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Increased amyloid β-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease

(phenotype/*APOE4* gene)

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ABSTRACT **Amyloid β-peptide (Aβ) deposition in senile plaques and cerebral vessels is a neuropathological feature of Alzheimer disease (AD). We examined the possibility that commonly observed variability in Aβ deposition in late-onset AD might be related to apolipoprotein E genotype (*APOE* gene; the two most common alleles are *3* and *4*), since *APOE4* is a susceptibility gene for late-onset AD and apolipoprotein E interacts strongly with Aβ *in vitro*. In an autopsy series of brains of late-onset AD patients, we found a strong association of *APOE4* allele with increased vascular and plaque Aβ deposits. Late-onset AD patients with one or two *APOE4* alleles have a distinct neuropathological phenotype compared with patients homozygous for *APOE3*.**

Amyloid deposition in cerebral vessels and in senile plaques is one of the recognized neuropathological features of both early- and late-onset Alzheimer disease (AD) (1–3). A major component of these amyloid deposits is the β-peptide (Aβ) fragment of amyloid precursor protein (APP, protease nexin II, encoded on chromosome 21) (4–11). The role of Aβ and APP in the pathogenesis of certain cases of early-onset AD is supported by the association of specific point mutations in APP with rare examples of autosomal dominant early-onset familial AD (12–16). In the much more common cases of late-onset AD, a direct role of Aβ and APP in the pathogenesis of AD is less likely. APP mutations have not been identified in sporadic and familial late-onset AD, and genetic linkage for late-onset AD has been excluded on chromosome 21 (17, 18). In addition, the degree of amyloid deposition in late-onset AD, particularly for cerebral vessels, can vary widely from case to case (18–22).

We have recently provided evidence that apolipoprotein E (apoE) allele ε4 (*APOE4* gene; located on chromosome 19) is a susceptibility gene or risk factor in both sporadic and familial late-onset AD (23–25) and is inherited as an autosomal codominant trait. The possible involvement of apoE in late-onset AD is based on several findings in addition to the reported increase of *APOE4* gene frequency (23, 24). apoE appears to play an important role in nerve cell injury and regeneration (26). The role of apoE isoforms in neuronal injury may involve not just facilitation of cholesterol transport but also interactions that result in targeting or sequestering of proteins (27). Furthermore, apoE is found in close association with amyloid deposits in vessels and plaques in AD as well as in other amyloidoses such as Creutzfeldt–Jakob disease and kuru (23, 28, 29). A specific role for apoE in AD-related pathology is suggested by the high-avidity binding of apoE isoforms *in vivo* with Aβ and the significantly different binding characteristics of the apoE4 isoform with Aβ compared with the apoE3 isoform (30, 31).

To test whether *APOE* genotype might be associated with a distinctive neuropathological phenotype, we examined the extent of amyloid deposition in autopsy-confirmed cases of sporadic late-onset AD in patients with defined *APOE* genotype, using routine neuropathological reports and examination of Congo red- and immunostained slides. We found that patients with sporadic late-onset AD who have one or two copies of *APOE4* have a distinctive neuropathological phenotype of greatly increased vascular and plaque amyloid deposits compared with patients homozygous for *APOE3*.

MATERIALS AND METHODS

Case Selection and *APOE* Genotyping. Frozen and formalin-fixed material from 143 patients with autopsy-confirmed cases of late-onset AD without affected family members and without other neurological disease in the period 1985–1992 was obtained through the Kathleen Bryan Brain Bank at Duke University. All cases satisfied National Institutes of Health and Consortium to Establish a Registry for Alzheimer's Disease criteria (1, 2). *APOE* genotyping for each patient was carried out as previously described, using amplification by polymerase chain reaction (24, 32, 33).

Neuropathological Analysis. Information on age at death, apparent duration of illness, gender, brain weight, mention of amyloid deposits in cerebral vessels (Congo red stain), and description of number of neuritic plaques (69 cases) and neurofibrillary tangles (95 cases) from modified King's silver-stained sections (34) were taken from the original neuropathological reports. Each count represented a single microscopic field felt to be the most affected area in that tissue section; a 10× objective was used for plaques (field of 2.92 mm^2) and a 20× objective for tangles (field of 0.72 mm^2). Counts were truncated at 100 per field.

