Neurofibrillary Tangles in Nondemented Elderly Subjects and Mild Alzheimer Disease

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Background: The relationship between neuropathological lesions and mild, “preclinical,” cognitive impairments of Alzheimer disease is poorly understood. Identification of the lesions that are most closely associated with the earliest symptoms of Alzheimer disease is crucial to the understanding of the disease process and the development of treatment strategies to affect its progression.

Design and Main Outcome Measures: We examined the extent of neurofibrillary tangles (NFTs) in 4 neocortical regions, the hippocampus, the entorhinal cortex, and the amygdala in 65 elderly subjects with no dementia, questionable dementia, mild dementia, or moderate dementia as assessed using the Clinical Dementia Rating Scale (CDR).

Setting and Patients: Postmortem study of nursing home residents.

Results: Neurofibrillary tangles were present in the entorhinal cortex and the hippocampus of all subjects, including those without cognitive deficits. Neocortical NFTs were mostly absent in the nondemented (CDR score, 0.0) subjects. The density of NFTs in the questionably demented (CDR score, 0.5) subjects was not significantly increased ($P > .20$) relative to the nondemented group in any of the brain regions studied. Significant increases ($P < .04$) in NFT density become apparent first in the amygdala and the temporal cortex in subjects rated to be mildly impaired (CDR score, 1.0). By the time that cognitive impairments were judged to be moderately severe (CDR score, 2.0), all regions of the brain examined, except for the occipital cortex, were significantly ($P < .05$) involved.

Conclusions: Some NFTs are present in the entorhinal cortex and hippocampus of most elderly individuals irrespective of their cognitive status, but the density of NFTs increases as a function of dementia severity.

Arch Neurol. 1999;56:713-718

A critical question in understanding the classic neuropathological features of Alzheimer disease (AD) (neuritic plaques [NPs] and neurofibrillary tangles [NFTs]) is whether they precede, follow, or occur in synchrony with the earliest and mildest signs of cognitive deterioration. Although the commonly used formal neuropathological diagnostic criteria for AD rely almost exclusively on the density of neocortical NPs, several studies have argued that the development of NFTs, especially in the entorhinal cortex, represents the earliest neuropathological change in AD. In fact, a recent consensus recommendation for the neuropathological diagnosis of AD has advised that in addition to the evaluation of NPs, the density and distribution of NFTs be considered in establishing a diagnosis of AD.

Frequently, diagnostic approaches to AD reflect the progression of the identified neuropathological lesions but overlook or minimize the role of the cognitive symptoms of the disease, especially when these deficits are mild or moderate. While the importance of NFTs to AD and its neuropathological diagnosis cannot be minimized, the relationship of NFTs to the earliest stages of AD and to the onset of dementia and cognitive dysfunction remains elusive. Some investigators have argued that the presence of NFTs is evidence of a pathological process and that, when they occur in the absence of cognitive deficits, they represent the preclinical or incipient manifestations of AD. Other investigators have suggested that since NFTs can be identified in the brains of nondemented elderly subjects and because their density can correlate with the age of the subjects, they should be treated as a consequence of the aging process. Furthermore, while NFTs may be necessary for the expression of dementia, by themselves, they are not sufficient for dementia. Thus, although the

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SUBJECTS AND METHODS

SUBJECTS

Sixty-five subjects constituted the principal cohort of this postmortem study. The subjects were selected from a group of 278 who had been residents of the Jewish Home and Hospital in Manhattan and Bronx, NY, an academic affiliate of the Mount Sinai School of Medicine, New York, NY. This cohort was part of a larger clinical and epidemiological study of early AD in which all consenting residents and new admissions to the Jewish Home and Hospital were given mental status screening tests, with more detailed clinical evaluations performed on patients without dementia or on patients with mild cognitive impairment. All assessment procedures were approved by the Jewish Home and Hospital and the Mount Sinai School of Medicine institutional review boards, and consent for autopsy was obtained from each subject's legal next of kin. Because the aim of this study was to identify the relationship between NFTs and early and mild dementia, only those subjects with CDR scores of 0.0 to 2.0 (see “Final Case Selection” subsection) were selected for inclusion. Many of the subjects included in this series died without undergoing neuropsychological testing because the testing began after the subjects had entered the Jewish Home and Hospital. However, detailed clinical records are available for all residents, and research staff conduct detailed interviews with staff and family caregivers to obtain information about the antemortem functional and cognitive status of the patients. The subject selection criteria were based on neuropathological and cognitive measures. Initially, all subjects with non–AD-related neuropathological lesions or AD-related neuropathological lesions complicated by other neuropathological lesions of sufficient magnitude to contribute to cognitive dysfunction were excluded from consideration. These neuropathological lesions included, but were not limited to, Pick disease, Lewy body inclusions, Parkinson disease, stroke, multi-infarct dementia, and severe cerebrovascular disease. Subjects with mild cerebrovascular disease judged to be insufficient in severity to affect cognitive function24-27 were not excluded.

