Specific Regional Transcription of Apolipoprotein E in Human Brain Neurons

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In central nervous system injury and disease, apolipoprotein E (APOE, gene; apoE, protein) might be involved in neuronal injury and death indirectly through extracellular effects and/or more directly through intracellular effects on neuronal metabolism. Although intracellular effects could clearly be mediated by neuronal uptake of extracellular apoE, recent experiments in injury models in normal rodents and in mice transgenic for the human APOE gene suggest the additional possibility of intraneuronal synthesis. To examine whether APOE might be synthesized by human neurons, we performed *in situ* hybridization on paraffin-embedded and frozen brain sections from three nondemented controls and five Alzheimer's disease (AD) patients using digoxigenin-labeled anti-sense and sense cRNA probes to human APOE. Using the antisense APOE probes, we found the expected strong hybridization signal in glial cells as well as a generally fainter signal in selected neurons in cerebral cortex and hippocampus. In hippocampus, many APOE mRNA-containing neurons were observed in sectors CA1 to CA4 and the granule cell layer of the dentate gyrus. In these regions, APOE mRNA containing neurons could be observed adjacent to nonhybridizing neurons of the same cell class. APOE mRNA transcription in neurons is regionally specific. In cerebellar cortex, APOE mRNA was seen only in Bergmann glial cells and scattered astrocytes but not in Purkinje cells or granule cell neurons. ApoE immunocytochemical localization in semi-adjacent sections supported the selectivity of APOE transcription. These results demonstrate the expected result that APOE mRNA is transcribed and expressed in glial cells in human brain. The important new finding is that APOE mRNA is also transcribed and expressed in many neurons in frontal cortex and human hippocampus but not in neurons of cerebellar cortex from the same brains. This regionally specific human APOE gene expression suggests that synthesis of apoE

might play a role in regional vulnerability of neurons in AD. These results also provide a direct anatomical context for hypotheses proposing a role for apoE isoforms on neuronal cytoskeletal stability and metabolism. (Am J Pathol 1999, 154:601–611)

Apolipoprotein E (apoE) is a major cholesterol transport protein expressed in two separate compartments: peripheral tissues and nervous system.^{1–7} The original hypothesis for the role of apoE in the peripheral and central nervous system (CNS) was that apoE facilitates reverse cholesterol transport from regions of nerve cell injury or repair.^{1–3,8–10} Many experimental studies in rodents have demonstrated local increase of apoE after injury to peripheral nerve or during synaptic remodeling in the central nervous system.^{7,10–16} After peripheral nerve injury, apoE is expressed in recruited macrophages during myelin breakdown and repair.^{7,11,12,17} In rodent models of CNS injury, APOE mRNA is present normally in astrocytes and is up-regulated after injury to entorhinal projections to hippocampus.^{18,19}

The three major alleles of human apoE (APOE2, APOE3, and APOE4) are associated with differences in the age of onset of Alzheimer's disease (AD).^{20–23} The APOE4 allele is associated with increased risk and earlier age of onset of late-onset AD, whereas the APOE2 allele decreases risk and delays AD onset in genetic studies compared with APOE3/3 individuals.^{24–26} In addition to its well documented role in AD, the APOE4 allele has been implicated in poorer neurological recovery to head injury, cerebral hemorrhage, and cognitive status after cardiac bypass surgery.^{27–33} These experiments provide epidemiological evidence for the close relationship of APOE alleles to age of onset and/or outcome in a common human neurodegenerative diseases and in nervous system injury but again do not provide evidence for how apoE may influence the recovery and survival of neurons.