Analysis of Congo Red-Stained Material for Vascular Amyloid. A subset of 53 patients was chosen by selecting in chronological order those cases for which Congo red-stained slides had previously been prepared. Forty of the 53 autopsy reports specifically mentioned vascular amyloid and formed one set of blinded observations completed before genotyping. Mention of vascular amyloid was graded as follows: no evident amyloid deposits, grade 0; trace amyloid deposits including one positive vessel, grade 1; and readily identifiable vascular amyloid, grade 2. In addition, a single Congo red-stained slide of frontal cortex and of hippocampus from each of the 53 patients was examined double-blind for extent

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Abbreviations: AD, Alzheimer disease; Aβ, amyloid β-peptide; APP, amyloid precursor protein.

of vascular amyloid by three observers (D.E.S., C.M.H., and B.J.C.), using the above grading system (average inter-rater agreement of 85%).

Immunocytochemical Methods. Six *APOE4/4* patients, eight *APOE3/3* patients, and five *APOE3/4* patients were randomly selected without knowledge of neuropathological report. Paraffin sections (6–8 μm thick) of hippocampal region and frontal lobe were treated to remove paraffin, treated with 90% (wt/wt) formic acid for 3 min, and washed, and then Aβ was localized by immunoreactions using either monoclonal antibody 4D12/2/6 to Aβ fragment 8–17 [courtesy of George Glenner and David Alsop (4, 5)] or 10D5 to Aβ-(1–28) [courtesy of Athena Neuroscience (San Francisco) (6)]. Both antibodies provided excellent localization of plaque and vascular amyloid (4–6). Sections from different patients were allowed to react in parallel with identical antibody dilution and enzymatic detection steps, using the ABC method (Vectorstain, Burlingame, CA). Controls allowed to react in parallel were unstained (for example, see figure 4*A* of ref. 23). Semiadjacent sections (untreated with formic acid) were used to demonstrate neuritic plaques with monoclonal antibody SMI-34 to 164-kDa-neurofilament protein (Sternberger–Meyer, Jarrettsville, MD). The apoE immunoreactivity was localized by using a polyclonal antibody to human apoE which recognizes apoE2, apoE3, and apoE4 isoforms on Western blots (courtesy of Joel Morrisett, Baylor College of Medicine).

Analysis of Immunocytochemical Material. Using a 16× contrast interference objective, we selected three fields in regions of maximal plaque density for each section and drew plaque outlines on a divided grid covering 0.25 mm^2 by using a camera lucida. Percentage of area occupied by Aβ deposits was estimated by percentage of grid boxes containing any portion of an immunoreactive plaque or vessel. All drawing and rating was done blinded to patient and genotype (inter-rater reliability was 85%). Representative fields were photographed with a Zeiss photomicroscope.

Statistical Analysis. Data were entered in a Statgraphics package and multiple analysis of variance was used to describe the relationships between variables. No significant differences existed between the set of 143 cases or the various subsets for age at death, duration of illness, or gender ratio.

RESULTS

Increased Aβ Deposits in Cerebral Cortex of Sporadic AD Patients Homozygous for *APOE4*. Immunocytochemistry for Aβ in brains of patients with sporadic late-onset AD demonstrated striking differences between immunostained sections of cerebral cortex from *APOE3/3* and *APOE4/4* patients (Fig. 1 *A* and *B*). Sections from *APOE3/3* patients usually show little to no vascular staining and faintly immunoreactive plaques (Fig. 1*A*), while sections from *APOE4/4* patients are commonly darkly stained with abundant immunoreactive vessels on the cerebral surface and strongly immunoreactive plaques and vessels in the parenchyma (Fig. 1*B*). Sections from *APOE4/4* brains can often be differentiated from sections from *APOE3/3* brains without a microscope.

apoE immunoreactivity was observed in cerebral vessels, neurons, glial cells, senile plaques, and neurofibrillary tangles as described in previous reports (22, 23, 28, 29). Like Aβ immunoreactivity, apoE immunoreactivity is enhanced after formic acid treatment (31). There was no major difference in the localization or intensity of apoE immunoreactivity in

