Intraneuronal ApoE in Human Visual Cortical Areas Reflects the Staging of Alzheimer Disease Pathology

GILLIAN EINSTEIN, PHD, VIKAS PATEL, MD, PHILLIP BAUTISTA, MS, MICHELLE KENNA, BA, LEAH MELONE, BA, ROHYN FADER, MD, KRIS KARSON, BA, STEPHANIE MANN, BA, ANN M. SAUNDERS, PHD, CHRISTINE HULETTE, MD, DEBORAH MASH, PHD, ALLEN D. ROSES, MD, AND DONALD E. SCHMECHEL, MD

Abstract. Alzheimer disease (AD) is marked by progressive loss of cortical neurons with associated cognitive decline. Multiple genetic and environmental factors likely contribute to this progressive loss. Such genetic factors include the polymorphic locus (APOE) that encodes apolipoprotein E (apoE). In order to test the hypothesis that intraneuronal apoE is present at all stages of AD, not just in visual cortical regions known to be vulnerable to AD. We found that intraneuronal apoE was present at all these stages, however, only in visual cortical regions known to be vulnerable to AD. The late stages, the laminar distribution of apoE-immunoreactivity matched the distribution of other markers of AD pathology, especially modified tau. These data support previous findings that intraneuronal apoE in neocortex is common in aged, nondemented controls and demonstrate that it may be more common in regions at risk for AD pathology. Thus, intraneuronal accumulation of apoE may be an attribute of cortical neurons that are more vulnerable to age-related injury with the presence of apoE anadating the classical indices of late-onset AD pathology.

Key Words: Aging; Alzheimer disease; Association cortex; Neuropathology; Pyramidal neurons; Tau.

INTRODUCTION

Alzheimer disease (AD), a progressive neurodegenerative disease, produces clinical and pathological changes that appear to antedate overt clinical signs of dementia. Specific brain regions that are vulnerable to AD can show signs of AD pathology before the onset of the disease (1). Performance on a number of cognitive tasks is significantly poorer in subjects who later develop AD (2-4). The origin of these changes remains unknown despite the recent discovery of a number of genetic susceptibility factors.

APOE, the polymorphic genetic locus for the cholesterol transport protein, apolipoprotein E (apoE) influences risk and onset age of late-onset familial and sporadic AD (5, 6). ApoE is present in glial cells, neurons, cerebral vessels, and plaques in the brain where it could interact with other factors in the disease process such as amyloid precursor protein (APP) or its fragment, β-amyloid (7-12). Patients homozygous for the ε4 allele compared with those homozygous for the ε3 allele have increased vascular and plaque amyloid deposits (11). Intraneuronal apoE has been described in brain regions known to be vulnerable in AD such as hippocampus in both AD and age-matched controls as well as neocortex in the temporal lobe of a patient with intractable epilepsy (12-13). ApoE has also been localized to neurons in the nucleus basalis of Meynert, entorhinal cortex (15), and frontal cortex (16) in aged controls and patients with AD. Thus, apoE is present in the vulnerable neurons in human brain in neurons with and without neurofibrillary changes (9, 5, 11, 14, 16). What is still unclear is how its presence is related to the broader issue of selective neuronal degeneration and regional pathology in AD.

In order to clarify these relationships, we investigated the regional and laminar distribution of intraneuronal apoE in nondemented controls and AD patients. We chose 3 neocortical regions known to represent 1 modality, vision, at different levels of the cortical hierarchy: area 17, a primary visual cortical area; area 18, a secondary visual cortical area; inferior temporal cortex, an association visual cortical area. These 3 visual cortical regions were also used originally to demonstrate differential vulnerability to the pathological changes of AD with area 17 the least vulnerable, area 18 the next most vulnerable, and inferior temporal cortex (area IT), the most vulnerable (1, 17). In order to determine how intraneuronal apoE would correspond to the occurrence and severity of neuropathological events and their staging, we coupled studying the distribution of apoE-immunoreactivity with the distribution of immunoreactivity for several different aspects of AD pathology (gliosis, plaque formation, modified tau (neurofibrillary tangles), and the
### Table 1: Autopsy Data and Diagnosis

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<th>Genotype</th>
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<th>Braak and Braak Stage</th>
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**AD**

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loss of large pyramidal neurons) as well as with the Braak and Braak staging of AD neuropathology.