The demonstration that the astrocytic protein apoE can also be found in human neurons is important in assessing

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Table 1. Summary of Clinical and Pathological Data of Case Material

Case	Age (years)	Sex	Postmortem delay (hours:minutes)	Clinical diagnosis	Pathological diagnosis
1	40	Male	2:55	ALS	ALS
2	95	Female	5:30	Normal	CERAD, normal
3	78	Male	6:00	Normal	CERAD, normal
4	86	Female	2:00	AD	AD Braak IV
5	57	Male	3:00	AD, PD	AD Braak IV, PD
6	78	Female	2:00	AD	AD Braak V
7	81	Female	3:16	AD	AD Braak VI
8	76	Female	NA	AD	AD Braak III

ALS, amyotrophic lateral sclerosis; NA, not available.

this newer role for apoE. In fact, apoE immunoreactivity is found not only in extracellular amyloid deposits but also associated with intracellular neurofibrillary tangles.^{34–41} In addition, apoE protein-protein interactions observed *in vitro* also include specific interactions with intracellular neuronal proteins: microtubule-associated proteins (tau, MAP2C).^{42,43} Moreover, elegant cell culture experiments have demonstrated receptor-mediated uptake of apoE in neurons and large effects on neuritic outgrowth and morphology.^{8,9,41–48} These results supported an intracellular role for apoE in neuronal pathology in AD and suggested that apoE might be more directly involved in synaptic loss and disruption of neuronal cytoskeleton in addition to postulated effects on β -amyloidosis.^{8,42–44} One important consideration in evaluating extracellular *versus* intracellular hypotheses of the action of apoE is the source of intraneuronal apoE: uptake *versus* synthesis. A second consideration is the distribution of neurons containing intraneuronal apoE, particularly in brains with little or no neurofibrillary tangle formation.

The apparent normal human and primate pattern of apoE immunolocalization includes many neurons in addition to glial cells. Several studies from our laboratory have shown the presence of immunoreactive apoE in both neurons and glial cells in normal human and primate CNS but essentially restricted to glial cells in normal rodent brain.^{36,49–51} In humanized transgenic lines carrying genomic sequences for each of the three *APOE* alleles, *in situ* hybridization and immunocytochemical localization have demonstrated that apoE has been transcribed and translated not only in glial cells but also in selected populations of cerebral cortical neurons, including hippocampal neurons.^{51,52} These results in humans, primates, and transgenic rodents suggest that the most important source of intraneuronal apoE may be direct synthesis and not uptake from the extracellular space and that intraneuronal synthesis of apoE may be related to regulatory sequences in the human *APOE* gene.

To examine the important issue of whether human brain neurons can synthesize apoE, we performed *in situ* hybridization for *APOE* mRNA and immunolocalization for apoE in brain tissues collected from five AD patients and three nondemented controls, using the same methods and reagents developed in the human *APOE* transgenic mice.⁵²

Materials and Methods

Cases

Human brains were collected 3 to 6 hours postmortem from five AD patients and three clinically examined nondemented patients with non-neurological disease. One index case was selected for short postmortem delay and presumed absence of AD pathology, and the other seven cases were selected randomly from patients enrolled in the Bryan Alzheimer's Disease Center rapid autopsy protocol.⁵³ Clinical information on all patients was collected from clinic and hospital records. The pathological diagnosis of AD was established according to CERAD criteria,⁵⁴ and the degree of AD pathological changes was staged according to Braak⁵⁵ (Table 1).

Preparation of Tissue Sections

For all cases, routinely prepared blocks of frontal lobe, temporal lobe, including hippocampal region, and cerebellar cortex taken at autopsy were fixed for 5 to 7 days in 10% formalin and then embedded in paraffin for pathological analysis. The paraffin blocks were cut at 8-mm thickness, and semi-adjacent were sections mounted on coated slides for immunocytochemistry and *in situ* hybridization.

In addition, specially fixed and cryoprotected blocks of liver and brain were prepared from the index case of a patient with clinical and pathologically confirmed amyotrophic lateral sclerosis (case 1) and used for frozen sections according to published protocol.⁵¹ These blocks of frontal lobe, hippocampal region, cerebellum, and liver were left in 10% formaldehyde in 0.1 mol/L phosphate buffer (pH 7.4) overnight at 4°C and transferred to a solution of 20% sucrose in phosphate buffer overnight at 4°C. The tissues were then placed in tissue-freezing medium (Triangle Biomedical Sciences, Durham, NC), frozen in liquid-nitrogen-cooled ethane, and stored at –70°C before sectioning. Cryostat sections were cut at 8 mm and collected on gelatin-coated slides for *in situ* hybridization and immunolocalization.

