Clinicopathologic Studies in Cognitively Healthy Aging and Alzheimer Disease

Relation of Histologic Markers to Dementia Severity, Age, Sex, and Apolipoprotein E Genotype

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Objective: To study differences between subjects with Alzheimer disease (AD) and cognitively intact control subjects, with respect to brain histologic markers of AD, and the relationship of those markers in the AD group to severity of dementia, age at death, sex, and apolipoprotein E genotype.

Setting: Washington University Alzheimer's Disease Research Center, St Louis, Mo.

Design and Subjects: Consecutive neuropathologic series of 224 prospectively studied volunteer research subjects, 186 with dementia of the Alzheimer type (DAT) or “incipient” DAT and confirmed to have AD by postmortem examination and 13 cognitively intact subjects, confirmed to lack postmortem findings of AD.

Main Outcome Measures: Brain densities (number per square millimeter) of senile plaques and neurofibrillary tangles, extent of cerebral amyloid angiopathy, cortical Lewy bodies, and apolipoprotein E genotype.

Results: Neocortical neurofibrillary tangle densities were substantially correlated with dementia severity, and to a greater degree than was true for senile plaque densities. When infarcts, hemorrhages, and Parkinson disease changes coexisted with AD, neurofibrillary tangle and senile plaque densities were lower. Plaque-predominant AD was found in a greater proportion of subjects with milder than more severe dementia. Entorhinal cortical Lewy bodies were no more frequent in plaque-predominant AD than in the remaining AD cases. Increasing age at death was negatively correlated with dementia severity and densities of senile plaques and neurofibrillary tangles. The apolipoprotein E ε4 allele frequency was greater in AD than in control subjects but decreased with increasing age. After controlling for dementia severity, senile plaque densities were only weakly related to ε4 allele frequency, and only in hippocampus. However, the degree of cerebral amyloid angiopathy was clearly related to ε4 allele frequency. Among subjects diagnosed during life as having DAT or incipient DAT, only 7% were found to have a neuropathologic disorder other than AD causing their dementia.

Conclusions: (1) The order of the strength of relationships between densities of histologic markers and dementia severity in AD is neurofibrillary tangles greater than cored senile plaques greater than total senile plaques. (2) Advanced age at death is associated with somewhat less severe dementia and fewer senile plaques and neurofibrillary tangles. (3) Plaque-predominant AD may represent a developmental stage in AD. (4) Despite a substantial effect of apolipoprotein E ε4 as a risk factor for AD, on decreasing the age at AD onset, and increasing the amount of cerebral amyloid angiopathy, its effect on senile plaque densities is variable and complex, being confounded with age, dementia severity, and methodologic differences. (5) Stringent clinical diagnostic criteria for DAT, even in the very mild stage, and senile plaque–based neuropathologic criteria for AD are highly accurate.

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SUBJECTS AND METHODS

SAMPLE

All subjects were volunteers seen for longitudinal research studies of DAT and cognitively healthy aging. None were members of AD kindreds with known genetic mutations. Those with dementia met clinical research inclusionary and exclusionary diagnostic criteria for DAT,18 corresponding to those for “probable AD.”19 Control subjects and those with “incipient” dementia (see next section for definition) met the same exclusionary criteria. All subjects meeting these criteria, examined during life and coming to autopsy at Washington University, St. Louis, Mo, from January 1, 1981, through May 31, 1996 (n=224), were considered for this report. Two of 17 control subjects were eliminated from analyses because they suffered massive cranioocular trauma that led to their death. As discussed previously,10 their brains had abundant SPs, interpretable either as induced by the trauma20,21 (a debatable point) or evidence of preclinical AD. Two other controls (aged 74 and 85 years at death) had brains with neocortical SP densities that met neuropathologic criteria for AD (see that section for definition). Thus, sample size for the controls in the analyses reported here was 13.

There were 207 subjects diagnosed as having DAT or incipient DAT. The presence of AD was confirmed neuropathologically in 192 of the 207, a clinical diagnostic accuracy rate of 93%. Twenty-one of the 207 subjects were eliminated from this series. In 14, a non-AD cause for the clinically diagnosed DAT was found (Table 1). In the brain of 1 subject diagnosed clinically as having incipient DAT, no abnormalities were observed on neuropathologic study. In 6 persons with autopsy-confirmed AD, the cognitive status near death could not be ascertained or major intervening comorbidity made it impossible to know much of the dementia was attributable to AD. Thus, the sample examined here (N=199) included 13 control subjects and 186 subjects with DAT or incipient DAT with autopsy-confirmed AD. Demographic information is provided in Table 2.