### MATERIALS AND METHODS

#### Cases

Tissue samples from 10 nondemented controls and 4 AD patients were collected at autopsies performed less than 9.5 hours (h) from the time of death (University of Miami, Johns Hopkins University, and Duke University); the average age of the controls was 82.5 ± 4.6 years and the average age of the AD cases was 71.0 ± 3.9 years (Table 1). To be enrolled as a "nondemented control", individuals were screened by a research nurse (Duke) or trained technician (University of Miami) at entry and had to be found to be (1) free of cognitive disorders leading to functional impairments in everyday life, (2) living independently without difficulty, and (3) not diagnosed as having a memory disorder. Most individuals (all the AD cases) also consented to being tested during life for cognitive performance with a battery of neuropsychological tests including the Mini-Mental Status Exam and tests of language (animal fluency, naming, verbal fluency), visuospatial abilities (constructional praxis), visuomotor/executive function (symbol-digit modalities test, Trailmaking part A, Trailmaking part B), and verbal and visual measures of learning and memory. After baseline performance was established patients were followed up annually.

At autopsy, blocks approximately 1-cm-thick were cut to include areas 17, 18, and IT (Fig. 1), and placed directly in fixative (4% paraformaldehyde in 0.1 M phosphate buffer). Adjacent blocks for each area were fixed and later embedded in paraffin, sectioned, and stained with hematoxylin and eosin and microwave King stains; these preparations were later used for diagnosis of AD pathology and for Braak and Braak staging (1, 17). Tissue was also obtained from frontal, parietal, entorhinal, subiculum, and hippocampal cortex, as well as from the amygdala, nucleus basalis of Meynert, caudate, and putamen and the

frequencies of plaques and tangles in those areas estimated. Diagnosis of AD was made according to CERAD criteria (18).

#### Immunohistochemistry

After 24 h fixation, 35-40-μm-thick serial sections were cut on a vibratome, rinsed in 0.1M phosphate buffer 3 times for 10 minutes (min) each, pretreated in 1% methanol-1% hydrogen peroxide, and again rinsed in 0.1 M phosphate buffer. They were then incubated with the various primary antisera at room temperature for 1 h and then at 4°C overnight (SMI-32 was incubated for 48 h). Primary antisera directed against the following were used: GFAP (Boehringer Mannheim 1:10), neurofilament antigens—Tau-2 (Leinco Technologies 1:1000), Aβ (A4, courtesy of Athena Neurosciences; Mouse Monoclonal 1:500). SMI-32 (Sternerberger Monoclonals; 1:7000), and apoE (2 goat polyclonal antibodies: Calbiochem 1:10,000 and GHE-3, gift of Dr. Karl Wiesgraber). The apoE antibodies react with human apoE on western blots giving a band at 34 kD.

After incubation in primary antisera, sections were rinsed 3 times (10 min each) in 0.1 M phosphate buffer and incubated at room temperature with the following biotinylated secondary antibodies: horse anti-mouse (GFAP 1:50, 30 min), goat anti-mouse (neurofilament antigens 1:400, 30 min), horse anti-mouse or goat anti-mouse (A4 1:300, 30 min), horse anti-mouse (SMI-32 1:300, 24 h at 4°C) and rabbit anti-goat (apoE 1:50, 30 min). The samples were then washed and incubated for 30 min in ABC standard reagent followed by 3 washes of 10 min each in 0.1-M phosphate buffer. Primary antisera binding was visualized using 3,3-diaminobenzidine (DAB). Sections were mounted on slides, air-dried overnight, dehydrated, cleared, and coverslipped.