A multistep approach was applied to the assignment of CDR scores based on cognitive and functional status during the last 6 months of life. Initially, a CDR score was obtained by careful review (K.K. and V.H.) of all information within each patient’s medical chart (admitting diagnoses, nurse’s notes, social work records, results of psychiatric and neurologic consultations, medication histories, results of mental status testing, and all other medical records and laboratory studies). These same records were then examined by a second reviewer to obtain a second independent CDR score. Subsequently, the second reviewer or another member of the cognitive assessment team conducted telephone interviews with at least 1 family member or caregiver for each subject and assigned a third CDR score. The intrarater reliability for 40 subjects undergoing consecutive assessment was high (interclass correlation, 0.88). All 3 CDR scores and all pertinent medical chart information were then presented to a senior clinician (D.M.), and a consensus CDR score was derived. The correlation between the initial postmortem medical chart review CDR scores and the final consensus CDR scores was r = 0.96 (P < .001). The reliability of the postmortem medical chart review procedure for CDR scoring was established in a pilot study. Clinical Dementia Rating Scale scores were determined by direct observation and patient interview, and by medical chart review alone for 35 subjects. An interclass correlation coefficient of 0.86 was obtained for the 2 independent assessments of CDR. A subset of the subjects (n = 22) had undergone neuropsychological assessment and had been assessed using the Mini-Mental State Examination28 and the Alzheimer’s Disease Assessment Scale.29 These assessments were also considered in deriving the final consensus CDR score. The correlation between the consensus CDR score assigned and the Mini-Mental State Examination score was r = 0.48 (P = .03). If only those subjects who had received a Mini-Mental State Examination score within 1 year of death were considered (n = 14), then the correlation between the consensus CDR score and the last Mini-Mental State Examination score rose to r = 0.73 (P = .003).

RESULTS

The median densities of NFTs in the 4 neocortical regions and in the hippocampus, entorhinal cortex, and amygdala are shown in Figure 1. To depict the occurrence of NFTs in the different brain regions more clearly, the results are plotted as the proportion of subjects receiving NFT ratings of none vs NFT ratings of sparse or
NEUROPATHOLOGICAL ASSESSMENT

Autopsy, tissue dissection, and neuropathological procedures were identical to those described earlier.28 All neuropathological studies were performed on the right hemisphere by 2 of us (D.P.P. and D.P.P.). The data on the density and distribution of neuropathological lesions were obtained without knowledge of the cognitive status of the subjects. Every subject underwent evaluation for the extent of neuropathological lesions using the Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) neuropathological battery.1 Although the full CERAD neuropathological battery (supplemented with additional regions of assessment) was performed for each subject, only data from examination of the NFTs in the hippocampus (CA1), entorhinal cortex, amygdala, middle frontal gyrus, superior and middle temporal gyrus, inferior parietal lobule, and occipital calcarine cortex (area 17) are presented. As part of the neuropathological assessment and diagnosis procedures, sections (3-8 μm thick) from paraffin-embedded blocks were stained using hematoxylin-eosin, modified Bielschowsky, modified thioflavine S, anti-β-amyloid, and anti-τ. Any case showing evidence of Lewy body formation (anti-ubiquitin [Daka Corp, Carpinteria, Calif] staining) was excluded from this study. Multiple (5 in general) high-power (200X, 0.5 mm²) fields were examined on each slide, and the density of NFTs was rated on a 4-point scale of absent, sparse, moderate, or severe according to the scoring criteria established by CERAD. When NFTs were unevenly distributed in a slide, NFTs in the region with the highest density were rated. Assessments of NFTs were based primarily on modified Bielschowsky staining.