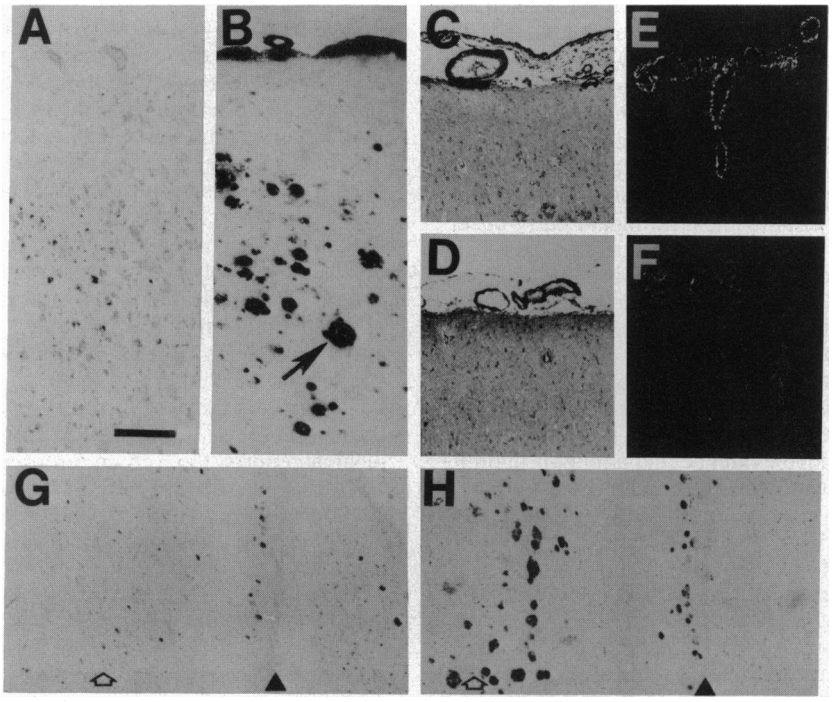

FIG. 1. Aβ and apoE immunolocalization in formic acid-treated paraffin sections of middle frontal gyrus (*A*, *B*, *C*, *D*) and hippocampus (*G*, *H*) of two patients with sporadic late-onset AD. Increased numbers of intensely Aβ-immunoreactive vessels and plaques are found in frontal cortex of the *APOE4/4* patient (*B*) compared with the *APOE3/3* patient (*A*). In hippocampus of the same patients, subiculum (open arrows) and molecular layer of dentate gyrus (arrowheads) also display more Aβ-immunoreactive plaques in the *APOE4/4* patient (*H*) compared with the *APOE3/3* patient (*G*). Cerebral vessels on the cortical surface and within the cortical plate (arrow, *B*) are strongly Aβ immunoreactive in the *APOE4/4* patient; the subpial region or layer I of cerebral cortex displays ribbonlike diffuse Aβ immunoreactivity. Congo red-stained sections from the above pair of patients show the same differences in vascular amyloid deposition. Viewed under polarized light, cerebral vessels from the *APOE4/4* patient have yellow-green birefringence typical of β-sheeted amyloid deposits (*E*), whereas there is an absence of birefringence in the *APOE3/3* patient (*F*). Despite the marked difference in Aβ immunoreactivity, the intensity of apoE immunoreactivity is similar in the two patients (non-formic-acid-treated sections of middle frontal gyrus: *C*, *APOE4/4* patient; *D*, *APOE3/3* patient). In particular, cerebral vessels are apoE immunoreactive in both genotypes. (All slides were photographed at ×68; bar in *A* indicates 200 μm.)

APOE3/3 cases compared with *APOE4/4*, including immunoreactive cerebral vessels observed in both genotypes (Fig. 1 *C* and *D*).

Retrospective Analysis of Vascular Amyloid in Autopsy Reports. In 40 available autopsy reports, retrospective grading revealed a significant dose-related increase of vascular amyloid with number of *APOE4* alleles ($P < 0.00001$). Typically, no vascular amyloid was mentioned for most *APOE3/3* cases, trace vascular amyloid was recorded for *APOE3/4* cases, and large amounts were observed in most *APOE4/4* cases (Table 1).

Prospective Blinded Analysis of Vascular Amyloid in Congo Red-Stained Material. We also examined prospectively Congo red-stained sections of hippocampus and frontal cortex from 53 patients and confirmed a highly significant association ($P < 0.00001$) between the extent of Congo red-positive amyloid angiopathy and dose of *APOE4* allele (Table 1). The typical difference in Congo red staining is illustrated in Fig. 1 *E* and *F*. Such amyloidotic vessels were not necessarily present throughout a given cortical region in *APOE4* homozygotes, but often they predominated in the depths of sulci such as the hippocampal fissure.