Figure 1. Specificity of DIG-labeled cRNA probes for human *APOE*. **A:** Southern blotting demonstrates that both antisense and sense *APOE* DIG-cRNA probes prepared from *APOE3* cDNA hybridize specifically with 1163-bp *APOE* cDNA fragments (illustrated for *APOE3* cDNA and *APOE2* cDNA fragments; *APOE4* not shown). **B:** Both antisense and sense *APOE* DIG-cRNA probes were able to detect 1.0 to 10 ng of *APOE2* plasmid cDNA in slot blot analysis. **C:** Northern blot analysis (15 μ g of total brain RNA loaded onto each lane) showed that only the antisense *APOE* DIG-cRNA probe (**left**) had specific hybridization with human *APOE* mRNA extracted from mouse brains of human *APOE* transgenic lines⁵¹ allele specific for *APOE3* (E3–437), *APOE2* (E2–205), and *APOE4* (E4–81) and human frontal cortex (labeled Human). No mRNA signal was detected in the *APOE* knockout (KO (–/–)) mouse⁵⁶ using antisense DIG-cRNA probe. Parallel blot hybridized with sense probe (**right**) did not yield any signal.

Immunocytochemistry

Immunocytochemistry was performed using avidin-biotin-peroxidase complex (ABC) methods using Elite Vector ABC kits (Vector Laboratories, Burlingame, CA). Sections were deparaffinized, treated with 90% formic acid for 3 to 5 minutes, washed, and then permeabilized with 0.1% Triton X-100 for 10 minutes. After washing with PBS three times, 10 to 15 minutes of methanol-peroxide pretreatment (10% methanol/3% hydrogen peroxide) was used to decrease endogenous peroxidase activity. The treated sections were blocked with avidin/biotin blocking reagent (Vector) and incubated with polyclonal goat antiserum to human apoE (Calbiochem, La Jolla, CA) diluted at 1:500 with Tris-buffered saline (TBS), pH 7.5, containing 0.1% Tween-20 for 30 minutes at 37°C. After thoroughly washing with TBS, the sections were exposed sequentially to biotinylated secondary antibodies and avidin-biotin-peroxidase complex for 30 minutes each at 37°C separated by washes. Vector VIP substrate kit was used to detect bound ABC complex.

Preparation of cRNA Probes

Human *APOE* cDNA fragments specific for *APOE3* allele (courtesy of Dr. D. Goldgaber, Stony Brook, NY) were cloned into Bluescript SK vector (Stratagene, La Jolla, CA) at the *EcoRI* restriction site. Digoxigenin-labeled cRNA (DIG-cRNA) probes in the antisense and sense orientation were synthesized from linearized templates by using bacterial T3 and T7 polymerases, respectively, in

the presence of DIG-11-UTP, as described by the manufacturer (Boehringer Mannheim, Indianapolis, IN).

Southern Blot Analysis, Southern Slot Blotting, and Northern Blot Analysis

Southern blot analysis was performed⁵¹ to test for specific hybridization of antisense and sense DIG-cRNA probes to *APOE* cDNA fragments. The sensitivity of the DIG-cRNA probes was analyzed by slot blotting for detection of *APOE* cDNA plasmids. Northern blotting⁵¹ was used to examine whether the antisense DIG-cRNA probe had specific hybridization to *APOE* mRNA but not to the similarly prepared sense probe. Total RNA was extracted from human brain frontal lobe as described previously⁵¹ and from similarly prepared mouse brain from *APOE* allele-specific transgenic⁵¹ lines *APOE2*–205, *APOE3*–437, and *APOE4*–81 and from *APOE* knockout mice⁵⁶ as positive and negative controls. Fifteen micrograms of each RNA sample was separated by 1.0% agarose gel and transferred to GeneScreen filters. The identical parallel filters were hybridized with the same antisense and sense DIG-cRNA *APOE* probes used for *in situ* hybridization. The hybridization signal on the GeneScreen filter was visualized using anti-DIG antibody conjugated with the alkaline phosphatase detection kit using the manufacturer's protocol (Boehringer Mannheim).