In some instances a preimmune serum was used as a control for apoE primary antibody (GHE-3 for apoE). Controls with the primary antibody omitted and with tissue incubated in only secondary antibody were always included. Sections from each
Fig. 1. Consecutive cross sections of a typical human brain from autopsy arranged from most rostral to most caudal (A–G). Boxed region denotes typical blocking for area IT. G. Boxed region denotes blocking for areas 17 and 18. The insert exemplifies 2 brain sections as they appeared once sectioned and processed for immunohistochemistry with the 3 strips in each showing typical...
subject were also Nissl stained to reveal regional and laminar boundaries based on cytoarchitecture.

Data Analysis

In order to plot the distribution of immunoreactive profiles in the same region for each antibody preparation, we first chose 2 sections and drew them at low magnification using a drawing tube and a 2X objective. These drawings were then used as "maps" to determine the optimal regions of analysis for each antibody preparation (i.e. sections in which all layers of cortex were represented with clearly delineated cytoarchitecture) (Fig. 1, insert). Once chosen, all the immunoreactive processes were drawn within a field as circumscribed by a 20X objective. We used a drawing tube to plot the distribution of immunoreactive neurons as stained by antibodies to tau, SMI-32, or apoE; glial cells as stained by anti-GFAP; and/or plaques as stained by antibodies to A4 or apoE within these fields from pial surface to white matter (magnification = 160X). Gial cells were marked by a "X"; plaques were outlined, and profiles of neurofibrillary tangles, SMI-32, and apoE-immunoreactive neurons were traced.

Identification of regional boundaries was determined by comparing cytoarchitecture as revealed by Nissl and SMI-32 immunoreactivity (19). Laminar boundaries within each area were determined by a combination of the distribution of large SMI-32-immunoreactive neurons and traditional soma size and cell packing density, as delineated in Nissl-stained sections. Our criteria were as follows: In all 3 regions, layer 1 was a neuron-free but often GFAP-rich region between the pial surface and the first layer of small pyramidal neurons; layer 2 was marked by small pyramidal neurons which could be observed in Nissl preparations but not by SMI-32-immunoreactivity except occasionally in area 18; layer 3 in all regions was marked by dense SMI-32 staining of large pyramidal neurons extending from the boundary of layer 2, with its small pyramidal neurons, to an SMI-32-free region interpreted as the layer 4 boundary; layer 4 was marked by the presence of small cell bodies in Nissl-stained sections and the absence of any staining in SMI-32 stained sections (19). Layer 4 varied in size from area to area; it was largest in area 17, smaller in area 18, and smallest in IT; layer 5 was marked in all areas by large pyramidal neurons densely-stained by SMI-32; layer 6 was marked by small cell bodies that stained lightly with SMI-32 in areas 18 and IT.

**APOE Genotyping**

For all cases, DNA was isolated from approximately 300 mg of frozen brain tissue. APOE genotyping was performed using restriction fragment isotyping followed by autoradiography (20).

**RESULTS**

Hematoxylin and eosin stained sections from both the control, and AD cases revealed the expected cytoarchitectonic features of areas 17, 18, and IT. Even in AD cases, IT was discernible by its characteristic distribution of SMI-32-immunoreactive neurons (Fig. 2A).

Paraffin-embedded sections from areas 17, 18, and IT were studied for neurofibrillary tangles in entorhinal, hippocampal, and primary visual cortex and staged according to the criteria of Braak and Braak (1, 17). These cases were also diagnosed for presence of AD according to CERAD criteria (18). Individuals with Braak and Braak stages I or II pathology were invariably controls and failed to meet CERAD criteria for AD pathology. Individuals in stage III represented either controls or patients with AD and PD. Individuals in stages IV-VI had invariably been diagnosed clinically with dementia, and also met CERAD criteria for AD (Tables 1, 2).