Immunochemical Detection of Vascular Amyloid. Aβ-immunoreacted sections from a subset of 19 of the 53 patients showed the same statistically significant association of amount of vascular amyloid and dose of *APOE4* allele (Table 1). Amyloidotic vessels in *APOE4* homozygotes often included leptomeningeal vessels and large and small vessels in the cerebral wall. Parenchymal vessels were often surrounded by a plaquelike accumulation of amyloid [plaquelike angiopathy of Scholz (19)].

Average Neuritic Plaque Counts Are Increased in *APOE4/4* Homozygotes. We examined 69 autopsy reports for possible association of *APOE* genotype with neuritic plaque count. In four of five cortical regions, the average number of neuritic plaques in silver-stained material was greater in *APOE4* than in *APOE3* homozygotes (Table 1). Neuritic plaque counts did not correlate with duration of illness. Average counts were increased and related to *APOE4* allele dosage in frontal, temporal, and parietal cortex and were borderline significant in the CA1 subfield of hippocampus.

Table 1. Neuropathological and immunochemical characteristics of brains of patients with sporadic AD

	APOE3/3	*APOE3/4*	*APOE4/4*
	$n = 47$	$n = 64$	$n = 23$
Average age at death, yr	76.4	79.1	75.8
Average illness duration, yr	7.7	8.5	9.0
Data from autopsy reports and routine pathological stains			
Congophilic amyloid angiopathy,			
average grade (0 = none, 1 = trace, 2 = present)			
40 cases by report	$n = 15$	$n = 11$	$n = 14$
	0.27	0.80	1.92*
53 cases by review	$n = 20$	$n = 16$	$n = 17$
	0.40	1.16	1.76*
Neuritic plaques,			
average number per low-power field (2.92 mm²)	$n = 25$	$n = 28$	$n = 16$
Middle frontal gyrus	55	82	87†
Superior temporal gyrus	59	77	86‡
Inferior parietal lobule	61	68	86§
Entorhinal cortex	27	34	27
Hippocampus (CA1)	10	13	15
Neurofibrillary tangles,			
average number per high-power field (0.72 mm²)	$n = 30$	$n = 44$	$n = 21$
Middle frontal gyrus	6	7	8
Superior temporal gyrus	7	9	13
Inferior parietal lobule	7	10	8
Entorhinal cortex	31	35	42
Hippocampus (CA1)	26	37	48
Data from immunocytochemical studies			
Aβ-immunoreactive vessels,			
average grade (0 = none, 1 = trace, 2 = present)	$n = 8$	$n = 5$	$n = 6$
Middle frontal gyrus/hippocampus	0.50	0.80	2.00¶
Aβ-immunoreactive plaques,			
% area occupied			
Middle frontal gyrus	$n = 7$	$n = 4$	$n = 6$
Strongly immunoreactive	4.5	9.7	31.4*
Weakly immunoreactive	12.5	4.2	5.8
Total plaques	16.9	13.9	37.2¶
Hippocampus	$n = 6$	$n = 3$	$n = 6$
Strongly immunoreactive	5.6	14.3	32.8‖
Weakly immunoreactive	7.5	2.6	5.3
Total plaques	13.2	16.9	38.2¶
Neuritic plaques,			
% area occupied			
Middle frontal gyrus	$n = 4$	—	$n = 4$
	6.5		20.2¶

Significant differences between the three allele groups are indicated by the following symbols: *, $P < 0.00001$; †, $P < 0.0002$; ‡, $P < 0.01$; §, $P < 0.02$; ¶, $P < 0.001$; ‖, $P < 0.0001$.

Average Neurofibrillary Tangle Counts Are Mildly Increased in *APOE4* Homozygotes. For 95 patients, the autopsy reports mentioned neurofibrillary tangle counts. In all five of the cortical regions presented above, the average number of neurofibrillary tangles was greater in *APOE4* compared with *APOE3* homozygotes (Table 1). However, the average neurofibrillary tangle count for these cortical regions is positively correlated ($P < 0.01$) with apparent duration of illness. The slight increase in average neurofibrillary tangle counts in *APOE4* homozygotes apparently represents the upward trend in duration of illness associated with *APOE4* (Table 1).