CLINICAL ASSESSMENT

At each clinical assessment, the subjects were assigned a Washington University Clinical Dementia Rating (CDR)23,24 in which 0 indicates no dementia and 0.5, 1, 2, and 3 signify questionable (or very mild), mild, moderate, and severe dementia, respectively. The CDR was assigned by an experienced physician (L.B., E.H.R., or J.C.M.) or nurse specialist (M.C., or J.N.) with physician review after standardized interview18,23 (no impairment) to 18 (maximum impairment). Interrater reliability of the CDR and sum of boxes among trained physicians and nurses has been good.25,26 (Results of psychometric testing were not disclosed to the physicians or nurses at the time the CDR was assigned.) Experienced nurse specialists (M.C. and J.N.) or physicians (L.B., E.H.R. and J.C.M.) interviewed a knowledgeable informant after the subject’s death, but before the results of autopsy were known, and derived a CDR and sum of boxes pertinent to the subject’s level of cognitive performance just before the terminal events leading to death. This interview was a portion of our validated postmortem interview protocol.28 In addition, a physician reviewed the records of all clinical assessments (usually multiple and videotaped for independent rating by a second clinician) and the postmortem interview to prepare an “expiration summary” with its own CDR and sum of boxes, also before results of autopsy were known. This method was useful to take into account discrepancies between ratings during life and those on postmortem interview with respect to CDR 0.5 vs CDR 0.10 When there were disagreements regarding CDR 0 or CDR 0.5 between clinical raters or between last clinical assessment and postmortem interview/expiration summary, for the present study the subject was assigned to a CDR 0/0.5 classification. The sum of boxes associated with the CDR 0.5 was then used for data analysis. By this procedure, the CDR 0 classification was preserved as one in which no rating other than CDR 0 was applied during life or post mortem. Our experience9,10 has led us to recognize that a CDR 0.5 designation is an indication of the beginning manifestation of DAT (and a predictor of postmortem confirmation of AD) when there is no other ready explanation. Whereas some subjects with CDR 0.5 satisfy our research criteria for DAT19 (impairment in memory plus 3 of the other CDR cognitive domains), others do not, because they are judged to be impaired in fewer domains. Similarly, those subjects with CDR 0.3 who do not meet our criteria for DAT would not satisfy other standard diagnostic criteria19,29 for DAT, often because the cognitive decline has not reached the threshold of interfering with social or occupational activity. In our studies, they are being labeled as having “incipient” DAT, CDR 0.5, or CDR 0/0.5. Because quantitative data on the neuropathologic markers in the brains of subjects with AD who had CDR 0/0.5 (n=6) were highly similar to those of subjects with AD who had CDR 0.5 (n=11), data on these 2 groups were combined and labeled CDR 0.5 in subsequent analyses. Estimated age at onset was based on reports from collateral sources when cognitive decline was first diagnosed. Duration of the severe dementia stage (CDR 3) was estimated for all subjects who reached that point before death. All procedures and the means for obtaining informed consent were approved by the institutional review board. Relevant clinicopathologic data on small groups of these subjects have been reported previously.19,20,22,30,31

apoE GENOTYPING

At Washington University, DNA was extracted from frozen brain or antemortem blood samples by means of QIamp kits (Qiagen, Chatsworth, Calif) as per the manufacturer’s protocols. Two hundred microliters of blood was placed in a tube with 25 µL of Qiagen proteinase K and 200 µL of buffer AL, then vortexed, incubated at 70°C for 10 minutes, and mixed
with 210 µL of ethanol. The mixture was spun through a QIAamp spin column at 6000g for 1 minute. The column was washed twice with 500 µL of buffer AL and then DNA was eluted with 200 µL of buffer AE. Genotypes were determined by polymerase chain reaction amplification, digestion with CfoI (Boehringer Mannheim, Indianapolis, Ind), and electrophoresis through 6% NuSieve 3:1 agarose (FMC Bioproducts, Rockland, Me). The electrophoresis conditions were 4.5 V/cm for 90 minutes. Products were visualized under UV light after the gel was incubated in ethidium bromide solution. Primer sequences and polymerase chain reaction conditions were as previously described.12,13 When blood or frozen brain samples were not available, paraffin-embedded fixed brain samples were sent for genotyping at both Emory University, Atlanta, Ga, and Duke University, Durham, NC, as a quality control measure. At Emory, DNA extracts were prepared from sections of these samples mounted on glass slides; 2 different methods were used as previously described.10 Polymerase chain reaction amplification and restriction enzyme (HhaI) digestion12 were then carried out, and the resulting DNA fragments were visualized with ethidium bromide. At Duke, a crude DNA extract was prepared from sections mounted on glass slides.14 Two microliters of the extract was used in the 15-µL radioactive apoE restriction fragment isotyping protocol.11 Genotype genotypes were successfully obtained in 10 of the 13 controls and 166 of the 186 subjects with autopsy-confirmed AD.

