American Journal of Pathology, Vol. 148, No. 6, June 1996

Apolipoprotein E-ε4 Alleles in Cerebral Amyloid Angiopathy and Cerebrovascular Pathology Associated with Alzheimer's Disease

Daniel R. D. Premkumar, Dawn L. Cohen, Peter Hedera, Robert P. Friedland, and Rajesh N. Kalaria

From the Departments of Neurology, Neurosciences, and Pathology, Case Western Reserve University, Cleveland, Ohio

The presence of apolipoprotein E-ε4 (APOE-ε4) allele has been implicated as a risk factor for Alzheimer's disease (AD). We examined the frequencies of APOE-ε4 alleles in age-matched controls and subgroups of 190 AD subjects exhibiting cerebral amyloid angiopathy (CAA) and other frequently associated lesions. CAA was evident in 96% of the AD subjects, which were divided into two groups, one bearing mild or no apparent CAA and the other with moderate to severe CAA. APOE-ε4 allele frequency (48%) in the latter advanced CAA group was six times higher than in those who exhibited mild CAA. In the advanced CAA subjects, the occurrence of an ε4 allele was increased by a factor of 17 (95% confidence interval, 7.56 to 38.9). This was despite the fact that neocortical amyloid-β plaque densities in the two groups were similar and that all of the AD subjects had met the accepted neuropathological criteria. We also observed that the degree of CAA severity was greatest in the group of subjects with the ε4/ε4 genotype. The association between CAA and APOE-ε4 was further implicated in two non-AD subjects among neurological controls with severe CAA. These two subjects, both homozygous for the APOE-ε4 allele, were primarily diagnosed as having Creutzfeldt-Jakob disease and Pick's disease in the absence of significant neocortical amyloid deposition. Allele frequency comparisons between neurological control subjects with CAA and those without likewise accorded a strong relationship between the APOE-ε4 allele and the presence of CAA. More remarkably, the ε4 allele frequency was highly associated with AD subjects exhibiting lobar or intracerebral hemorrhage, all of whom had advanced CAA. We observed that 36% of the AD subjects had concomitant cerebrovascular pathology resulting from single infarcts, multiple microinfarcts, ischemic white matter lesions, or petechial hemorrhages. Although the difference in APOE genotype distribution between subjects with and without cerebrovascular lesions did not reach statistical significance, we did note that the frequency of the ε4 allele was significantly higher in subjects with such pathology as compared with those without. However, we found no evidence to suggest that the acquisition of an APOE-ε4 allele or one of the alleles, ε2 or ε3, was a factor in the occurrence of atherosclerosis localized in the basal surface arteries. Analyses of our sample also confirm that there was a lower frequency of the APOE-ε2 allele in AD subjects and that the frequency of the ε4 allele in AD subjects with concomitant diffuse Lewy body disease was intermediate between controls and AD subjects. Our results suggest that the APOE-ε4 allele is a significant factor in the development of CAA in AD and reveal the possibility that APOE is an independent factor in CAA and other vascular abnormalities associated with AD. (Am J Pathol 1996, 148:2083–2095)

It is now clear that a number of proteins or factors are associated with amyloid-β (Aβ) deposits and the neurofibrillary lesions in Alzheimer's disease (AD). Some of these include the heparan sulfate glycosaminoglycans, amyloid P component, complement

Supported by National Institutes of Health grants RO1AG10030, POAG08012B2, and R5AG08992A2 and a grant from Philip Morris, Inc.

Accepted for publication February 12, 1996.

Address reprint requests to Dr. R. N. Kalaria, Department of Neurology (BRB5), Case Western Reserve University, 10900 Euclid Avenue, Cleveland, Ohio 44106-4938.

Table 1. *Distribution of* APOE *Genotypes in AD Subjects and Normal and Non-AD Controls*

Groups	n	Age (years)	Brain weight (g)	*APOE* genotypes					χ^2 statistics*	
				ε2/ε3	ε2/ε4	ε3/ε3	ε3/ε4	ε4/ε4	χ^2	*P* value
Normal controls (9 M/7 F)	16	70 ± 1	1239 ± 55	5 (31%)	2 (12%)	6 (38%)	3 (19%)	0 (0%)		
Neurological controls (17 M/17 F)	34	73 ± 2	1197 ± 35	3 (9%)	2 (6%)	16 (47%)	11 (32%)	2 (6%)	5.91	0.207
All AD (82 M/108 F)	190	79 ± 1	1136 ± 13	9 (5%)	0 (0%)	68 (36%)	89 (46%)	24 (13%)	43.7 15.5	0.000 0.003

