Pathologic and Nicotinic Receptor Binding Differences Between Mild Cognitive Impairment, Alzheimer Disease, and Normal Aging

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Background: Neurochemical and pathologic studies show that mild cognitive impairment (MCI) is frequently a transitional state between normal aging and Alzheimer disease (AD). Neuropathologic sample sizes have been limited because relatively few individuals with MCI die before dementia develops. Decreased neocortical nicotinic receptor binding is characteristic of AD but has not been investigated in subjects with MCI.

Objective: To assess nicotinic receptor binding and pathologic differences in control subjects with no dementia (ND) and in subjects with clinically and pathologically described MCI or Alzheimer disease.

Design: This was a clinicopathologic analysis. Subjects with ND had no demonstrable cognitive or functional impairment. Subjects with MCI met Petersen clinical criteria for single- or multiple-domain amnestic MCI and died before the disorder progressed to AD. Subjects with AD met National Institute for Neurological Diseases and Stroke/Alzheimer’s Disease and Related Disorders Association clinical criteria for AD. All subjects underwent a complete diagnostic and semiquantitative neuropathologic examination. Data were examined after both clinical and histopathologic classification of subjects.

Setting: Sun Health Research Institute Brain Donation Program, and Arizona Alzheimer Disease Center.

Participants: Twenty-one control subjects with ND, 8 subjects with MCI, and 70 subjects with AD, prospectively followed up to autopsy.

Main Outcome Measures: Nicotinic acetylcholine receptor binding value, total tangle density, total plaque density, and Braak stage.

Results: At the last examination before death, subjects with AD were significantly younger, less educated, and more cognitively and globally impaired compared with subjects with ND. When categorized by clinical diagnosis, MCI was always intermediate between ND and AD. On the whole, MCI was pathologically intermediate between ND and AD for senile plaque density, neurofibrillary tangle density, and Braak stage, but some subjects with MCI lacked neuritic plaques entirely. Binding for nicotinic acetylcholine receptors did not differ between the ND and MCI groups, but it was significantly less in the AD group.

Conclusions: Most MCI may be considered a transitional state between ND and AD clinically and neuropathologically, but in some MCI cases there is lack of neuritic plaques, and, therefore, it cannot be considered early AD. Nicotinic receptor binding seems to be lost during the transition from MCI to AD.

Mild Cognitive Impairment (MCI) has been represented as a condition occurring between normal aging and Alzheimer disease (AD). While there continues to be debate about classification of the various subtypes of MCI, it is generally agreed that MCI involves some degree of cognitive impairment but of insufficient severity to constitute dementia. If we assume the clinical construct of MCI as a transitional state between normal aging and AD, the conclusion might be that MCI represents a transitional phase pathologically, radiologically, and neurochemically. For this reason, postmortem studies of MCI should subclassify subjects by histopathologic stage of AD rather than by clinical measures alone.

We investigated whether MCI is not only a transitional state between no dementia (ND) and AD clinically and pathologically but also whether it is a transitional state for nicotinic acetylcholine receptor (nAChR) loss. Further, we in-
NEUROPATHOLOGIC EXAMINATION

The mean postmortem interval was 2.8 hours. Paraffin blocks containing brain tissue were cut at 5-µm intervals and stained with hematoxylin-eosin for analysis. Additional paraffin sections containing tissue from the anterior cingulate gyrus, amygdala, entorhinal cortex, middle frontal gyrus, middle temporal gyrus, inferior parietal lobule, and anterior medulla were stained for immunohistochemical analysis of α-synuclein (LB309 monoclonal antibody) to identify Lewy bodies and Lewy body-related neurites. Sections from frozen blocks were stained with Campbells-Switzer, Gallyas, and thioflavine S methods to identify plaques, tangles, and other inclusions. Large 4 × 3-cm frozen sections containing tissue from the coronal planes through most of the frontal, temporal, parietal, and occipital lobes were stained with hematoxylin-eosin and Luxol fast blue to detect cerebral white matter rarefaction. Additional immunohistochemical procedures were used as needed, including those for ubiquitin to detect intraneuronal inclusions of motoneuron disease with dementia, and αβ-crystallin and phosphorylated neurofilament to detect swollen neurons in corticobasal degeneration. For all stains except hematoxylin-eosin and Luxol fast blue, both positive and negative control sections were processed with every batch of slides.