NEUROPATHOLOGIC ASSESSMENT

The following regions from the left cerebral hemisphere were sampled for microscopic morphometric analyses: middle frontal gyrus, anterior third of superior temporal gyrus, inferior parietal lobule, CA1 portion of the hippocampus and subiculum, and entorhinal cortex between the levels of the mamillary and lateral geniculate bodies. Sections (6 µm) were cut from each paraffin-embedded block perpendicularly to the pial surface. The staining and counting procedures for NFTs and SPs were detailed previously1 and are summarized here. Two modifications of the Bielschowsky ammoniacal silver method were used. One was optimized to demonstrate neurofibrillary abnormalities, including extracellular and intracellular NFTs and cored plus noncored neuritic plaques. The other, adapted from Hedreen and colleagues,16 provided optimal detection of all SPs, including diffuse and neuritic plaques.

In the microscopic sections from each region, densities of NFTs and SPs (total and subtypes), expressed as average number per square millimeter, were determined in a standardized method detailed previously1 without selection bias based on variations of lesion densities or distributions in individual cases. Counts were taken in 10 consecutive 1×1-mm cortical fields per slide, 5 along the pial surface and 5 along the white matter–cortex junction. Both intracellular and extracellular tangles were included in the NFT counts. Total SPs included all varieties of argyrophilic diffuse and neuritic plaques. Diffuse plaques are amorphous or finely fibrillar deposits and lack abnormal argyrophilic neurites or central cores. Neuritic plaques contain abnormal swollen argyrophilic neurites. Cored SPs are a subset of neuritic plaques and contain central compact cores. Neuritic plaque densities were determined by means of one silver method on a section of frontal cortex within 50 µm of the section used for total SP densities with the other silver method.16 A previous publication from this laboratory provided evidence that total SP densities by this method are highly correlated with percentage of cortical area occupied by SPs and therefore with “amyloid burden.”16 It also provided evidence for cross-validation of the manual method used here with the automated counting. Because there were 8- to 10-fold more severely demented (CDR 3) subjects with AD than those in any of the less severe CDR stages (Table 2), complete NFT and SP morphometric studies were performed on all subjects with CDR less than 3 related to the degree and duration of dementia.17 Densities (counts per square millimeter) of total neocortical SPs were not related to severity of dementia in the subjects with AD, but cored neocortical plaque densities were.

In the last few years, there have been 2 major advances pertinent to such clinicopathologic studies. The apolipoprotein E (apoE) genotype, specifically the ε4 allele, has been recognized as a major risk factor for late-onset AD that lowers the age at onset and has been reported to increase the extent of β-amyloid deposition in brain parenchyma and in cerebral and meningeal blood vessels.12-16 As Olichney and colleagues16 pointed out, “the potential confounding factors of the ε4 allele . . . being associated with an earlier age of onset and possible apoE-gender interactions also need to be addressed since these factors have not been controlled for in most neuropathologic studies of apoE in AD.” The second recent advance is the recognition that cortical Lewy bodies are often found in the brains of those older individuals with “plaque-only” or “plaque-predominant” AD (“Lewy body variant of AD”).17 This report extends the scope of the previous communication. It is based on a much larger series, 224 sub-
and on 78 of the 144 CDR 3 subjects. (Power considerations on comparing data on CDR 3 subjects with those on any of the less severely demented groups indicated that the gain from providing NFT and SP data on 144 instead of 78 CDR 3 subjects would be minimal.) The 78 were chosen to include the youngest and oldest subjects to analyze for age effect. No other selection criteria were used. Neuritic plaque densities were determined on all 144 CDR 3 subjects.

Because of their quantitative nature, the consensus criteria reported by Khachaturian38 were used for the diagnosis of AD, with the added requirement that the average total SP density of the 10 microscopic fields exceeded the age-adjusted criteria in at least 1 neocortical region. “Plaque-predominant” AD (PPAD) was defined as having no more than 1 NFT per square millimeter averaged across the 10 fields in any neocortical region.