Figure 2. *In situ* hybridization for *APOE* mRNA in frozen human liver sections of nondemented control (case 1) showing relative lack of signal with sense *APOE* DIG-cRNA probe (**A**) compared with strong signal in hepatocytes with antisense probe (**B**). s, sinusoid spaces with unstained red blood cells; cords of hepatocytes are indicated by arrows. Presence or absence of signal for Kupffer cells in **B** could not be determined due to strong hepatocyte signal. Bar, 20 μ m.

In Situ Hybridization

In situ hybridization for human *APOE* mRNA was performed using the same methods developed in human *APOE* transgenic mice.⁵² In initial experiments, hybridiza-

tion was tried at 55°C, 65°C, and 70°C. Hybridization at high temperature (70°C) yielded the best signal and lowest background compared with higher background at lower hybridization temperatures as human *APOE* mRNA has a high GC content.^{52,57,58} Prehybridization was carried out for 1 to 3 hours at room temperature (RT) in prehybridization buffer consisting of 50% formamide, 5X SSC, 10% dextran sulfate, 100 mmol/L dithiothreitol, 250 μ g/ml Baker yeast tRNA, and 10 U/ml RNase inhibitor (Gibco-BRL, Gaithersburg, MD). The hybridization mixtures were prepared by adding 50 to 100 ng/ml DIG-cRNA probes to the prehybridization buffer and then heated 5 minutes at 95°C to denature the probes. Hybridization was allowed to proceed for 16 hours at 70°C in a humid chamber. After washing three times with 2X SSC at 60°C and once with 0.2X SSC at room temperature for 15 minutes each wash, the sections were incubated for 30 minutes at room temperature with alkaline-phosphatase-conjugated anti-DIG antibody diluted at 1:1000. After three to five washes, the sections were developed using an alkaline phosphatase detection kit following the manufacturer's protocols (Boehringer Mannheim).

Results

Tests for Specificity of *APOE* DIG-cRNA Probes

To confirm the specific hybridization of *APOE* cRNA probes with human *APOE* cDNA and mRNA, Southern blot, Southern slot blot, and Northern blot analyses were performed using antisense and sense digoxigenin-labeled *APOE* cRNA (DIG-cRNA) probes. Southern blot results showed that both antisense and sense DIG-cRNA probes hybridized specifically with expected the 1163-bp *APOE* cDNA fragments (Figure 1A). Both the sense and antisense DIG-cRNA probes were able to detect between 1.0 and 10 ng of *APOE* plasmid cDNA in Southern slot blot analysis (Figure 1B). Northern blot analysis demonstrated that only the antisense *APOE* DIG-cRNA probe showed specific hybridization with human *APOE* mRNA in total RNA extracted from human brains and *APOE* transgenic mouse brains under the same conditions (Figure 1C). Sense DIG-cRNA probes did not hybridize with *APOE* mRNA in the parallel Northern blot (Figure 1C). No hybridization signal was detected in RNA extracted from *APOE* knockout mouse brain using antisense DIG-cRNA probe. These results support specific hybridization of antisense *APOE* DIG-cRNA probes to blotted *APOE* mRNA.

To further analyze the specificity of the *APOE* DIG-cRNA probes, *in situ* hybridization was performed on sections from frozen human liver and brain and from paraffin-embedded brain tissues. In each experiment, both antisense and sense *APOE* DIG-cRNA probes were used in semi-adjacent sections processed in parallel. *APOE* transgenic^{51,52} and *APOE* knockout⁵⁶ mouse brain

Figure 3. *In situ* hybridization for *APOE* mRNA in glial cells of cerebellar cortex corresponds to apoE immunolocalization. **A:** Relative lack of hybridization signal with sense *APOE* DIG-cRNA probes in paraffin sections of cerebellar cortex of AD patient (case 5). **B:** Strong hybridization signal along Purkinje cell/Bergmann glial cell layer with antisense probe for *APOE* mRNA in semi-adjacent sections of case 5 represents Bergmann glial cells (see below). **C:** Restriction of *APOE* mRNA hybridization pattern to glial cells in cerebellum was also observed clearly in frozen sections of nondemented control with ALS (case 1). **D:** Higher magnification of area demarcated above in **B** demonstrates signal in radial glial fibers (RGF) and Bergmann glial cell bodies (arrows) and lack of any signal in Purkinje cells (P). **E:** Immunocytochemical localization of apoE demonstrates presence of apoE immunoreactivity in similar distribution to mRNA localization, although Bergmann glial cell bodies and entire extent of radial glial fiber are more clearly seen. The faint background staining of Purkinje cell (P) shows relationship of unseen Purkinje cells to Bergmann glial cells in **A** to **D**.^{6,57} Bar, 60 μ m (**A** and **B**) and 20 μ m (**C** to **E**).