Density of neuritic plaques was determined in the hippocampus, entorhinal cortex, temporal lobes, parietal lobes, and frontal lobes using CERAD criteria. Scoring was on a scale of 0 (none) to 3 (frequent), based on the highest score attained in any lobe. In addition, all plaques, both neuritic and diffuse, in the same 3 necrotopical regions and in the hippocampus and entorhinal cortex were rated in the same manner (termed “total plaque density”) except that scores were assigned as the perceived mean score for each area. Total scores for the 5 areas ranged from 0 to a possible maximum of 15. Neurofibrillary tangle density was calculated in a similar manner (termed “total tangle density”) and also using the Braak staging system.

[¹H](±)EPIBATIDINE BINDING

Midfrontal cortex (Brodmann areas 38, 39, and 46) was homogenized and assayed for [¹H](±)epibatidine binding as previously described. In brief, nAChR levels were determined by incubating 0.75 to 2.5 mg of protein in 1 mL of assay buffer containing 5 mmol/L of [¹H](±)epibatidine (45-65 Ci/mmol [1.7±2.4 GBq/mol]; New England Nuclear Corp, Boston, Mass). Nonspecific binding was determined in the presence of 1 µmol/L of unlabeled (±)epibatidine (RBI, Natick, Mass). Samples were incubated on ice for 2 hours, and the assay was terminated by rapid filtration through GF/C filters (Whatman, Florham Park, NJ), presoaked in 0.5% polyethylene amine for at least 1 hour, using a cell harvester (Brandell Instruments, Gaithersburg, Md), and radioactivity was quantified by liquid scintillation spectrometry (Tri-Carb 1600TR; Packard Instruments Co, Meriden, Conn). There were 3 replicates per experiment, with the data representing the average of 1 to 3 separate experiments per sample. Protein content in the samples was determined using a protein assay (BCA; Pierce Chemical Co, Rockford, Ill) with bovine serum albumin as the standard.

STATISTICAL ANALYSIS

Comparison of means was done using 1-way analysis of variance (ANOVA). Post hoc tests were done using the least significant difference (LSD) test. All analyses were conducted with Excel 2003 (Microsoft Corp, Redmond, Wash) and Instat (GraphPad Software Inc, San Diego, Calif) software. Subjects were examined by diagnostic category and also by the clinical
and histopathologic staging of AD according to NIA-Reagan criteria. Spearman rank correlations of nicotinic receptor binding density with CERAD neuritic plaque density and Braak stage were done both within the 3 categories and across the 3 diagnostic groups.

**RESULTS**

Ninety-nine subjects were recruited for the study for data collection and interpretation. Mean MMSE, FAST, and GDS scores, and mean ages and disease duration are given in **Table 1**. Subjects with AD had a significantly lower mean age at death compared with subjects with MCI. They also had significantly fewer years of education compared with subjects with ND. Disease duration differed significantly between subjects with MCI and AD. The MMSE, FAST, and GDS scores differed significantly between the MCI and AD groups and between the ND and AD groups.

The total plaque and neurofibrillary tangle (NFT) densities and the Braak stages are given in **Table 2**. The total plaque and neurofibrillary tangle (NFT) densities and the Braak stages are given in **Table 2**. Total plaque, NFT, and Braak stages differed significantly between the ND and AD groups and between the ND and MCI groups (Table 2). Subjects with AD had more total plaque and NFT and higher Braak stages compared with subjects with ND and MCI. The nAChR binding did not differ between the ND and MCI groups. However, nAChR binding was significantly lower in the AD group compared with the ND group.

The sample was subdivided by clinical diagnosis and histopathologic findings. Clinically, subjects with AD were younger at death, were less educated, had longer disease duration, and were more cognitively, functionally, and globally impaired at death compared with subjects in both the ND and MCI groups. Nevertheless, there were no significant clinical differences in age at death, years of education, disease duration, and MMSE, FAST, and GDS scores between subjects in the ND group with or without AD pathology, and no differences between subjects in the MCI group with or without AD pathology.