**ApoE Immunoreactivity in Neurons**

The frequency of apoE-immunoreactive neurons varied between regions in all subjects and between nondemented control and AD brains. Neurons were considered apoE-immunoreactive if their staining was clearly above background, as defined by degree of neuronal staining in adjacent sections reacted with preimmune serum or secondary antibody alone (Fig. 2B, C). We collected 14 cases, total. Three of the 10 controls were excluded from analysis because background staining was high. The remaining 7 controls had low enough background to be analyzed extensively (cases HClCN, HClDK, HClDF; A95-06, HClCY, HClDA, and HClDG). All 4 AD cases had low background and strong intraneuronal apoE-immunoreactive and were included in the analysis. In all samples, glia in all cortical layers were also apoE-immunoreactive. In all cortical areas examined, apoE-immunoreactive neurons were distinct from other apoE-immunoreactive profiles such as plaques and glia (Fig. 3).

When intraneuronal apoE was present, it was most noticeable in pyramidal neurons as identified by their triangular or pear-shaped cell bodies and their stout apical dendrites (Fig. 4A, B). Within pyramidal neurons, apoE staining differed qualitatively between neurons from nondemented control and those from AD brains. In neurons from control brains, the staining was usually light, diffuse, and localized to the region of the soma (Fig. 4A). In neurons from AD brains, the staining was more dense and tangle-like in its intracellular pattern, spreading further into the apical dendrites (Fig. 3B).

**Braak and Braak Stages I-III**

Seven individuals were staged at Braak and Braak stages I-III. All of these were nondemented controls.

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regions from which 20X plots were made of neurons immunoreactive for each marker. Photograph of brain sections courtesy of Dr. El Mufson.
Fig. 2. Photomicrographs exemplifying cytoarchitecture and apoE-immunoreactivity in area IT of AD brain (A94-225). A: SMI-32-immunoreactivity reveals typical cytoarchitecture for area IT. A few, small immunoreactive neurons in layer 2 and large immunoreactive neurons in layers 3 and 5. B. Incubation in secondary antibody (link control) reveals the absence of apoE-immunoreactivity. C. ApoE immunoreactivity reveals dense apoE-positive plaques in layers 2–3 and, although difficult to discern at low magnification, immunopositive neurons in layers 3 and 5 (see also Figs. 3 and 4). Numbers on the left margin denote cortical layers.

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<th>Area 18</th>
<th>IT</th>
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<td>–/–</td>
<td>+/–</td>
</tr>
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<td>3/3</td>
<td>–/–</td>
<td>–/–</td>
</tr>
<tr>
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<td>–/–</td>
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<td></td>
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<td>4/4</td>
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<td></td>
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The Relationship of Braak and Braak Stages to ApoE Immunoreactive Neurons and Tau Across Neocortical Areas (ApoE/Tau)

Intraneuronal apoE: ApoE-immunoreactive plaques were present in nondemented control brains but not consistently so; they could be found in any of our 3 cortical areas. ApoE-immunoreactive neurons were present in 4 out of the 7 cases: One at stage II and 3 at stage III (Table 2).

Regional Distribution of Intraneuronal apoE: In the latter 4 brains, the presence of intraneuronal apoE in the 3 cortical areas was as follows: Little, if any, in area 17; often in area 18 (4 of the 4); and frequently in area IT (3 of the 4) (Table 2; Fig. 5, top).

Laminar Distribution of Intraneuronal apoE: When intraneuronal apoE was present, area IT was populated densely with apoE-immunoreactive neurons. In this region, apoE-immunoreactive neurons were variably distributed to several cortical layers. In 1 subject they were distributed to the superficial layers; in another, to the deep layers; and in another, to all cortical layers, 2–6 (Fig. 5, top).

Comparison of the Distribution of Intraneuronal apoE with Other Markers of Pathology: GFAP-positive astrocytes were always present in layer 1 and in the white matter. In some cases, they were also numerous in gray matter. Dense gliosis of the gray matter tended to correlate with the presence of apoE-immunoreactive neurons (Fig. 6). Aβ-immunoreactivity of senile plaques ranged from light to heavy in all regions and could be observed in any of the 3 cortical areas (Fig. 6). Aβ-immunoreactivity of plaques correlated positively with the presence of apoE-immunoreactive plaques, but not with the presence of apoE-immunoreactive neurons.