Cerebral amyloid angiopathy was assessed by means of thioflavine S–stained tissue sections.1 The investigator (D.W.M.) scanned the entire section (measuring approximately 2.5 cm²) to ensure detecting all thioflavine S–positive meningeal and parenchymal blood vessels. The maximum number of positive blood vessel profiles in any single 1-mm² field was measured for a given section as follows: grade 0 (none), grade 1 (1-2 positive profiles), grade 2 (3-5 positive), and grade 3 (>3 positive). This sampling strategy was adopted because of our experience that AD brains with widespread CAA also have high CAA scores in individual fields.

Cortical Lewy bodies (LB) were assessed with an anti-ubiquitin–stained section of entorhinal cortex, chosen to be representative of the limbic system where cortical LBs tend to be prevalent.39 (In an unpublished consecutive series of 30 AD subjects with cortical LBs in which at least 5 neocortical regions were studied by 2 of us [S.S.M. and M.G.], all had entorhinal LBs; 29 had neocortical LBs.) The LBs were noted as present or absent after search through consecutive 1-mm² fields from the medial edge of entorhinal cortex to the depth of the collateral sulcus. The LBs were distinguished from NFTs by being circular or oval, nonfibrillar, and usually associated with an eccentric nucleus. On hematoxylin-eosin preparations of the substantia nigra, LBs were noted as present or absent. In addition, nigral neuronal loss, gliosis, and free neuremeline pigment were each assessed on a scale of 0 to 3+. When there was a combined score of 2.5 or higher on those scales, substantia nigral degeneration was diagnosed. All histologic data reported here were acquired by 1 of us (D.W.M.) without knowledge of clinical data, genotype, or prior neuropathologic diagnostic assessment.

### NEUROPATHOLOGIC DIAGNOSES

The 13 control subjects (CDR 0) had brains with very few, if any, SPs or NFTs (exemplified in Figure 1, Figure 2, and Figure 3). Of the 186 AD cases, 114 (61.3%) had pure AD. A substantial number had both AD and Parkinson disease changes (substantia nigral degeneration plus nigral LBs [PD], 14.0%) or nonspecific substantia nigral degeneration (SND, 8.1%). Only 8.6% of the subjects had AD plus vascular lesions, defined as acute, subacute, or remote infarcts (including microinfarcts) or hemorrhage but excluding CAA. The remaining 8.1% had AD, vascular lesions, and either PD or SND. (None had signs of stroke or parkinsonism at onset of dementia.) Of the 13 controls, 9 had vascular lesions, 1 had PD, and 4 others had SND (1 control subject had vascular lesions and SND).

### EFFECT OF AGE

Older subjects had less severe dementia as indicated by CDR at death \((r = -0.33)\). Similarly, the correlations between age and lesion density shown in the last column of Table 3 were also negative, except for a non-significant positive age correlation with entorhinal cored SPs. For these reasons, age at death was used as a covariate in the next analyses.

### STATISTICAL ANALYSES

The distributions of plaque and tangle densities in the 10 fields for each of the 5 regions for each subject showed substantial intrasubject variability with distributions skewed to the right. By taking the logarithm of each density (adding 0.5 to each density to avoid logarithms of 0) and taking the average of the logarithms, a “typical” density was computed for each lesion, for each region, for each subject. This transformation also reduced the skew of the distribution of counts for a particular lesion and region across subjects. An average across the 3 neocortical regions was also computed for each subject. Average values were transformed back to densities (number per square millimeter) for greater clarity in presentation. Other treatments of the raw densities, including taking the average of the 3 fields with the highest densities, using the maximum of the 10 densities, and using the arithmetic average of the 10 densities, were also subjected to statistical analyses with results similar to those presented here, except that the resulting analyses were more suspect because of failure to meet the ordinary distributional assumptions. All calculations were performed with SAS.40 Analyses of covariance, adjusting for age at death, were used to compare neuropathologic lesion densities across the 4 severity groups (CDRs 0.5, 1, 2, and 3). Pairwise comparisons between least square means were adjusted for multiple comparisons by the Tukey-Kramer method.41
RELATIONSHIP OF NFT AND SP TO DEMENTIA SEVERITY