Neuropathologic classification was performed in subjects in the ND, MCI, and AD groups (**Table 3** and **Table 4**). In Table 3, subjects in the control group (ND) not having AD were classified as ND-ND, and those possibly having AD were classified as ND-AD. Using the same criteria, subjects with MCI were classified in the same manner: MCI-ND and MCI-AD, respectively. All 21 subjects in the ND group were classified as not having AD, and 9 as possibly having AD, because of the presence of moderate density of neuritic plaques. All of the subjects in the MCI group were classified as not meeting NIA-Reagan criteria because they did not have clinical dementia. By neuropathologically based CERAD criteria, 12 subjects were classified as not having AD, and 9 as possibly having AD, because of the presence of moderate density of neuritic plaques. All of the subjects in the MCI group were classified as not meeting NIA-Reagan criteria, but 3 of the 8 had sufficient neuritic plaque density to meet CERAD criteria for possible AD. All subjects with AD met NIA-Reagan criteria for AD, with most
classified as having a high probability of dementia. All subjects with AD were classified as definitely or probably having AD by CERAD criteria.

There was a significant difference in age at death (P = .02, ANOVA), with the AD group being significantly younger than the MCI-ND group (post hoc LSD) (Table 3). There were no significant differences in mean educational achievement. There was a significant difference in disease duration (P < .001, ANOVA). There was a significant difference in mean FAST scores (P < .001, ANOVA), mean GDS scores (P < .001, ANOVA), and mean MMSE scores (P < .001, ANOVA). The differences were mainly between subjects in the ND and AD groups (post hoc LSD).

The total plaque counts differed significantly (P < .001, ANOVA) between groups, with significant differences between ND-ND and AD, ND-AD and AD, and MCI-AD and AD groups (post hoc LSD) (Table 4). Total NFT counts differed significantly (P < .001, ANOVA), with significant differences between ND-ND and AD, ND-AD and AD, and MCI-AD and AD groups (post hoc LSD). Braak stages differed significantly (P < .001 ANOVA), with significant differences between ND-ND and AD, ND-AD and AD, MCI-ND and AD, and MCI-AD and AD groups (post hoc LSD). The nAChR binding differed significantly (P < .05, ANOVA), with significant differences between ND-AD and AD groups (post hoc LSD).

Spearman rank correlations of nAChR binding density with CERAD neuritic plaque density and Braak stage were done both within the 3 categories and across the 3 diagnostic categories. Overall, no correlations were found between nAChR binding and neuropathologic measures. Only correlations done across all groups were near the sig-

### Table 3. Clinical Characteristics in Subjects With ND, MCI, and AD According to Whether They Met CERAD Criteria for Possible AD

<table>
<thead>
<tr>
<th>Variable</th>
<th>ND-ND (n = 12)</th>
<th>N/C-AD (n = 9)</th>
<th>MCI-ND (n = 5)</th>
<th>MCI-AD (n = 3)</th>
<th>AD (n = 16)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at death, y</td>
<td>84.1 ± 5.4</td>
<td>86.0 ± 6.2</td>
<td>92.0 ± 6.3</td>
<td>88.3 ± 4.9</td>
<td>80.0 ± 12.7</td>
<td>&lt; .05†</td>
</tr>
<tr>
<td>Disease duration, y</td>
<td>15.6 ± 2.5</td>
<td>14.9 ± 2.0</td>
<td>15.0 ± 3.8</td>
<td>14.7 ± 2.3</td>
<td>10.9 ± 6.6</td>
<td>&lt; .001§</td>
</tr>
<tr>
<td>Educational achievement, y</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>3.3 ± 2.5</td>
<td>1.5 ± 0.7</td>
<td>9.0 ± 5.1</td>
<td>&lt; .01,‡ &lt; .001§</td>
</tr>
<tr>
<td>FAST score</td>
<td>2.0 ± 0.0</td>
<td>2.0 ± 0.6</td>
<td>3.0 ± 0.0</td>
<td>2.0 ± 0.0</td>
<td>6.0 ± 1.8</td>
<td>&lt; .05,‡ &lt; .001§,§ &lt; .01</td>
</tr>
<tr>
<td>GDS score</td>
<td>2.0 ± 0.4</td>
<td>1.9 ± 0.4</td>
<td>3.3 ± 0.6</td>
<td>2.5 ± 0.7</td>
<td>5.9 ± 1.2</td>
<td>&lt; .001§</td>
</tr>
<tr>
<td>MMSE score</td>
<td>28.1 ± 1.2</td>
<td>28.9 ± 1.3</td>
<td>25.6 ± 1.5</td>
<td>27.7 ± 1.5</td>
<td>12.7 ± 9.5</td>
<td>&lt; .001,‡ &lt; .001§</td>
</tr>
</tbody>
</table>