Neurofibrillary tangle-bearing neurons were also present in 4 of the 7 nondemented control cases. When pres-
Fig. 3. Low magnification photomicrographs showing distribution of apoE-immunoreactivity in layers 1–6 of areas 17, 18, and IT of an AD brain (A94–225). A. In area 17, apoE is present in plaques only. B. In area 18, apoE is present in plaques and neurons in layers 3 and 5. C. In area IT, apoE is present in plaques and neurons staining them densely. Arrows point to immunoreactive neurons.

tent, tangle bearing neurons were more often in area IT (3 cases) than area 18 (1 case). Tangle bearing neurons were never present in area 17. The presence of neurofibrillary tangles did not correspond necessarily to the presence of apoE-immunoreactive neurons. Neurofibrillary tangles could be present in a cortical region where there were no apoE-immunoreactive neurons (2 of the 4 cases) and apoE-immunoreactive neurons could be present in regions where there were no neurofibrillary tangles (other 2 cases). Tangle-bearing neurons corresponded occasionally to the presence of apoE-immunoreactive neurons in only 2 of the 4 cases (Table 2; Fig. 6). SMI-32 staining revealed the presence of large pyramidal neurons in layers 3 and 5 of all cortical areas (Fig. 6).

Braak and Braak Stages III–VI

INTRANEURONAL apoE: At these Braak and Braak stages of definite AD pathology intraneuronal apoE and apoE-immunoreactive plaques were always observed (Figs. 4, 5, bottom).

Regional Distribution of Intraneuronal apoE: As in earlier Braak and Braak stages, the distribution of apoE-immunoreactive neurons differed in each of the cortical regions. ApoE-immunoreactive neurons were never present in area 17. ApoE-immunoreactive neurons, were always present in large number in areas 18 and IT with the exception of 1 case (A94–227) which contained apoE-immunoreactive neurons in area 18 but not IT (Fig. 7. See Discussion).

Laminar Distribution of Intraneuronal apoE: The distribution of apoE-immunoreactive neurons in areas 18 and IT had a distinct pattern in all of these cases. ApoE-immunoreactive neurons were numerous in layers 2/3 and 5 with some examples in layer 6. One case (A94–216) had a sparse distribution of apoE-immunoreactive neurons in layers 1 and 4 (Fig. 5, bottom).

Comparison of the Distribution of Intraneuronal apoE Other Markers of Pathology: GFAP-positive glial cells were observed throughout the cortex and white matter, often with a more dense distribution to layers 2/3 and 5/6 (Fig. 7). This dense gliosis correlated closely with the presence of apoE-immunoreactive neurons. Aβ-immunoreactive plaques and vessels were distributed fairly evenly to all cortical regions, corresponding only slightly, if at all, to the distribution of apoE-immunoreactive neurons. Neurofibrillary tangles were present in areas 18 and IT where they were distributed most densely to layers 2/3 and 5; they could also be present in layers 1, 4 and 6, coinciding with the distribution of apoE-immunoreactive neurons. SMI-32 staining revealed a diminished population of large pyramidal neurons in layers 2/3 and 5 in areas 18 and IT. The distribution of SMI-32-positive neurons correlated positively with the distribution of apoE-immunoreactive neurons (Fig. 7) with the exception of 1
Fig. 4. High magnification photomicrographs of apoE-immunoreactive neurons in layer 5 of area IT. A, upper panel. In this example of apoE immunoreactivity in layer 5 of area IT of a control brain (HC/HC), immunoreactivity is present primarily in pyramidal neurons with staining quality light and punctate. B, lower panel. In contrast to the upper panel, in layer 5 of area IT of an AD brain (A94–225) staining is present in plaques and pyramidal neurons with the staining quality dense, appearing like neurofibrillary tangles. Arrows point to cell bodies of apoE-immunoreactive neurons.