Values show mean ± SEM and number (n) of subjects. Brain weights between normal controls and AD subjects were significantly different at $P < 0.03$. There were no subjects with ε2/ε2 genotype. The frequency of *APOE* genotypes of the total pool in men was similar to that in women ($\chi^2 = 1.18$, $P = 0.882$). The mean (± SEM) age of men was 77 ± 1 years and that of women was 79 ± 1 years. Neurological controls represent non-AD cases including amyotrophic lateral sclerosis, corticobasal ganglionic degeneration, Creutzfeldt-Jakob disease, dementia with indistinctive pathology, hereditary dysphasic dementia, Huntington's disease, Parkinson's disease, Pick's disease, subcortical gliosis, glioblastoma multiforme, and cerebral ischemic disease with Binswanger's. M, male; F, female.

*Pearson's χ^2 test *versus* normal controls (upper numbers) and neurological controls (lower numbers, where shown).

proteins, serine protease inhibitors, and recently the apolipoproteins.[1–3] The precise significance of the associated factors is unknown, but among these apolipoprotein E (*APOE,* gene; apoE, protein) is being intensively studied in the central nervous system.[4] ApoE is a polymorphic protein that plays a central part in the metabolism of cholesterol and the triglycerides. In the human body, the synthesis of apoE is controlled by a single gene locus on chromosome 19 that determines three co-dominant alleles, ε2, ε3, and ε4. The polymorphism results in six apoE phenotypes, E2/2, E3/3, and E4/4 as homozygotes and E2/3, E3/4, and E2/4 as heterozygotes. Specific *APOE* alleles were first implicated in atherosclerosis.[5] Recent studies have shown the *APOE*-ε4 allele to be highly associated with late-onset familial and sporadic AD.[6–8] The frequency of the *APOE*-ε4 allele in AD from living and autopsy series was reported to be between 30 and 40%, which is approximately three times that in the general population[6] and other neurological disorders.[9–12] However, some studies[13–19] have variably implicated an increased inheritance of the *APOE*-ε4 allele in other amyloid-forming disorders[13–17] besides vascular dementia.[18,19] For example, the frequency of the *APOE*-ε4 allele has been reported to be comparable to or higher than that of AD subjects in diffuse Lewy body disease (DLBD)[13,15,16] and Creutzfeldt-Jakob disease.[17] Interestingly, the ε4 allele frequency was also found to be increased in subjects who had accrued brain Aβ deposits subsequent to head injury.[20] These foregoing studies implicate an important functional role for apoE isoforms in the pathophysiology of AD. ApoE may influence the accumulation of brain amyloid including that in the cerebral vasculature.[21]

Vascular amyloid deposition resulting in cerebral amyloid angiopathy (CAA) is a prominent feature of AD.[22,23] CAA differs from generalized amyloidosis because of its almost exclusive cerebral distribution involving large and small leptomeningeal as well as intraparenchymal microvessels.[24] Although CAA appears to be most severe in AD, it sometimes occurs in the normal elderly[23] and is the principal feature of some rare hereditary disorders linked to mutations in genes of proteins deposited in the vascular wall.[22] Advanced CAA may often lead to lobar or intracerebral hemorrhages.[22–24] However, the factors responsible for such vascular amyloid accumulation are unclear.

In this study, we assessed the inheritance of the *APOE* genotype as a factor for developing CAA associated with AD. We specifically examined the frequencies of the *APOE*-ε4 allele in AD subjects exhibiting advanced CAA and intracerebral hemorrhage in comparison with controls and AD subjects with mild or negligible CAA. In an attempt to delineate the vascular pathology[25–27] often associated with AD, we also assessed *APOE* genotypes in subgroups of AD subjects with and without cerebrovascular lesions.

Materials and Methods

Subjects and Brain Tissue

Human brain tissue from a total of 240 subjects with either Alzheimer type of dementia, no neuropathological diagnosis (normal controls), or other neurological diseases (neurological controls) was obtained at autopsy immediately upon removal within 2 to 7 hours after death (age range, 55 to 90 years). Tissues were either frozen or used fresh for microvessel isolation. Table 1 gives details of subject age, sex, brain weight, and the disorder at death. Autopsies were performed between 1988 and 1995 at the Institute of Pathology, Case Western Reserve University and University Hospitals. In this largely