**Abbreviations:** AD, Alzheimer disease; CERAD, Consortium to Establish a Registry for Alzheimer’s Disease; FAST, Functional Assessment Staging Scale; GDS, Global Deterioration Scale; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; ND, no dementia.

<table>
<thead>
<tr>
<th>Variable</th>
<th>ND-ND (n = 12)</th>
<th>N/C-AD (n = 9)</th>
<th>MCI-ND (n = 5)</th>
<th>MCI-AD (n = 3)</th>
<th>AD (n = 16)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaque total†</td>
<td>3.4 ± 4.8</td>
<td>7.9 ± 2.9</td>
<td>4.5 ± 4.8</td>
<td>9.1 ± 2.4</td>
<td>13.1 ± 1.3</td>
<td>&lt; .001,‡ &lt; .001,§ &lt; .01</td>
</tr>
<tr>
<td>Tangle total†</td>
<td>3.3 ± 1.7</td>
<td>4.6 ± 3.3</td>
<td>5.9 ± 1.9</td>
<td>4.6 ± 0.5</td>
<td>12.7 ± 2.9</td>
<td>&lt; .001,‡ &lt; .001,§ &lt; .01</td>
</tr>
<tr>
<td>Braak stage</td>
<td>2.5 ± 0.8</td>
<td>3.1 ± 0.6</td>
<td>3.6 ± 0.5</td>
<td>3.0 ± 0.0</td>
<td>5.2 ± 0.8</td>
<td>&lt; .001,‡ &lt; .001,§ &lt; .01,</td>
</tr>
<tr>
<td>nAChR binding</td>
<td>10.1 ± 3.0</td>
<td>13.4 ± 3.7</td>
<td>12.5 ± 4.5</td>
<td>10.1 ± 2.2</td>
<td>8.5 ± 2.6</td>
<td>&lt; .05§</td>
</tr>
</tbody>
</table>

**Abbreviations:** AD, Alzheimer disease; CERAD, Consortium to Establish a Registry for Alzheimer’s Disease; MCI, mild cognitive impairment; nAChR, nicotinic acetylcholine receptor; ND, no dementia.

*Data are given as mean ± SD.
†ND-ND vs AD.
‡ND-AD vs AD.
§MCI-ND vs AD.
||MCI-AD vs AD.
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and with Braak stage (clinically classified MCI that progresses to AD. Studies of cerebrospinal fluid show increased tau and -amyloid(42) protein levels in subjects with AD clinically, fewer than half of subjects have sufficient AD pathology to meet a diagnosis of AD by CERAD criteria. Cognitive impairment in these subjects is, therefore, questionably attributable solely to AD pathology. Second, nAChRs are lost mainly during the transition from MCI to AD. Finally, we found that, after separation by histopathology, subjects with ND and MCI with and without AD pathology cannot be differentiated clinically.