tissues were routinely included as positive and negative controls, respectively. High-stringency hybridization at 70°C overnight (~16 hours) yielded the best hybridization signal and lowest background staining⁵² due to the high GC content of the *APOE* gene.⁵⁸ Using antisense *APOE* DIG-cRNA probes under these conditions, specific hybridization signal was observed in human hepatocytes as expected (Figure 2B). Chromogen development times were controlled to produce minimal signal in sections of human liver (Figure 2A) and brain (Figures 3A, 4, A and D, and 6A) processed in parallel using sense *APOE* DIG-cRNA probe. These reagents and methods have been described in *APOE* transgenic mice where antisense *APOE* DIG-cRNA probe permits specific detection of human *APOE* mRNA.⁵² The present and published *in situ* hybridization results support the conclusion that the antisense *APOE* DIG-cRNA probe specific hybridizes with intracellular human *APOE* mRNA in brain and liver tissues.

Glial APOE mRNA Transcription and Expression in Cerebellar Cortex

In human cerebellar cortex, very strong *APOE* mRNA hybridization signal was observed in Bergmann radial glial cells and in some scattered astrocytes (Figure 3). Similar localization was observed in both frozen (Figure 3C) and paraffin-embedded sections (Figure 3, B and D) in nondemented control and AD patients. Localization of *in situ* hybridization signal was entirely consistent with the pattern of immunoreactive apoE localization in sections from the same brains (Figure 3E). No apparent evidence for neuronal *APOE* mRNA hybridization or neuronal apoE immunolocalization was observed in cerebellar cortex of these human brains (Figure 3, B–E). The expression of the human *APOE* gene in Bergmann glial cells was very strong, and hybridization signal often extended for considerable distances into their radial processes extending outward in the molecular layer. In normal and transgenic

Figure 4. *In situ* hybridization for *APOE* mRNA in glial cells and neurons of cerebral cortex corresponds to apoE immunolocalization. Hybridization with sense *APOE* DIG-cRNA probe results in some faint cell staining (arrows indicate background signal in neurons) in paraffin section from frontal cortex of AD patient (case 4; A) and frozen section from temporal lobe of nondemented control with ALS (case 1; D). B: Field from parallel processed paraffin section of frontal cortex of AD patient (case 4) hybridized with antisense *APOE* DIG-cRNA probe showing *APOE* mRNA-positive neurons (arrow) and an example of satellite glial cell (arrowhead). C: Hybridization signal was observed in scattered cells in paraffin section of frontal cortex from AD patient (case 5). Size and morphology of these cells was consistent with pyramidal cortical neurons (arrows). The eccentric location of signal in some cases suggests possible additional staining of satellite glial cells (arrowheads). E: Particularly distinctive hybridization signal was observed in frozen section of temporal lobe of nondemented control with ALS (case 1), whose parallel sections processed with sense probe showed low background sense probe signal (see D). Several *APOE* mRNA-positive neurons (arrows) and presumptive astrocytes (arrowheads) are indicated. F: Immunocytochemical localization of apoE in the parallel paraffin section of frontal cortex from AD patient (case 5; compared with *in situ* hybridization in C) demonstrated immunoreactive apoE in many neurons (arrows), glial cells (arrowheads), and senile plaques (SP). Bar, 20 μ m.

rodent cerebellar cortex, apoE immunoreactivity in Bergmann glia and their long radial fiber processes is typically intense.^{6,51} This normal glial-specific apoE immunolocalization and *APOE* mRNA hybridization pattern in human cerebellar cortex corresponds to that observed in humanized mice transgenic for genomic fragments of human *APOE* gene and in normal wild-type mice.⁵²