Immunocytochemical Analysis of Amyloid Deposition in Sporadic AD Patients. The large increase in amyloid deposition noted in *APOE4* compared with *APOE3* homozygotes with sporadic AD includes both vascular amyloid deposition (Fig. 1, Table 1) and deposition in senile plaques (Fig. 1 *A* and *B*). To confirm these differences qualitatively and quantitatively, we compared immunoreacted material from six patients homozygous for *APOE4*, seven patients homozygous for *APOE3*, and four patients who were heterozygous (*APOE3/4*). Low-power views of cerebral cortex reveal the typical increased overall Aβ immunoreactivity observed in paired sections of frontal cortex from five *APOE4* homozygotes (Fig. 2 *Right*) and five *APOE3* homozygotes (Fig. 2 *Left*). Plaques in *APOE4/4* cases were more numerous and somewhat larger, often including a vessel, and were darker than those observed in *APOE3/3* sections processed in parallel under identical conditions.

Quantitative Analysis of Aβ-Immunoreactive Plaques. Three microscopic fields from immunoreacted sections of frontal cortex and hippocampus from the above patients were chosen to represent the maximal density of Aβ-immunoreactive plaques observed in that section and quantitated (see lower part of Table 1). There was a highly significant, 5- to 7-fold greater average area covered by strongly Aβ-immunoreactive plaques ($P < 0.00001$) in *APOE4* compared with *APOE3* homozygotes in both cortical areas. *APOE3/4* patients are intermediate.

In several *APOE3/3* patients, very weakly immunoreactive plaques were present and their number tended to be higher than in *APOE4/4* cases. These lighter plaques can be seen in the background of several of the *APOE3* homozygote cases in Fig. 2 (Fig. 2 *A*, *E*, and *G*). Nevertheless, the average total area covered by all Aβ-immunoreactive plaques, both weakly and strongly immunoreactive, was 2- to 3-fold greater ($P < 0.001$) in *APOE4* homozygotes compared with *APOE3* homozygotes in both cortical areas (Table 1).

Comparison with Neuritic Plaques. Additional semiadjacent sections of frontal cortex from eight of the above patients were immunostained with an antibody to neurofilament protein (SMI-34) to test whether plaque density identified by neuritic content also varied between the *APOE3* and *APOE4* homozygotes as suggested by autopsy report counts. Although the number and total area covered by neuritic plaques (as defined by SMI-34 immunoreactivity) is less than that for amyloid plaques (as defined by Aβ immunoreactivity) (see bottom of Table 1), comparison of area covered by plaques in semiadjacent sections shows a significant and linear correlation for the two methods of plaque detection in these eight patients ($P < 0.006$, $r = 0.73$). The area covered by neuritic plaques is significantly greater in *APOE4* than in *APOE3* homozygotes ($P < 0.001$).

DISCUSSION

Extracellular Aβ deposits are considered characteristic neuropathological features of AD (1–6, 22, 35). Several studies have noted that the amount of Aβ deposits in cerebral vessels and the density of senile or neuritic plaques can vary widely from case to case (2, 22). The results of the present study