Other clinicopathologic studies have investigated subjects with MCI compared with subjects with ND and AD. One recently published study investigated 37 subjects with MCI proximate to death and compared them with subjects with ND and AD. This investigation found that most (25/37) subjects with MCI met CERAD criteria for possible, probable, or definite AD. Another clinicopathologic study revealed that subjects with clinically classified MCI had higher NFT density compared with subjects with ND and that NFT density but not amyloid plaque density correlated with memory scores. That study was limited by a small number of subjects with MCI (n=3). Like Guillozet et al, we find that subjects with MCI, in general, had more tangle pathology and higher Braak stages compared with subjects with ND. We did not perform correlations with specific instruments of memory function. Two recent clinicopathologic studies provide additional information. A study by Markesbery et al compares the pathologic findings of ND, MCI, and early AD by region. These investigators found that, in MCI, neuritic plaques were significantly elevated in the frontal, temporal, parietal, amygdala, and posterior cingulate regions compared with ND, and were not different from early AD. In MCI, NFTs were increased in the parietal lobe, amygdala, entorhinal cortex, hippocampal CA1 neurons, and subiculum compared with ND. Similar to our study finding, mean Braak stage was 3.3. When their data are analyzed in the context of pathologic diagnoses, many subjects did not meet pathologic criteria for AD. Similarly, the other recent study, by Petersen et al, with 67 subjects with MCI, found that most subjects with amnestic MCI do not meet the pathologic criteria for AD and that 19 (29%) of subjects had primary neuropathologic findings other than AD. Our findings, therefore, agree with these studies in that, while MCI is a transitional clinical state between normal aging and dementia, it is neuropathologically heterogeneous. Future clinicopathologic studies of MCI should classify subjects by neuropathologic findings to prevent mixing different pathologic entities.

Biochemical studies of MCI are still in a preliminary stage. Studies of cerebrospinal fluid show increased tau and decreased -amyloid(42) protein levels in subjects with clinically classified MCI that progresses to AD. In some studies of subjects with clinically defined ND, MCI, and AD, it has been concluded that the cholinergic deficit is not yet present at the MCI stage. These studies may be difficult to interpret, however, because clinical, rather than histopathologic, staging of AD was used, resulting in the mixing of subjects with and without significant AD histopathology. When subjects with ND are classified on the basis of AD histopathology, it has been shown that choline acetyltransferase activity declines commensurate with plaque development and accumulation, placing the cholinergic deficit at the preclinical stage.

Another marker of cholinergic activity is nAChR binding. Nicotine binding levels are modified as a result of altered synthesis, transcription, translation, and transport of nicotine receptors. Thus, extensive investigations have found nAChRs to be substantially lost in the neocortex in AD, but it has not yet been investigated in MCI. In this study, we found that nAChR loss does not occur in the transition from ND to MCI but during the transition from MCI to AD. Since the subjects with MCI were older, on average, than subjects with ND, it is unlikely that the absence of nAChR binding changes between ND and MCI is the result of age, since nAChRs decrease with age. This suggests that nAChR loss may be secondary to the accumulation of AD pathology. Alternatively, subjects with AD may be compensating for receptor density loss from cholinergic postsynaptic terminals by increasing nicotinic receptor density on postsynaptic neurons. One caveat to consider is that our nAChR binding is of the midfrontal cortex only and the temporal neocortex and hippocampus were not examined. Nicotinic receptor density may not be so closely related to AD pathology as choline acetyltransferase activity, since choline acetyltransferase activity is purely postsynaptic, whereas nicotinic receptors are both presynaptic and postsynaptic.

Limitations of this study include the small sample size of the subjects with MCI. This is apparent especially when groups are divided by histopathologic findings. Only subjects with single- or multiple-domain amnesia were examined to ensure less heterogeneity. Subjects were selected by diagnostic categories. Because of the small sample in the MCI group, it is difficult to match groups by age and educational achievement. Similar to Petersen et al, we found that subjects with MCI were very old. We excluded subjects who had Lewy body pathology.

Understanding the biochemical changes that occur during the transition from ND to MCI and from MCI to AD may provide insight into the changes in the brain that are responsible for the clinical phenotype of the progressive cognitive decline.

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Author Contributions: Dr Sabbagh had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Sabbagh, Reid, Peterson, and Beach. Acquisition of data: Sabbagh, Sue, Connor, Peter-


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son, and Beach. *Analysis and interpretation of data: Sabbagb, Shah, and Beach. Drafting of the manuscript: Sabbagb, Shah, Sue, and Beach. Critical revision of the manuscript for important intellectual content: Sabbagb, Reid, Connor, Peterson, and Beach. Statistical analysis: Sabbagb and Beach.*

**References**