Neuronal and Glial *APOE* mRNA Transcription and Expression in Frontal Cortex

In frontal cortex and hippocampus (Figure 4), the pattern of *in situ* hybridization for human *APOE* mRNA was remarkably different from that observed in cerebellar cortex of the same cases (Figure 3). *APOE* mRNA hybridization

signal could be observed in selected populations of large neurons in frontal lobe (Figure 4, B and C) and in frozen sections of hippocampus (Figure 4E). The *APOE* mRNA hybridization signal intensity for neurons varied markedly. The number and distribution of neurons with *APOE* mRNA hybridization signal was similar to the pattern of immunocytochemical localization of neuronal apoE in parallel sections (case 5, Figure 4, C and F). Often, the relative *APOE* mRNA hybridization signal was stronger in presumptive astrocytes or glial cells compared with neurons (Figure 4E). The number and distribution of *APOE* mRNA-positive neurons and relative glial/neuronal intensity differences were qualitatively similar to the pattern observed in transgenic mice carrying human *APOE* genomic fragments.^{51,52} We observed *APOE* mRNA-pos-

Figure 5. Paraffin sections of hippocampus from nondemented control (case 3) showed consistent pattern of apoE immunoreactivity (A) and *in situ* hybridization signal (B) for neuronal *APOE* translation and transcription in granule cell layer of dentate gyrus. Scattered apoE-immunoreactive neurons (arrows in A) and *APOE* mRNA-positive neurons (arrows in B) are located within the granule cell layer. From both immunolocalization and mRNA localization results, there are also clearly many granule cell neurons that do not contain signal. Staining of smaller non-neuronal cells (arrowheads) may variably represent astrocytes (arrowhead 1 in A), microglial cells (arrowhead 2 in A and arrowhead 2 in B), and satellite glial cells (arrowhead 1 in B). Bar, 20 μ m.

itive and apoE-immunoreactive neurons in cerebral regions with AD pathology (case 5), including apoE-immunoreactive senile plaques (Figure 4, C and F). This result was consistent with our previous reports on apoE immunoreactivity of neurons and senile plaques.³⁶

Neuronal and Glial *APOE* mRNA Transcription and Expression in Hippocampus

In sections of human hippocampus, *APOE* mRNA hybridization signals were observed in neurons in all of the cases, including nondemented controls and AD patients (Figures 5 and 6 and in presumptive glial cells. The typical appearance of apoE-immunoreactive granule cell neurons is illustrated in Figure 5A for the granular cell layer of the dentate gyrus, similar to our previous report.³⁶ *APOE* mRNA hybridization signal was present in a similar number of neurons in the granule cell layer of the dentate gyrus (Figure 5B). *APOE* mRNA-positive neurons were observed in the granule cell layer of the dentate

gyrus adjacent to more numerous nonhybridizing neurons (Figure 5B). *APOE* mRNA-positive neurons were observed in all sectors of the hippocampus, illustrated for CA1–2 (Figure 6B), CA3 (Figure 6, D and E), and CA4 (Figure 6, G and H) and adjoining temporal cortex (Figure 4E). No appreciable hybridization signal was visualized in the sections probed in parallel with the sense DIG-cRNA probe as illustrated for the CA1–2 sector (Figure 6A). These observations support the proposition that the antisense probe hybridization signal was specific for cells containing *APOE* mRNA sequence.

Discussion

Our *in situ* hybridization results support the conclusion that human *APOE* mRNA is transcribed and expressed not only as expected in glial cells but also in selected populations of neurons in frontal cortex and hippocampus in normal controls and AD patients (Figures 4 to 6). This is a new finding, and we have been careful to exclude as best as possible other reasons for these observations. We have verified that both antisense and sense *APOE* DIG-cRNA probes used in these experiments can specifically hybridize with *APOE* cDNA on Southern blot (Figure 1A) and that only the antisense probe hybridizes with human *APOE* mRNA on Northern blot (Figure 1C). In addition, no hybridization signal is visualized on human brain and liver sections processed in parallel and probed with the sense probe (Figures 3A, 4A, and 6A). The antisense probe detects human *APOE* mRNA in transgenic mice,⁵² but no hybridization signal is observed for sense or antisense probe in *APOE* knockout mice.⁵² The observation of regional specificity in terms of neuronal hybridization (see below) further supports the conclusion that neuronal hybridization is specific for the presence of *APOE* mRNA. Thus, the present reagents and methods apparently provide a reliable assay for localization of *APOE* mRNA in human brain.