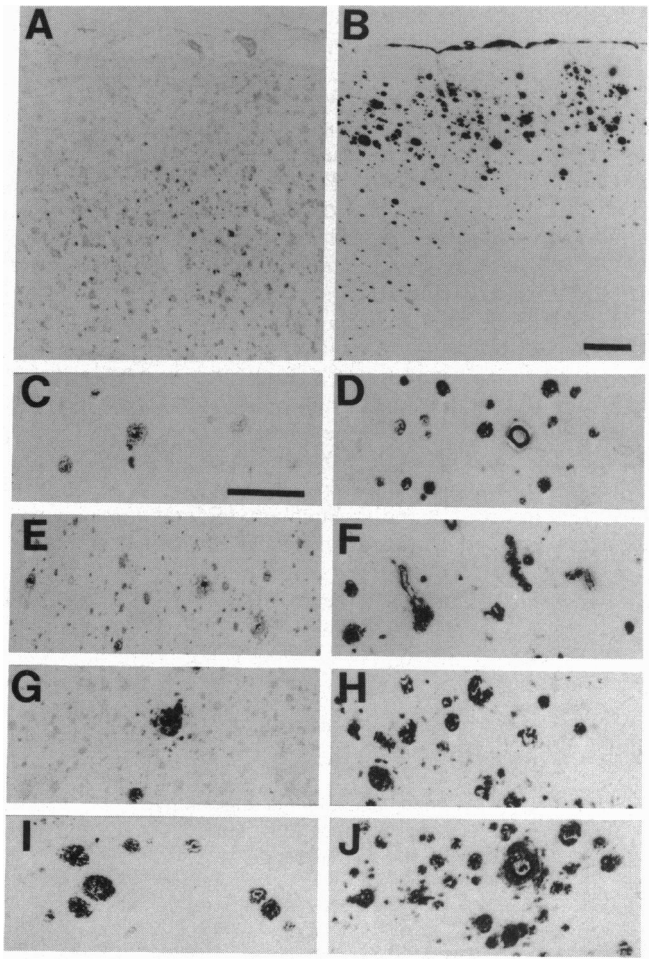

FIG. 2. Aβ immunoreactivity in formic acid-treated paraffin sections of middle frontal gyrus stained from five AD patients with *APOE3/3* genotype (*A*, *C*, *E*, *G*, *I*) and five AD patients with *APOE4/4* genotype (*B*, *D*, *F*, *H*, *J*). Pairs are matched for duration of illness. *APOE3/3* brain sections have few darkly stained plaques, although some patients show numerous weakly stained diffuse plaques (*A* and *E*). In contrast, Aβ immunoreactivity in *APOE4/4* patients is more intense and extensive than in *APOE3/3* patients, with numerous darkly stained plaques, vessels, and subpial deposits (*B*, *D*, *F*, *H*, *J*). (*A* and *B* were photographed at 25×; *C–J*, at 100×. Bar indicates 500 μm in *B* and 200 μm in *C*.)

demonstrate that the amount of histologically identified Aβ in vessels and plaques in the cerebral cortex of patients with sporadic late-onset AD is a direct function of their APOE genotype. We found increased amyloid deposits in vessels by using both Congo red staining and specific immunocytochemistry for Aβ, and increased density of strongly Aβ-immunoreactive plaques in patients homozygous for *APOE4* compared with patients homozygous for *APOE3*. *APOE3/4* patients were intermediate. There is a statistically significant association between the degree of Aβ deposition in both vessels and plaques and the dosage of *APOE4* (Table 1). Thus, *APOE* genotype in sporadic late-onset AD explains much of the variability in extent of amyloid deposition previously noted in AD patients, particularly for vascular amyloid (19–22). Differences in brain weight, gender ratio, apparent duration of illness, and age at death were not factors. The present study addresses only Aβ immunoreactivity in two regions (hippocampus and middle frontal gyrus) of cerebral cortex of patients dying with AD. The high amount of Aβ observed in some *APOE4/4* and *-3/4* patients with short duration of illness suggests that increased Aβ

deposition may be present in some persons with *APOE4* allele who are not demented.

The observed association of increased Aβ deposits with *APOE4* may reflect a direct pathogenetic role for apoE isoforms in amyloid deposition (23, 24, 27, 30, 31). Recent *in vitro* biochemical studies have demonstrated isoform-specific interactions of apoE with Aβ that might account for greater aggregation of Aβ with apoE4 isoform *in vivo* (30, 31). apoE immunolocalization in our cases confirms previous studies of AD that suggest that apoE is present in cells, cerebral vessels, and plaques, where it could interact with either APP or fragments of APP such as Aβ (23, 28, 29). In normal human brain and nervous tissue, apoE is localized in astrocytes and microglial cells (36). In brains of patients with AD as well as other amyloidoses, apoE immunoreactivity is also observed in vascular endothelial cells, neurons, and neuritic processes and associated with neurofibrillary tangles (23, 28, 29).

Routine neuropathological diagnosis of AD relies on silver impregnation techniques and not on direct detection of amyloid to visualize plaques containing neuritic processes and neurons with neurofibrillary tangles (1, 2, 35). It is important to note, therefore, that the relationship between *APOE* genotype and Aβ deposition was also reflected in routine neuritic plaque counts taken from the neuropathology reports of our cases (Table 1). In a subset of patients, immunocytochemical identification of neuritic plaques supported the findings in the diagnostic autopsy series. Thus, observed variation in an independently measured index of extracellular pathological feature of AD (plaques identified by neurite content) may also be closely associated with *APOE* genotype.

In contrast to the strong association of *APOE* genotype with extent of extracellular amyloid and plaque pathology of cerebral cortex (Aβ deposition in vessels and plaques and neuritic plaque density), the number of neurofibrillary tangles at the time of death may be less dependent on *APOE* genotype. There were no statistically significant differences in the numbers of neurofibrillary tangles in several cortical regions among *APOE3/3*, *-3/4*, and *-4/4* genotypes. The literature suggests that neurofibrillary tangles are endpoints of neuronal injury in a variety of neurodegenerative illnesses (37–39). The counts in the various cortical regions examined correlated strongly with duration of illness ($P < 0.01$) in the patients in this study.