There was considerable intersubject variability in marker densities within a given CDR group, exemplified in Figures 1, 2, and 3, no greater for older than younger subjects. Nevertheless, there was a significant relationship between dementia severity (both CDR and its sum of boxes) and NFT densities in all regions (Table 3). The NFTs were less dense in neocortical compared with hippocampal regions. Furthermore, the relation of NFTs to dementia severity varied for neocortical compared with hippocampal regions. The NFTs in the neocortex were substantially correlated with dementia severity (r values with sum of boxes ranged from 0.44-0.49). Analysis of variance comparing the 4 CDR groups and subsequent post hoc tests showed that this resulted primarily from increased NFTs in severe dementia (CDR 3). The correlations with dementia severity were more modest in the hippocampal and entorhinal regions (0.30 and 0.21). In these regions, the same approach disclosed that the increase in NFTs occurred in the milder stages of the disease.

| Table 1. Fourteen Subjects With DAT With Non-AD Autopsy Diagnoses |
|--------------------------|------------------|
| Final Diagnoses* | No. |
| Mesolimbocortical dementia | 1 |
| Corticobasal degeneration; ε2/3, 3/3, 3/3 | 3 |
| Progressive supranuclear palsy; ε3/3 | 1 |
| Vascular dementia; ε2/3, 3/4, 3/3, 4/4 | 4 |
| Hippocampal sclerosis, severe CAA; ε2/3 | 1 |
| Severe cortical degeneration, CAA, insufficient AD lesions; ε3/3 | 1 |
| Diffuse Lewy body disease; ε2/3 | 1 |
| Diffuse Lewy body disease, white matter gliosis | 1 |
| Severe nigral degeneration, nigral and cortical LB, small infarcts, hippocampal sclerosis, insufficient AD lesions | 1 |

*Results of the successful apoE (apolipoprotein E) genotyping follow the neuropathologic diagnoses. DAT indicates dementia of the Alzheimer type; CAA, cerebral amyloid angiopathy; AD, Alzheimer disease; and LB, Lewy bodies.
†This subject was reported as fulfilling neuropathologic criteria for AD but did not meet the more stringent criteria used in the present study.

Table 2. Demographic Characteristics, Clinical Data, and apoE Genotype of the Controls and Subjects With DAT With Confirmed AD*

<table>
<thead>
<tr>
<th>Final Diagnosis</th>
<th>Clinical Dementia Rating†</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>0</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>13</td>
</tr>
<tr>
<td>Mean (± SD) age at death, y</td>
<td>82.4±5.7</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>8/5</td>
</tr>
<tr>
<td>Estimated age at onset of AD, y</td>
<td>84.3±9.9</td>
</tr>
<tr>
<td>Sum of boxes at death</td>
<td>0.0±0.1</td>
</tr>
<tr>
<td>apoE genotype (n=176)</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>3</td>
</tr>
<tr>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>33</td>
<td>5</td>
</tr>
<tr>
<td>34</td>
<td>1</td>
</tr>
<tr>
<td>44</td>
<td>1</td>
</tr>
<tr>
<td>% With 1 or 2 ε4</td>
<td>20</td>
</tr>
<tr>
<td>ε4 Allele frequency</td>
<td>0.15</td>
</tr>
</tbody>
</table>

*DAT indicates dementia of the Alzheimer type; AD, Alzheimer disease; and apoE, apolipoprotein E.
†The Clinical Dementia Rating 0/0.5 and 0.5 groups were combined; see “Clinical Assessment” subsection in the “Subjects and Methods” section. 0 indicates no dementia; 0.5, questionable (or very mild) dementia; 1, mild dementia; 2, moderate dementia; and 3, severe dementia.

OTHER NEUROPATHOLOGIC FEATURES

Mean±SD brain weight for the men who were CDR 3 at death was 1174±146 g, whereas it was 1040±115 g in the women. Brain weight decreased with increasing duration in the CDR 3 stage (r=-0.39 for each sex).
Plaque-predominant AD (PPAD) was identified in 37 subjects (mean±SD age, 83.7±9.6 years at death) of the 120 with complete morphometric analysis listed in Table 3. Plaque-predominant AD was present in a greater proportion of subjects with milder dementia than among those more severely affected (71%, 63%, 29%, and 19% of subjects with CDR 0.5, 1, 2, and 3, respectively) (Mantel-Haenszel $x^2$ [1] = 20.361, $P < .001$). In PPAD, total and neuritic cored and noncored plaque densities in almost all regions examined were significantly lower than in the remaining subjects with AD when severity of dementia was controlled. There was no relation between presence of PPAD and age at death. Of the 36 PPAD cases with antiubiquitin preparations, 22% had cortical LBs. Of the 82 cases of AD that were not plaque-predominant and that were assessed for cortical LBs, 32% were positive. This difference in percentages was not significant. Age did not influence the presence or absence of cortical LBs.

