and education. The dotted line represents the relationship after including a term for tangles. Note that the relationship represented by the dotted line slope is much less steep, indicating a weaker association between amyloid load and AD after controlling for tangles. By contrast, the solid line representing the relationship between tangles and the probability of AD is essentially unchanged after including a term for amyloid (Figure 4B).

**RELATIONSHIP OF AMYLOID LOAD AND TANGLES TO LEVEL OF COGNITIVE FUNCTION**

To ensure that our results did not depend on the diagnostic classification approach used in the study, we conducted a similar set of analyses with level of global cognition assessed proximate to death. The crude association between amyloid load and tangles to level of cognition is shown in Figure 5. In separate analyses controlling for age, sex, and education, each 1% increase in amyloid load was associated with about a 0.16 \( P = .001 \) standard unit lower cognitive score (Table 3, model 1), and each tangle was associated with a 0.06 \( P < .001 \) standard unit lower cognitive score (Table 3, model 2). When a term for tangles was added to the regression analysis with amyloid, the association of amyloid load with clinical disease was reduced by more than 80% and was no longer significant, whereas the association of tangles with clinical disease was essentially unchanged (amyloid = −0.028 units; \( P = .46 \); tangles = −0.057 units; \( P < .001 \)) (Table 3, model 3). Figure 6A shows how the relationship between amy-
laid load and level of cognition is modified by inclusion of a term for tangles in the model. The solid line shows the relationship between amyloid load and level of cognition from a model adjusted for age, sex, and education. The dotted line represents the relationship after including a term for tangles. Note that the dotted line is nearly straight. By contrast, the solid line representing the relationship between tangles and level of cognition is essentially unchanged after including a term for amyloid (Figure 6B).

**COMMENT**

We quantified extracellular deposits of amyloid-β peptide plaque deposits and phosphorylated tau-immunoreactive neurofibrillary tangles from multiple brain regions from persons with and without clinically diagnosed AD. Both lesions were related to the presence of clinical AD and to the level of cognitive impairment when examined separately. However, when a term for tangles was added to the regression model with amyloid, the association of amyloid load with clinical disease was markedly attenuated and no longer significant, whereas the effect of tangles was essentially unchanged. These data are consistent with a sequence of events whereby neurofibrillary tangles mediate the association of amyloid deposition with clinical disease.

These data are in agreement with and extend the results of 3 small prior studies that used immunocytochemical techniques to assess plaques and tangles from persons with and without dementia and employed multivariate analytic techniques to examine their relative contribution to clinical status proximate to death.6–8 Two of those studies relied on semiquantitative measures of deposition to clinical status proximate to death.6–8 Two of those studies relied on semiquantitative measures of dementia severity, plaques and tangles.6,7 One of these 2 studies reported that plaques and tangles were related to a single cognitive measure proximate to death but that the plaques were no longer significant after controlling for tangles.8

Our findings are most consistent with a sequence of pathologic events whereby the effect of amyloid deposition on cognitive impairment is mediated primarily through the formation of neurofibrillary tangles. The precise molecular mechanisms linking amyloid deposition with tangle formation remains to be elucidated. However, recent data suggest that amyloid deposition may be required for tangles to develop and that both lesions may be required for neurotoxicity. For example, double transgenic mice overexpressing both human amyloid precursor protein and human tau appear to develop more tau abnormalities than single transgenics with tau alone.18 Similarly, injecting amyloid into tau transgenic mice appears to result in enhanced tau changes.19 Finally, recent in vitro studies suggest that amyloid can promote tau aggregation and phosphorylation,20,21 perhaps through the cleavage of caspase.22

There are several strengths to this study. Amyloid load and tangles were linked to both clinical diagnosis and level of global cognitive function assessed proximate to death. All analyses were performed on comparable persons from a single cohort, with high rates of follow-up participation and, finally, brain autopsy. This cohort controls for other potentially confounding variables such as occupation and lifestyle, and the sample size was sufficiently large to control for other important and potentially confounding variables such as age, sex, and education. Finally, uniform structured procedures were followed, examiners were blinded to previously collected data, and all postmortem data were collected by personnel blinded to clinical data, further reducing the potential for bias.

The study has several limitations. Most important, clinicopathologic analyses are inherently cross-sectional. Therefore, a sequence can be inferred but cannot be proved. Thus, although we tested an a priori hypothesis supported by the literature, we need to be cautious about the inferences that can be made from the analytic approach in this study. Likewise, one must also be cautious when drawing inferences regarding mechanisms of disease to humans from in vitro studies and animal models. A complete understanding of disease will come from the convergence of findings from several types of studies, all with their particular strengths and weaknesses. There are other limitations. We only quantified the fibrillar form of amyloid-β and paired helical filament tau–positive tangles identified by antibodies to a single epitope. Different epitopes of amyloid-β or tau or soluble amyloid-β or tau may be important to the development of clinical disease. To simplify the analyses, we combined data from multiple brain regions into a single measure of each pathologic index. It is possible that region-specific analyses would further illuminate the association of amyloid-β and tau with clinical status. Other pathologic findings in the brains of older persons could affect the relationship between amyloid-β and tau or modify their relationship to clinical disease. Finally, participants are not representative of the US population as a whole in terms of education and lifestyle. It is possible that these factors could alter the relationship of amyloid load and tau to clinical disease. Therefore, the findings will need to be replicated in similar studies of laypersons.

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Author contributions: Study concept and design (Drs Bennett, Wilson, and Arnold); acquisition of data (Drs Bennett, Schneider, Wilson, Arnold); analysis and interpretation of data (Drs Bennett, Wilson, Arnold, and Bienias); drafting of the manuscript (Dr Bennett); critical revision of the manuscript for important intellectual content (Drs Schneider, Wilson, Arnold, and Bienias); statistical expertise (Drs Bennett and Bienias); obtained funding (Dr Bennett); administrative, technical, and material support (Drs Bennett, Schneider, Wilson, and Arnold); study supervision (Drs Bennett and Arnold).

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REFERENCES