The pattern of transcription and expression of *APOE* mRNA in human brain neurons is apparently regionally specific based on examination of cerebellar cortex, frontal cortex, and hippocampus. This pattern is entirely similar to our findings in mice transgenic for genomic fragments of the human *APOE* gene.^{51,52} *APOE* mRNA is present in some, but not all, neurons in frontal cortex and hippocampus in the cases in our series (Figures 4E, 5B, and 6, D and E) apparently unrelated to extent of AD pathology. The apparent number and distribution of neurons containing *APOE* mRNA is qualitatively similar to the number and distribution of apoE-immunoreactive neurons (Figures 4F, 5A, and 6, C and F). In striking contrast, neither *APOE* mRNA nor apoE immunoreactivity is detected in cerebellar cortical neurons (Purkinje cells, stellate-basket cells, and granule cells) in these same cases (Figure 3). The pattern of intense apoE immunoreactivity of Bergmann glial cells and their long radial processes and the lack of neuronal apoE immunoreactivity seen in human cerebellar cortex is similar to the pattern observed in normal rat and mouse.^{6,51,52} The presence of apoE-immunoreactive neurons in human frontal cortex and hip-

Figure 6. *In situ* hybridization demonstrated *APOE* mRNA signal in numerous neurons in CA1–2 (B), CA3 (C to E), and CA4 (F to H) sectors of human hippocampus. **A:** *In situ* hybridization with sense *APOE* DIG-cRNA probe hybridization with paraffin section of hippocampus of AD patient (case 8) showed faint background signal in some neurons (arrow) in CA1–2 sector. **B:** Hybridization with antisense *APOE* DIG-cRNA probe in parallel processed paraffin section of same region showed numerous *APOE* mRNA-positive neurons (arrows). In some cases, eccentric signal close to neurons (arrowhead) suggested signal in satellite glial cells. Comparison of apoE immunolocalization and *APOE* mRNA hybridization pattern of CA3 sector of another AD patient (case 5) demonstrated similar distribution of apoE immunoreactivity (C) and *APOE* mRNA hybridization signal (D) in neurons (arrows) and glial cells (arrowheads). **E:** *APOE* mRNA hybridization signal in paraffin-embedded section of CA3 sector in another AD patient (case 6) showed examples of *APOE* mRNA-negative or low-signal neurons (arrow 1), medium-signal, presumably positive neurons (arrow 2), and strong-signal neurons (arrow 3). Nearby are some presumptive glial cells (arrowheads.) with strong hybridization signal. **F:** ApoE immunoreactivity pattern in paraffin section of CA4 sector of nondemented control (case 2) showing immunoreactive neurons (arrow) and glial cells (arrowhead). **G:** *APOE* mRNA hybridization signal in paraffin section of CA4 sector of AD patient (case 4) showing numerous positive neurons (arrows) and glial cells (arrowheads). **H:** *APOE* mRNA hybridization signal in frozen section of CA4 sector of nondemented control with ALS (case 1) showing *APOE* mRNA-positive neurons (arrows) and glial cells (presumptive astrocytes, arrowhead). Bar, 20 μ m.

pocampus in the present series confirms our previous published results.^{36,42,50} ApoE-immunoreactive neurons are also observed in a regionally specific pattern in mice transgenic for genomic fragments of human *APOE* gene.^{51,52} We have not yet examined other human brain regions or examined the issue of influence of *APOE* genotype on these results.

The pattern of *APOE* mRNA localization and apoE immunoreactivity is qualitatively similar in the present series, particularly in the striking regional differences between cerebral cortex and cerebellum. However, we observed relatively fewer *APOE* mRNA-positive neurons than apoE-immunoreactive neurons in cerebral cortex and hippocampus of these AD patients and normal controls. In the frozen sections, we observed typical relatively weaker *APOE* mRNA signals in neurons than adjacent astrocytes (Figures 4E and 6H). This may reflect a sensitivity threshold of our antisense *APOE* DIG-cRNA probe, or more likely a lesser amount of *APOE* mRNA in neurons. The limitation of detection for *APOE* mRNA in neurons may depend on several factors. Failure to detect *APOE* mRNA in neurons in other studies to date^{18,19,59} could be due to less sensitive or specific probes used, loss of mRNA during agonal events, tissue preparation or cutting and mounting of sections, too high a stringency of hybridization and/or prolongation of washing steps, and the relative signal-to-noise characteristics of the detection system.