After controlling for duration in the CDR 3 stage, NFT and SP regional densities were higher in the pure AD CDR 3 group than in the various CDR 3 mixed groups (AD+PD, AD+SND, AD+vascular, AD+vascular+PD or SND), as exemplified in Figures 1 through 3. The significant comparisons were for NFTs in each neocortical region as well as hippocampal and entorhinal regions, for parietal total SPs, and for frontal and mean neocortical cored SPs (all $P < .05$). There were no differences in marker densities among the various mixed AD groups.

**apoE GENOTYPE**

The numbers of each apoE genotype and the $e4$ allele frequencies in control and AD-confirmed subjects are given in Table 2. (The $e4$ allele frequency is the ratio of the number of $e4$ alleles in a group of persons to the total number of apoE alleles, 2 per person, in that group.) An increase in $e4$ allele frequency in the AD group is clear. The Mantel-Haenszel $x^2$ was significant ($P = .04$). The $e4$ allele frequency in the AD-confirmed group was slightly higher for men than for women ($P = .06$). It was also related to age at death ($F_{2,163} = 7.10$, $P = .001$). Average age at death was 84, 80, and 77 years for those with 0, 1, or 2 $e4$ alleles, respectively. Similarly, estimated average age at onset of AD was 75, 70, and 67 years for those with 0, 1, or 2 $e4$ alleles, respectively. Expressed another way, 78% of those with AD onset before 70 years of age but only 28% of those with onset after 80 years had 1 or 2 $e4$ alleles. Continuous estimates of the relationship between $e4$ allele frequency and age at onset indicated an allele frequency of about 0.6 at age 50 years, declining to about 0.2 at age 85 years and beyond, with little difference between men and women. The increasing $e4$ allele frequencies across the CDR groups (CDR 0.5 to 3) is related to the decreasing mean ages across the groups (Table 2).

After controlling for dementia severity, hippocampal NFT and SP marker densities were weakly related to $e4$ allele frequency, sometimes to a degree that reached statistical significance. No significant correlations ($P > .05$) of $e4$
Table 3. Mean Density of Markers in AD: Relation to CDR, Sum of Boxes, and Age at Death*

<table>
<thead>
<tr>
<th>CDR</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>P†</th>
<th>Correlations With Sum of Boxes</th>
<th>Correlations With Age at Death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=17)</td>
<td>(n=8)</td>
<td>(n=17)</td>
<td>(n=8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neurofibrillary Tangles</td>
<td>Total Senile Plaques</td>
<td>Cored Senile Plaques</td>
<td>Neuritic Plaques¶ (Cored and Noncored)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal</td>
<td>0.4‡</td>
<td>1.2</td>
<td>0.7‡</td>
<td>3.2</td>
<td>.001</td>
<td>.46</td>
<td>–.37</td>
</tr>
<tr>
<td>Temporal</td>
<td>0.5‡§</td>
<td>0.8‡§</td>
<td>3.3</td>
<td>4.6</td>
<td>.001</td>
<td>.44</td>
<td>–.23</td>
</tr>
<tr>
<td>Parietal</td>
<td>0.3‡</td>
<td>0.8‡</td>
<td>1.1‡</td>
<td>3.5</td>
<td>.001</td>
<td>.48</td>
<td>–.29</td>
</tr>
<tr>
<td>Mean neocortical</td>
<td>0.4‡</td>
<td>0.9‡</td>
<td>1.5‡</td>
<td>3.8</td>
<td>.001</td>
<td>.49</td>
<td>–.32</td>
</tr>
<tr>
<td>Hippocampal</td>
<td>4.5‡§</td>
<td>18.3</td>
<td>17.7</td>
<td>19.5</td>
<td>.004</td>
<td>.30</td>
<td>–.08</td>
</tr>
<tr>
<td>Entorhinal</td>
<td>3.2‡§</td>
<td>9.3</td>
<td>11.2</td>
<td>9.1</td>
<td>.02</td>
<td>.21</td>
<td>–.04</td>
</tr>
</tbody>
</table>

* Density indicates number per square millimeter; CDR, Clinical Dementia Rating. Based on least square means for mean of log counts for each region with age as a covariate. Significant differences between severity stages based on 1-tail Tukey-Kramer adjusted comparisons. Correlations are simple Pearson correlations; those with associated P values of <.05 are shown in boldface. See “Statistical Analyses” subsection of the “Subjects and Methods” section for details. Statistical tests for correlations with sum of boxes are 1-tailed. See dagger footnote, Table 2, for explanation of CDR scores.