DISCUSSION

This study examined the presence of intraneuronal apoE relative to the continuum of pathological changes observed in human brain with aging and AD, as described by Braak and Braak (1, 17). By studying 3 visual cortical areas of differing vulnerability to the disease pro-
Braak and Braak Stage II (non-demented control)

Braak and Braak Stage VI (AD)

Fig. 5. Plots showing distribution of apoE-immunoreactive neurons in areas 17, 18, and IT of a non-demented control and AD brain. Top panel: This example of Braak and Braak stage II (HCIDY) shows no plaques and virtually no apoE-immunoreactive neurons in area 17, apoE-immunoreactive neurons in area IT are present in layers 2–6. Bottom panel: This example of Braak and Braak stage VI (A94–225) shows numerous apoE-immunoreactive plaques but as above, no neurons in area 17, apoE-immunoreactive plaques (not shown) and in layers 3 and 5, immunoreactive neurons in areas 18 and IT. Numbers on the left margin denote cortical layers.

In contrast, we have demonstrated that apoE-immunoreactive neurons (1) are present at every Braak and Braak stage of pathology from stage I through stage VI, in non-demented controls as well as in AD brains; (2) are often neocortical pyramidal neurons; (3) follow the regional distribution of AD pathology. Unfortunately, there were not enough subjects in this study to be able to address the related question of the relationship of apoE-immunoreactivity and apoE genotype. However, the presence of apoE-immunoreactive neurons at the earliest stages of neuropathological changes suggests that intraneuronal apoE is a normally encountered characteristic of human neocortical neurons. However, its regional and laminar distribution at the later stages of AD neuropathology suggests that, in AD, it may be an important component of the disease process.

Presence of Intraneuronal apoE at the Earliest Braak and Braak Stages

We have determined that intraneuronal apoE is present in non-demented control brains at the earliest Braak and Braak stages. Even at these earliest stages, the distribution of intraneuronal apoE parallels the regional distribution of pathological changes that occur later in AD. It is unclear whether the presence of prominent intraneuronal apoE in vulnerable neocortical regions is normal or represents a very early pathological change. In addition to its presence in non-demented control brains, intraneuronal apoE has been demonstrated in neurological disorders other than AD such as Parkinson disease and epilepsy (13, 14) and at sites of neuronal degeneration in the CNS of rodents with ischemia-induced neuronal excitotoxicity (21). ApoE synthesis is upregulated in regions of axon regrowth in the periphery and sprouting in the CNS (22–24) and is present at sites of neuronal repair in the CNS of rodents with perforant path lesions (25, 26). The present evidence cannot differentiate between the hypothesis that intraneuronal apoE is a normal characteristic of neocortical neurons or the hypothesis that intraneuronal apoE represents neuronal response to injury or age-related synaptic remodeling. The fact that it is present in both the early and late stages of AD pathology suggests that it might be indicative of some pathological process which, in the case of someone with AD, might start long before obvious cognitive and neuropathological changes are evident. This would not be surprising since performance on a number of cognitive tasks is poorer in subjects who later develop AD suggesting that neuronal problems antedate the more obvious cognitive measures (2–4).

Presence of apoE in Pyramidal Neurons

At all Braak and Braak stages, intraneuronal apoE was most prominent in neocortical pyramidal neurons (15, 16, 27). Whether or not intraneuronal apoE is confined to pyramidal neurons is still not clear. However, since large, neocortical pyramidal neurons are a vulnerable neuronal type in AD and a neuronal population that degenerates selectively in AD (28, 29), the presence of intraneuronal apoE in this population at the earliest Braak and Braak stages raises the question of whether intraneuronal apoE marks a prodromal stage of AD neuronal pathology. A single AD case (A94–97) at Braak and Braak stage VI had no apoE-immunoreactive neurons in area IT also had a complete lack of SMI-32-immunoreactive neurons in the same region. However, there were apoE-immunoreactive neurons in area 18 of the same case suggesting that apoE-immunoreactive neurons were not present in IT because AD pathology was so advanced that most pyramidal neurons in this region had degenerated. This
finding may provide an explanation for Metzinger et al finding that the presence of apoE-immunoreactive neurons is less in AD than in controls (1996); in advanced stages of AD, one might actually observe less intraneuronal apoE due to the loss of the very neuronal population that may use apoE preferentially throughout the course of the disease.