FINAL CASE SELECTION

After the completion of the neuropathological studies and assignment of consensus CDR scores, a final consensus conference was held (V.H., D.P.P., D.P.P., D.M., K.L.D., and R.C.M.) for the selection of subjects for inclusion in the study. Using the criteria for selection previously outlined, the original cohort of potential subjects was reduced to 65 subjects who met all of the inclusion and exclusion criteria described. Four groups of subjects were formed, consisting of subjects with CDR scores of 0.0 (cognitively intact), 0.5 (questionable dementia), 1.0 (mildly impaired), and 2.0 (moderately impaired). A fifth group was formed from a cohort with CDR scores of 4.0 and 5.0 (severely demented)29 to provide an extreme comparison group (Table 1). Because the focus of the study was on the association between NFTs and cognitive deficits, the study groups were formed based purely on their CDR scores without regard to the extent of NFTs or NPs present. However, the distribution of the subjects for neuropathological diagnoses within each CDR category is presented in Table 2. Groups formed based on CDR scores did not differ significantly on age (F3,62 = 1.8, P = .15). There were significantly (P = .006) more women (n = 51) than men (n = 14) in the study cohort (Table 1), but the proportion of men and women in the different CDR groups did not differ significantly (χ² = 0.8, P = .82).

DATA ANALYSES

The principal study cohort consisted of the subjects with CDR scores of 0.0, 0.5, 1.0, and 2.0. The 4 CDR categories were used as the independent variable for subsequent analyses. Since the study examined the neuropathological features of early AD or mild dementia, results from the extreme comparison group (CDR scores of 4.0 and 5.0) were not used in any of the statistical tests, but their data are presented in the tables and figures for illustrative purposes. The dependent variables consisted of the CERAD ratings of NFT density in the regions specified. Because the measures were based on ordinal CERAD ratings, they were analyzed by Kruskal-Wallis analyses of variance (ANOVAs) for each region. For individual group comparisons, Mann-Whitney U tests were performed when significant overall differences were revealed by the Kruskal-Wallis tests. Differences in NFT densities across brain regions were analyzed by Friedman ANOVAs for related samples. Wilcoxon matched pairs tests were used for post hoc analysis of differences between brain regions within any given CDR grouping. To determine the correlation between CDR scores and NFT density, Spearman rank order procedures were used.
gyri than in the inferior parietal lobule, which had a greater density of NFTs than the occipital cortex \((P, 0.03)\).

Comparison of the median NFT density scores of subjects with CDR scores of 4.0 and 5.0\(^2\) (Figure 1) suggests that even when cognitive deficits were rated as severe or terminal, NFT densities in the neocortical regions were less profound than in the hippocampus, entorhinal cortex, and amygdala \((P, 0.05)\). The density of NFTs in the frontal, temporal, and parietal neocortical regions and the amygdala (but not in the hippocampus and entorhinal cortex) increased in the severely demented group (CDR scores, 4.0 and 5.0) relative to the moderately impaired group (CDR scores, 2.0) (Mann-Whitney \(U\) tests, \(P, 0.03\)).

The density of NFTs in the different brain regions examined correlated significantly with the level of cognitive impairment. With the exception of the occipital cortex, NFT ratings were significantly and positively correlated with the CDR scores in each of the brain regions (Spearman rank order \(r\) range, 0.34-0.54; \(P, .001\)). The correlation of NFT density with CDR scores was highest for the superior and medial temporal cortical region \((r = 0.54, P < .001)\).

The age of the subjects was also positively correlated with the density of NFTs in all of the regions examined, except for the occipital cortex (Spearman rank order \(r\) range, 0.34-0.25; \(P < .05\)). Analysis of partial correlations controlling for age showed that the correlation of CDR scores with NFT density was not accounted for by the age of the subjects. The range of correlations of CDR with NFT density ratings remained significant for all regions \((r\) range, 0.50-0.27; \(P < .04\)), except for the occipital cortex \((r = 0.18, P = .16)\), even after controlling for age.