†P values for relationships between CDR and marker densities.
‡P < .05 from CDR 3.
§P < .05 from CDR 2.
¶ P < .05 from CDR 1.
¶A total of 144 subjects with CDR 3 were available for analysis of neuritic plaques.

Neuritic plaques, a criterion used to recognize neuritic SPs. The slightly stronger relationship of NFT densities to dementia severity in AD as compared with that of SP densities, and stronger relationship in neocortical than hippocampal or entorhinal regions, are confirmed. The NFT correlations were driven mainly by NFT increases in severe dementia for neocortical NFTs, but by increases in the mild stage for hippocampal and entorhinal NFTs. Neocortical cored plaque densities were substantially related to dementia severity, more so than densities of total plaques or neuritic plaques, of which the cored plaques are a subset. Diffuse plaque densities were not related to degree of dementia. This conclusion is tempered by the recognition that absolute histopathologic criteria for distinguishing among SP subtypes (especially diffuse vs neuritic) are not yet available. For instance, most neocortical “diffuse” SPs in AD can be shown45 to contain PHF-1 immunoreactive dystrophic neurites, a criterion used to recognize neuritic SPs.

In an earlier report, there was a substantial relationship of dementia severity and duration to neocortical NFT and cored SP densities but not to neocortical total SP densities. The differences in sample size between the 2 studies may have accounted for the finding of a relationship between neocortical total SP densities and dementia severity in the present report. The consensus in the recent literature is that dementia severity is substantially more likely to be related to NFT than to SP densities.42,51 Hyman and colleagues pointed to their evidence that total SP and amyloid burden did not appear to accumulate across the stages of dementia severity in AD (plaque subtypes not exam-
Contrary results have been published, more in keeping with the pioneering report of Blessed et al. Differences in sample size, selection criteria, and histologic methods may well contribute to the continuing controversy. Indeed, intersubject and intrasubject variability reduces the strength of the relationships between lesion densities and dementia severity. Biologic variability is likely to play an important role, but there is still a substantial problem of sampling error in assessing densities of lesions as well as other relevant measures, such as neuronal numbers. We have focused on SPs and NFTs, the hallmarks of AD. They are appropriate for diagnostic criteria but far from ideal as markers of severity of brain dysfunction. Cytoskeletal destabilization or loss of synapses or critical neuronal populations may well be more central to mechanisms of dementia in AD than are SPs or NFTs.

Not surprisingly, additional neuropathologic lesions, such as those of Parkinson disease and infarcts, add to the dementia of AD and, on average, SP and NFT densities for a given degree of dementia are lower in AD mixed with other brain disorders than in pure AD. Also not surprising is that older subjects with AD at death have somewhat less severe dementia and lower densities of SPs and NFTs, given the likelihood of more frequent fatal comorbidity than is true at younger ages. (The measures of dementia severity chosen for this report were the CDR and its sum of boxes. They are highly correlated with and have performed as well as brief clinical scales or psychometric measures in DAT.)

**OTHER NEUROPATHOLOGIC FEATURES**

A larger percentage of subjects in the milder than in the more severe stages of dementia in this series had PPAD. Others have commented that this AD subtype may represent a developmental stage of AD preceding the appearance of more abundant NFTs. One can argue, therefore, that the amyloid-containing plaque is a more relevant lesion in early AD. The presence of PPAD has been reported to be accompanied by cortical LBs (“Lewy body variant of AD”) in most cases, but the present study failed to confirm that finding.

**apoE GENOTYPE**

Consistent with many published reports, this study found an association of apoE ε4 with AD and a relationship between apoE ε4 alleles and age at AD onset, with ε4 more prevalent in those persons having a younger age at onset. There was only a weak male–female difference. An association of the ε4 allele with increased β-amyloid deposition and numbers of diffuse and neuritic SPs has been noted in reports from several laboratories. However, consistent with our results on neocortex, others have noted no effect of ε4 on SP numbers in silver preparations or with β-amyloid immunostains. In this study, a confounding of age at death with dementia severity and a relation between age at onset and apoE genotype were noted. These factors make the correlation between genotype and SP density complex. In addition, varying methods may contribute to the discrepant results, with β-amyloid immunostains more likely than silver methods to show a relationship to ε4. In contrast, with thioflavine S we found a major effect of the ε4 allele on amyloid angiopathy, consistent with other observations in the literature.

There was not much power in the study of this sample to examine a possible “protective effect” of apoE ε2, but we noted a trend to lower NFT and SP densities with ε2, even when controlling for ε4 alleles and severity of dementia.