APOE allele ε4 (*APOE4*) is a proposed susceptibility gene or biological risk factor in late-onset familial and sporadic AD (23, 24). The association of *APOE4* with a distinct neuropathological phenotype is consistent with the proposed role of apoE4 as an autosomal codominant genetic trait in late-onset AD (24, 25). APP, Aβ, and other proteins must all be considered as possible substrates for the effect of apoE genotype on susceptibility to AD. Other genetic and environmental factors may also be discovered for late-onset AD.

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1. Khachaturian, Z. S. (1985) *Arch. Neurol.* **52,** 1097–1105.
2. Mirra, S. S., Heyman, A., McKeel, S., Sumi, S. M., Crain, B. J., Brownlee, L. M., Vogel, F. S., Hughes, J. P., van Belle, G. & Berg, L. (1991) *Neurology* **41,** 479–486.
3. Selkoe, D. J., Bell, D. S., Podlisny, M. B., Price, D. L. & Cork, L. C. (1987) *Science* **235,** 873–877.
4. Allsop, D., Landau, M., Kidd, M., Lowe, J. S., Reynolds, G. P. & Gardner, A. (1986) *Neurosci. Lett.* **68,** 252–256.
5. Ikeda, S., Allsop, D. & Glenner, G. G. (1989) *Prog. Clin. Biol. Res.* **317,** 313–323.
6. Hyman, B. T., Tanzi, R. E., Marzloff, K., Barbour, R. & Schenk, D. (1992) *J. Neuropathol. Exp. Neurol.* **51,** 76–83.
7. Goldgaber, D., Lerman, M. I., McBride, O. W., Saffioti, U. & Gajdusek, D. C. (1987) *Science* **235,** 877–880.
8. Tanzi, R. E., Gusella, J. F., Watkins, P. C., Bruns, G. A., St. George-Hyslop, P., Van Keuren, M. L., Patterson, D., Pagan, S., Kurnit, D. M. & Neve, R. L. (1987) *Science* **235,** 880–884.
9. Kang, J., Lemaire, H. G., Unterbeck, A., Salbaum, J. M., Masters, C. L., Grzeschik, K. H., Multhaup, G., Beyreuther, K. & Muller-Hill, B. (1987) *Nature (London)* **325,** 733–736.
10. Oltersdorf, T., Fritz, L. C., Schenk, D. B., Lieberburg, I., Johnson-Wood, K. L., Beattie, E. C., Ward, P. J., Blacher, R. W., Dovery, H. F. & Sinha, S. (1989) *Nature (London)* **341,** 144–147.
11. Van Nostrand, W. E., Wagner, S. L., Suzuki, M., Choi, B. H., Farrow, J. S., Geddes, J. W., Cotman, C. W. & Cunningham, D. D. (1989) *Nature (London)* **341,** 546–549.
12. Goate, A., Chartier-Harlin, M.-C., Mullan, M., Brown, J., Crawford, F., Fidani, L., Giuffra, L., Haynes, A., Irving, N., James, L., Mant, R., Newton, P., Rooke, K., Roques, P., Talbot, C., Pericak-Vance, M., Roses, A., Williamson, R., Rossor, M., Owen, M. & Hardy, J. (1991) *Nature (London)* **349,** 704–706.
13. Chartier-Harlin, M.-C., Crawford, F., Houlden, H., Warren, A., Hughes, D., Fidani, L., Goate, A., Rossor, M., Roques, P., Hardy, J. & Mullan, M. (1991) *Nature (London)* **353,** 844–846.
14. Murrell, J., Farlow, M., Ghetti, B. & Benson, M. D. (1991) *Science* **254,** 97–99.
15. Hendriks, L., van Duijn, C. M., Cras, P., Cruts, M., van Hul, W., van Harskamp, F., Warren, A., McInnis, M. G., Antonarakis, S. F., Martin, J.-J., Hofman, A. & van Broeckhoven, C. (1992) *Nat. Genet.* **1,** 218–221.
16. Mullan, M., Crawford, F., Axelman, K., Houlden, H., Lilius, L., Winblad, B. & Lannfelt, L. (1992) *Nat. Genet.* **1,** 345–347.