Whether apoE can be synthesized by neurons in normal human brain is fundamental to understanding the demonstrated role of the human *APOE* gene and its alleles as a susceptibility gene in late-onset AD^{20–23,43} and the additional role of *APOE* alleles in some forms of CNS injury. Cognitive decline in AD is most directly associated with neuronal loss and cytoskeletal abnormality, including neurofibrillary tangles, in which the principal constituent is hyperphosphorylated tau, a microtubule-associated protein.³⁸ One hypothesis for the role of *APOE* alleles in the pathogenesis of AD is a proposed influence on intraneuronal metabolism through interaction with neuronal cytoskeletal proteins, microtubule-associated proteins tau and MAP2.^{42,43} In the brains of patients with AD pathology, it is generally accepted that apoE is present in many neurons with neurofibrillary tangles.^{34,39,60,61} In normal controls, AD patients and nonhuman primates, apoE-immunoreactive neurons without apparent cytoskeletal pathology are also observed in hippocampus and cerebral cortex.^{49,50} In rodent experiments with brain injury induced by ischemia and kainic acid, apoE mRNA and apoE immunoreactivity are seen in neurons in injured areas and might reflect neuronal synthesis of apoE.^{14–16} *APOE* alleles have been proposed to affect the degree of cholinergic injury in Alzheimer's disease through mechanisms of altering synaptic plasticity.^{62,63} Although we cannot rule out the expected receptor-mediated neuronal uptake of apoE,^{45–48} the findings reported here describing specific neuronal hybridization for human *APOE* mRNA do not support the commonly held idea that apoE is synthesized and secreted only by non-neuronal cell classes in human CNS.^{4–7} We observed *APOE* mRNA-positive neurons in cerebral regions from normal controls

as well as cases with AD pathology, including apoE-immunoreactive neuritic plaques (Figure 4, C and F). This suggests that the previously described presence of apoE-immunoreactive neurons in human brain, both in normal and AD cases, may represent neuronal synthesis.^{34,36,49,60,61} The cerebellum, with very strong glial and lack of neuronal *APOE* transcription in all of the cases, is a relatively spared structure in AD compared with cerebral cortex. This same *in situ* hybridization pattern is also seen in our humanized *APOE* allele-specific transgenic mice.^{51,52} These results in cerebellum and cerebral cortex indicate that human *APOE* gene expression in neurons may be one factor or marker for selective vulnerability of cerebral cortical neurons observed in AD.^{54,64}

In this report, we have examined a limited series of recent nondemented control cases and AD patients for *in situ* hybridization for *APOE* mRNA. We have seen *APOE* mRNA transcription in selected neurons in all brains examined to date. These results cannot speak to possible quantitative difference or to effects of representative *APOE* alleles and many other factors. Our results do not permit any conclusions about whether more subtle or systematic differences in degree of neuronal *APOE* transcription and expression may exist between normal nondemented controls and AD patients. The regionally specific pattern of human *APOE* gene expression observed in the brains of transgenic mice^{51,52} is confirmed for human frontal cortex, hippocampus, and cerebellum, but we have not yet examined other brain regions. In addition, additional experiments with more extensive series comparing nondemented controls and AD patients with the various *APOE* genotypes will be necessary to comment on possible differences related to *APOE* allelic variation. These experiments will be necessary to define the limit conditions for neuronal transcription and expression of the *APOE* gene and the significance for neuronal metabolism, aging, and response to injury. Regionally specific intraneuronal synthesis of apoE may be a significant factor in regional vulnerability of neurons to cytoskeletal pathology and in interactions of apoE with neuronal proteins. For both intraneuronal synthesis and receptor-mediated uptake of apoE, there are significant questions as to the conditions under which this protein might gain access to the cytoplasmic compartment to participate in such interactions.

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