The results of this study show that the density of NFTs in most regions of the cerebral cortex, hippocampus, and amygdala increases significantly as a function of increas-

### Table 1. Demographic Characteristics of the Sample*

<table>
<thead>
<tr>
<th>CDR Group</th>
<th>0.0</th>
<th>0.5</th>
<th>1.0</th>
<th>2.0</th>
<th>4.0 + 5.0</th>
<th>All</th>
</tr>
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<tbody>
<tr>
<td>Sample size</td>
<td>18</td>
<td>11</td>
<td>22</td>
<td>15</td>
<td>15</td>
<td>81</td>
</tr>
<tr>
<td>Mean ± SD PMI, h</td>
<td>8.29 ± 5.96</td>
<td>5.59 ± 4.63</td>
<td>4.79 ± 3.97</td>
<td>6.09 ± 6.04</td>
<td>5.84 ± 7.39</td>
<td>6.17 ± 5.07</td>
</tr>
<tr>
<td>Age, y</td>
<td>Mean ± SD (range)</td>
<td>83.8 ± 9.9 (64-99)</td>
<td>85.8 ± 8.3 (69-94)</td>
<td>82.9 ± 8.2 (74-103)</td>
<td>89.1 ± 5.7 (74-97)</td>
<td>85.4 ± 10.3 (62-103)</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
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<td>9</td>
<td>16</td>
<td>12</td>
<td>12</td>
<td>64</td>
</tr>
</tbody>
</table>

* CDR indicates Clinical Dementia Rating Scale; PMI, postmortem interval. CDR group 0.0 indicates cognitively intact; 0.5, questionable dementia; 1.0, mildly impaired; 2.0, moderately impaired; and 4.0 and 5.0, severely demented.

### Table 2. Neuropathological Diagnoses and Neurofibrillary Tangle Characteristics of Study Subjects*

<table>
<thead>
<tr>
<th>CDR Group</th>
<th>0.0</th>
<th>0.5</th>
<th>1.0</th>
<th>2.0</th>
<th>5.0</th>
</tr>
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<tbody>
<tr>
<td>Normal</td>
<td>13</td>
<td>4</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Alzheimer disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Definite</td>
<td>0</td>
<td>1</td>
<td>11</td>
<td>11</td>
<td>15</td>
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<tr>
<td>Probable</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>0</td>
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<tr>
<td>Possible</td>
<td>3</td>
<td>6</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>11</td>
<td>23</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>

* CDR indicates Clinical Dementia Rating Scale. An explanation of the CDR groups is given in the footnote to Table 1.
†None indicates a Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) score of 0; sparse, CERAD score of 1; moderate, CERAD score of 3; and severe, CERAD score of 5.

### Figure 1. Density of neurofibrillary tangles in the 4 neocortical regions and in the entorhinal cortex, hippocampus, and amygdala in nondemented (Clinical Dementia Rating Scale [CDR] score, 0.0), questionably demented (CDR score, 0.5), mildly demented (CDR score, 1.0), and moderately demented (CDR score, 2.0) subjects. Data on severely demented subjects (CDR score, 4.0 and 5.0) are provided for comparison. The y-axis represents the median of Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) neurofibrillary tangle ratings (0 indicates none; 1, sparse; 3, moderate; and 5, severe).
ing cognitive impairment in elderly subjects. Unlike our previously observed increase in the density of NPs in the neocortex of subjects with CDR scores of 0.5, the density of NFTs in the questionably demented (CDR score, 0.5) subjects was not significantly increased relative to the nondemented group in any of the brain regions studied. Significant increases in NFT density, relative to nondemented subjects, become apparent first in the amygdala and the temporal cortex in subjects rated to be mildly impaired (CDR score, 1.0). By the time that cognitive impairments were judged to be moderately severe (CDR score, 2.0), all regions of the brain examined, except for the occipital cortex, were significantly involved. These results are in general agreement with the recently published work of Berg et al.