Distribution of apoE Immunoreactivity to Cortical Laminae

The laminar distribution of apoE-immunoreactive neurons differed at early and late Braak and Braak stages. The major differences were (1) the variability of its presence and laminar distribution at the early stages and (2) the sparing of layer 4 in advanced stages. The variability of its presence and its laminar distribution in the early Braak and Braak stages suggests that apoE may be a sign of normal cellular function depending on which neuronal populations need it at a given time. The lack of intraneuronal apoE in layer 4 in the presence of other signs of AD pathology is consistent with the general observation that layer 4 in AD suffers neither the loss of synapses nor neurons (30–32) and that, in AD, its presence is related to the disease stage.

Correspondence Between Intraneuronal apoE and Other Pathological Markers

Intraneuronal apoE showed no consistent pattern of correspondence with Aβ-plaque and vessel immunoreactivity either in early or late Braak and Braak stages.
Fig. 8. Composite representation of figure taken from Braak and Braak (1991) combining the staging of AD pathology with the distribution of apoE-immunoreactive neurons. Note that while the distribution of neurofibrillary tangles at Braak and Braak stage III does not correspond to regional distribution and density of apoE-immunoreactive neurons, in Braak and Braak stage V, it does.

Aβ deposition was distributed to all cortical laminae in all regions. This is in concert with other reports showing that the density and distribution of plaques is not correlated with the extent of cognitive decline in AD (31, 32).

In early Braak and Braak stages, there was also no correspondence between intraneuronal apoE and neurofibrillary tangles. This finding is in contrast to another study in which intraneuronal apoE was found only in neurons that had either neurofibrillary tangles- or PHF-immunoreactivity (15). The present study has demonstrated the independence of neurofibrillary tangles- and apoE-immunoreactivity in visual association cortex of nondemented brains and is consistent with the suggestion that the intraneuronal presence of apoE precedes or is independent of neurofibrillary tangles in primates and humans (11, 12, 14, 16). While the difference between the 2 studies may be methodological, another possibility is that AD pathology starts in different brain regions, and since the basal nucleus of Meynert and entorhinal cortex (regions examined in the earlier study) suffer AD pathology earlier than neocortical association areas (regions examined in the present study), regions examined in the earlier study would contain neurofibrillary changes even in the earliest Braak and Braak stages. To observe intraneuronal apoE without coincident neurofibrillary tangles staining in those regions would require looking at them even before Braak and Braak stage 1. To this point, 1 of our cases with AD and PD pathology (A94–216) characteristically had mild AD pathology (Braak and Braak III–IV) (33), while the regional and laminar distribution...
of intraneuronal apoE matched that of the cases with more severe AD pathology. This observation supports the suggestion that apoE is either recruited to or expressed by neurons independently of other markers of AD pathology.

Summary
We have used tissue from 3 visual cortical areas that are affected differently by AD pathology and found that intraneuronal apoE is present at the earliest Braak and Braak stages in the brains of non-demented subjects. At both early and late stages, apoE is present primarily in pyramidal neurons. The regional distribution of intraneuronal apoE follows Braak and Braak staging of AD pathology. In early Braak and Braak stages, the laminar distribution does not have a consistent pattern and is not coincident with other markers of AD pathology. However, in brains with significant AD pathology, the laminar distribution of intraneuronal apoE follows the pattern of neuronal vulnerability and is coincident with other markers of AD pathology. The demonstration that intraneuronal apoE is present in nondemented control brains at early Braak and Braak stages suggest that intraneuronal apoE is part of the normal cell biology of neurons. Its presence in AD brains at late Braak and Braak stages suggests that its presence may be a sign of neuronal challenge or injury and that it may be an independent marker of AD-related pathology, marking preclinical AD (Fig. 8). To determine whether both, or only 1 of these possibilities is true will require further studies that map the presence and distribution of intraneuronal apoE in other disease states as well as in the normal, young human brain.

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