17. Pericak-Vance, M. A., Yamaoka, L. H., Haynes, C. S., Speer, M. C., Haines, J. L., Gaskell, P. C., Jr., Hung, W.-Y., Clark, C. M., Heyman, A. L., Trofatter, J. A., Eisenmenger, J. P., Gilbert, J. R., Lee, J. E., Alberts, M. J., Dawson, D. V., Bartlett, R. J., Earl, N. J., Siddique, T., Vance, J. M., Conneally, P. M. & Roses, A. D. (1988) *Exp. Neurol.* **102,** 271–279.
18. Pericak-Vance, M. A., Bebout, J. L., Gaskell, P. C., Jr., Yamaoka, L., Hung, W.-Y., Alberts, M. J., Walker, A. P., Bartlett, R. J., Hanes, C. A., Welsh, K. A., Earl, N. L., Heyman, A., Clark, C. M. & Roses, A. D. (1991) *Am. J. Hum. Genet.* **48,** 1034–1050.
19. Scholz, W. (1938) *Z. Gesamte Neurol. Psychiatr.* **162,** 694–715.
20. Mandybur, T. I. (1975) *Neurology* **25,** 120–126.
21. Glenner, G. G. (1981) *Ann. Pathol.* **1,** 105–108.
22. Joachim, C. L., Morris, J. H. & Selkoe, D. J. (1988) *Ann. Neurol.* **24,** 50–56.
23. Strittmatter, W. J., Saunders, A. M., Schmechel, D., Pericak-Vance, M., Enghild, J., Salvesen, G. & Roses, A. D. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 1977–1981.
24. Saunders, A. M., Strittmatter, W. J., Schmechel, D., St. George-Hyslop, P. H., Pericak-Vance, M. A., Joo, S. H., Rosi, B. L., Gusella, J. F., Crapper-McLachlan, D. R., Alberts, M. J., Hulette, C., Crain, B., Goldgaber, D. & Roses, A. D. (1993) *Neurology* **43,** 1467–1472.
25. Corder, E., Saunders, A. M., Strittmatter, W. J., Schmechel, D. E., Gaskell, P. C., Small, G. W., Roses, A. D., Haines, J. L. & Pericak-Vance, M. A. (1993) *Science* **261,** 921–923.
26. Gelman, B. B., Goodrum, J. & Bouldin, T. W. (1991) *J. Neuropathol. Exp. Neurol.* **50,** 383–407.
27. Wisniewski, T. & Frangione, B. (1992) *Neurosci. Lett.* **135,** 235–238.
28. Diedrich, J. F., Minnigan, H., Carp, R. I., Whitaker, J. N., Race, R., Frey, W., II, & Haase, A. T. (1991) *J. Virol.* **65,** 4759–4768.
29. Namba, Y., Tomonaga, M., Kawasaki, H., Otomo, E. & Ikeda, K. (1991) *Brain Res.* **541,** 163–166.
30. Strittmatter, W. J., Weisgraber, K. H., Huang, D. Y., Dong, L.-M., Salvesen, G. S., Pericak-Vance, M., Schmechel, D., Saunders, A. M., Goldgaber, D. & Roses, A. D. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 8098–8102.
31. Wisniewski, T., Golabek, A., Matsubara, E., Ghiso, J. & Frangione, B. (1993) *Biochem. Biophys. Res. Commun.* **192,** 359–365.
32. Wenham, P. R., Price, W. H. & Blundell, G. (1991) *Lancet* **337,** 1158–1159.
33. Hixson, J. E. & Vernier, D. T. (1990) *J. Lipid Res.* **31,** 545–548.
34. Lloyd, B., Brinn, N. & Burger, P. C. (1985) *J. Histotech.* **8,** 155–156.
35. Tomlinson, B. E. & Corsellis, J. A. N. (1984) in *Greenfield's Neuropathology, 4th Edition*, eds. Adams, J. H., Corsellis, J. A. N. & Duclen, L. W. (Wiley, New York), pp. 951–981.
36. Murakami, M., Ushio, Y., Morino, Y., Ohta, J. & Matsukado, Y. (1989) *J. Clin. Invest.* **82,** 177–188.
37. Vallet, P. G., Gunterm, R., Hof, P. R., Golaz, J., Delacourte, A., Robakis, N. K. & Bouras, C. (1992) *Acta Neuropathol.* **83,** 170–178.
38. Terry, R. D., Masliah, E., Salmon, D. P., Butters, N., DeTeresa, R., Hill, R., Hansen, L. A. & Katzman, R. (1991) *Ann. Neurol.* **30,** 572–580.
39. DeKosky, S. T. & Scheff, S. W. (1990) *Ann. Neurol.* **27,** 457–464.