Increases in the density of NFTs beyond those present in the cognitively intact group became evident only in the subjects with CDR scores of 1.0, suggesting that NFTs are not associated with the first signs of cognitive impairment. It is possible that increased densities of NFTs in other brain regions may more closely reflect the earliest stages of cognitive dysfunction. Initial exploratory analyses failed to reveal any differences in NFT densities between the group with a CDR score of 0.0 and the group with a CDR score of 2.0 in the nucleus basalis of Meynert, substantia nigra, dorsal raphe nucleus, locus ceruleus, dorsal vagus nucleus, lateral cerebellum, cerebellar vermis, thalamus, hypothalamus, and mamillary bodies. Although it is possible that more quantitative techniques for NFT assessment, such as direct counting of NFT-bearing neurons or measures of neurofibrillary threads, would have allowed discrimination of the group with a CDR score of 0.5 from the group with a CDR score of 0.0, the results did not suggest a significant trend in this direction. It is unlikely that the failure to observe differences between the group with a CDR score of 0.0 and the group with a CDR score of 0.5 was due to a failure to accurately assign subjects to these CDR groups, since in our earlier study this same grouping of the same subjects showed clear differences in NP density between these 2 groups.

Several inferences can be derived from these observations. The fact that NFTs were observed in the hippocampus and entorhinal cortex of most of the nondemented subjects (>94%) suggests that some NFT pathological features in these regions may be relatively benign, occurring as a consequence of advanced age without causing discernable cognitive impairments. Furthermore, as illustrated in Figures 1 and 2, the density of NFTs in the entorhinal cortex of more than 50% of the subjects in the group with a CDR score of 0.0 was rated as moderate or severe. Other investigators have made similar observations about the presence of NFTs in the entorhinal cortex of clinically nondemented subjects, but Berg et al had discordant findings. Although the argument that these nondemented subjects with NFTs in the hippocampus and in the entorhinal cortex may represent cases of early AD or incipient AD cannot be denied, this proposition would imply that most of the nondemented subjects in our study cohort suffered from incipient AD. Given the robust correlation of entorhinal cortex NFT densities with the age of the subjects, a more parsimonious interpretation is that entorhinal cortex and hippocampal NFTs are associated with advanced age and by themselves are not sufficient for the expression of dementia.

The observed differences in the density of NFTs in the hippocampus and entorhinal cortex vs the neocortical regions examined suggest that the distribution of NFTs within the brain varies as a function of the degree of cognitive impairment (Figure 1). The hippocampus and entorhinal cortex are involved in the elderly even in the absence of cognitive deficits, but the degree of their involvement increases with increasing dementia severity. In the neocortex, NFTs are absent in nonimpaired and questionably impaired subjects, but increasing cognitive impairment is associated with the appearance of NFTs with densities that are in the sparse to moderate range. In this respect, the results reported are similar to those presented by Braak and Braak and suggest a regional progression of NFT pathological features from the hippocampus and entorhinal cortex to the neocortex.

In general, our observations suggest that the presence of NFTs in the brain is not diagnostic of AD, but that NFTs should be viewed as pathological markers that may be associated with the evolution of dementia. These results raise the question of what other features of AD
may be more closely associated with the initial onset of dementia. Clearly, neuronal loss, decreases in the density of synapses, deficits in neurotransmitter systems, and the density of NPs and β-amyloid burden, among other AD-related neuropathological features, are all potential candidates for close correlates of early dementia. In fact, in a study, the density of NPs in the same brain regions and subjects as those described in this article, the density of neocortical NPs was significantly higher in subjects with a CDR rating of 0.5 relative to subjects in the nondemented (CDR rating, 0.0) group. On the other hand, the density of NPs in the neocortex reached a plateau by the time subjects were classified as moderately demented (CDR rating, 2.0). These results, viewed in light of the current findings, suggest that increasing NP densities may be good markers of increasing dementia severity in early phases of AD, while increasing densities of NFTs may represent good markers of dementia severity during the late phases of AD.

Accepted for publication September 3, 1998.

This study was supported by grant P01-AG02219 from the National Institute on Aging, Bethesda, Md.

We thank Nicolaos Robakis, PhD, Mount Sinai School of Medicine, New York, NY, for the anti-β-amyloid, and Andre Delacourte, PhD, Unite INSERM, Lille, France, for the anti-t. We also thank James Schmieder, PhD, Tricia Shreve, Maria Paredes, Anne Peterson, Waseem Ahmed, MD, and Gregory Austin for their help with medical chart reviews, informant interviews, statistical analyses, autopsies, and specimen preparation.

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