**CLINICOPATHOLOGIC CORRELATION**

In this consecutive series of volunteer subjects, those who were judged clinically to be cognitively healthy (CDR 0) had brains with very few SPs or NFTs, except for 4 subjects with sufficient neocortical SP densities to meet neuropathologic criteria for AD, 2 of whom suffered severe craniocerebral trauma shortly before death. The 4 may well have represented true preclinical AD, expected to be present in at least a small proportion of cognitively intact elders. Some might propose for them the term *pathologic aging.* The Consortium to Establish a Registry for Alzheimer’s Disease designation would have been “possible AD” based on age-adjusted neuritic plaque score and absence of clinical history of dementia.

Subjects judged clinically to show slight cognitive decline (CDR 0.5 or 0/0.5), too mild for standard definitions of dementia, were almost always found to have abundant and widely distributed argyrophilic neocortical SPs. In attempts to detect clinically the mildest and earliest manifestations of AD, it is not surprising that an individual older person may be rated as cognitively healthy by one clinician and in slight cognitive decline by another clinician, as was true in the 1 subject in this series rated CDR 0/0.5 whose brain had no neuropathologic abnormalities. We interpret this as an instance of the diagnosis of incipient DAT, CDR 0/0.5, in error. With 17 of 18 people judged to have slight cognitive decline showing abundant neocortical SPs and 13 of 17 people judged cognitively healthy showing few if any neocortical SPs, we submit that there is validity in 2 conclusions: (1) slight cognitive decline in subjects who meet rigorous exclusion criteria suggests the presence of sufficient AD lesions in the brain to support a diagnosis of AD, and (2) abundant neocortical SPs are likely to be associated with at least slight cognitive decline. These numbers speak to the accuracy of both the clinical diagnostic and neuropathologic criteria for DAT and AD used in this study. The 13 remaining CDR 0 elderly adults whose brains were largely free of AD markers suggest that at least some persons in samples reported by others to have had plentiful SPs in the absence of dementia would have been rated as having at least incipient dementia by methods used in this study. There is still merit in the 1983 statement of Katzman and Terry that “there should be no question but that the generalization is a very strong one: many plaques and even a few tangles in the neocortex indicate dementia.”

Subjects with CDR 0/0.5 or 0.5 who fail to reach standard criteria for DAT have been discussed by many other groups and have been labeled with various terms, such as *mild cognitive impairment* or *cognitive impairment in the nondemented elderly.* We are adding to evidence that minimal cognitive decline signifies that AD is highly likely to be present when no other cause is readily apparent.
Neuropathologic criteria for AD are still subject to periodic review and debate. One of the relevant issues is the potential importance of SP subtypes, for example, diffuse vs neuritic SP. In other reports, we have commented on the predominance of diffuse over neuritic plaques in very mild AD, whereas the reverse is true in more severe disease. However, uncertainties regarding differentiation of diffuse from neuritic plaques suggest caution in evaluating apparent densities of those 2 plaque subtypes. Nevertheless, our data support the utility (for clinicopathologic correlation) of the consensus criteria published by Khachaturian, based on neocortical total SP densities.

Subjects in this series who met clinical research criteria for DAT ("probable AD") almost always proved to have AD on histologic examination of their brains; in only 7% was a brain disease other than AD responsible for the dementia. As reviewed elsewhere, clinical Research Diagnostic Criteria for DAT continue to be highly accurate; rarely non-AD causes are found in persons who fulfill clinical criteria for DAT in the present series. There were no examples of "tangle-only" AD and only 2 instances of diffuse LB disease (lacking SPs). The exclusion of persons with signs of parkinsonism preceding cognitive decline may explain the rarity of the second category in this series.

CONCLUSION

The results of this study support 5 main conclusions: (1) neocortical NFTs and, to a lesser degree, neocortical total and cored plaque densities are related to dementia severity in AD, but biologic and methodologic variability are important, and better histologic or biochemical markers should be sought for this purpose; (2) PPAD is much more prevalent in the milder than the more severe stages of dementia; (3) advanced age in AD is associated with somewhat lesser severity of dementia at death and lower densities of SPs and NFTs in most regions studied; (4) the robust effect of apoE 4 alleles on SP densities reported by others was not found in this study, despite confirmation of an effect of e4 as a risk factor for AD with onset before age 75 or 80 years and for the development of CAA; and (5) stringent clinical diagnostic criteria for DAT, even in the very mild or "incipient" stage, and neuropathologic criteria for AD based on total SP densities are highly accurate.

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