retrospective study, although precise clinical information on duration and magnitude of illness was not accessible, the majority of subjects (cases), both controls and AD, had died from a similar cause, which was bronchopneumonia, unless otherwise mentioned. The probable clinical diagnosis of AD was confirmed by histological examination that revealed the presence of established hallmarks in the four cortical lobes including neuritic plaques and neurofibrillary tangles and granulovacuolar degeneration in the hippocampus.[28] In addition, whereas all cases diagnosed as AD generally met the criteria of the consortium to establish a registry for AD (CERAD) for probable AD, more than 95% were definite AD.[29] These criteria for the pathological diagnosis of AD were used irrespective of the presence of CAA and other related lesions. To study the influence of *APOE* genotype on co-existing neuropathological lesions, AD cases were divided into four subgroups as follows: AD with CAA, AD with CAA-related intracerebral hemorrhage, AD with cerebrovascular lesions (CVLs), and AD with co-existent DLBD.

To analyze effects on CAA, the 190 AD subjects were divided into two subgroups, one consisting of 55 cases with minimal or no CAA (group A) and the other (135 cases) limited to only moderate to severe CAA (group B). The CVL subgroup with 69 AD cases exhibiting at least one CVL in the form of a remote or recent infarct, multiple microinfarcts, white matter lucencies, leukoariosis, or petechial or hemorrhagic infarcts was also noted and analyzed against those without such lesions. These designations were made irrespective of any history of cardiovascular disease or hypertension.[16] We also compared a group of 13 AD subjects as intracerebral hemorrhage (CH) cases characterized by lobar, cortical, or subcortical with remote or recurrent bleeds. All of these CH cases were found to have moderate to severe CAA. There were 35 AD subjects who exhibited diffuse cortical Lewy bodies and were classed as AD subjects with concomitant DLBD. In this group, we did not encounter any pure DLBD cases in the absence of cerebral amyloid or neurofibrillary tangles.[15] However, we did examine the influence of *APOE* genotype on CAA in the AD subjects with DLBD, 19 (58%) of whom had moderate to severe CAA.[30]

Unless otherwise stated, all control subjects diagnosed without AD were analyzed as two separate groups comprising normal controls and neurological controls. The 16 normal control subjects had neither apparent clinical nor any gross or histopathological evidence of AD. None of the normal controls exhibited CAA or CVL. Thirty-four patients came to autopsy with a clinical diagnosis of questionable dementia. Upon careful histopathological examination,[28] these did not fulfill any of the established criteria for a diagnosis of AD.[29] Notwithstanding, 9 of these controls were found to have mild to moderate CAA and 2 others exhibited severe CAA. The 34 subjects were assigned as neurological controls and given the following final neuropathological diagnosis (number of cases): amyotrophic lateral sclerosis (1), corticobasal ganglionic degeneration (2), Creutzfeldt-Jakob disease (2), DLBD (1), frontal lobe dementia (4), glioblastoma multiforme and metastasis cases (3), hereditary dysphasic dementia (1), hereditary sensory neuropathy (1), Huntington's disease (1), cerebral ischemic disease including multi-infarct dementia and Binswanger's (6), Parkinson's disease (3), Pick's disease (1), subcortical gliosis (1), and dementia with indistinctive pathology including gliosis (7).

To confirm that the degree of CAA was compatible with deposition of Aβ protein, cerebral microvessels with and without smooth muscle were isolated from the frontal and occipital lobes as described previously.[31] Aliquots of purified vessel fractions were spotted on polylysine-coated glass slides and prepared for staining to assess vascular amyloid deposition.[32]

Tissue Staining and Immunocytochemical Analyses

In addition to the conventional histopathological stains such as Congo Red, patterns of vascular amyloid deposition and degree of CAA were examined by immunostaining[33,34] with antibodies to Aβ protein (4G8 and 3160 from K. S. Kim, New York State Brain Research Institute, and S. Younkin, Case Western Reserve University, respectively) and apoE (Biodesign, Bar Harbor, ME) or by thioflavin S staining in 20- to 25-μm neocortical tissue sections and in isolated vessel fractions. Tissues from the occipital and frontal cortex of a randomly selected sample of 70 AD subjects were examined in this manner.

Assessment of CAA, Cerebrovascular Lesions, and Aβ Protein Plaque Density

CAA was graded by examination of conventionally stained neocortical tissue sections from the four lobes, frontal (Brodmann 10 or 32), temporal (areas 20 to 22), parietal (areas 39 and 40), and occipital (areas 17 or 18). Briefly, at least two sections, 2 to 3 cm in length, with complete neocortical ribbon from the four cortical areas were examined and graded for CAA severity similar to the procedure described

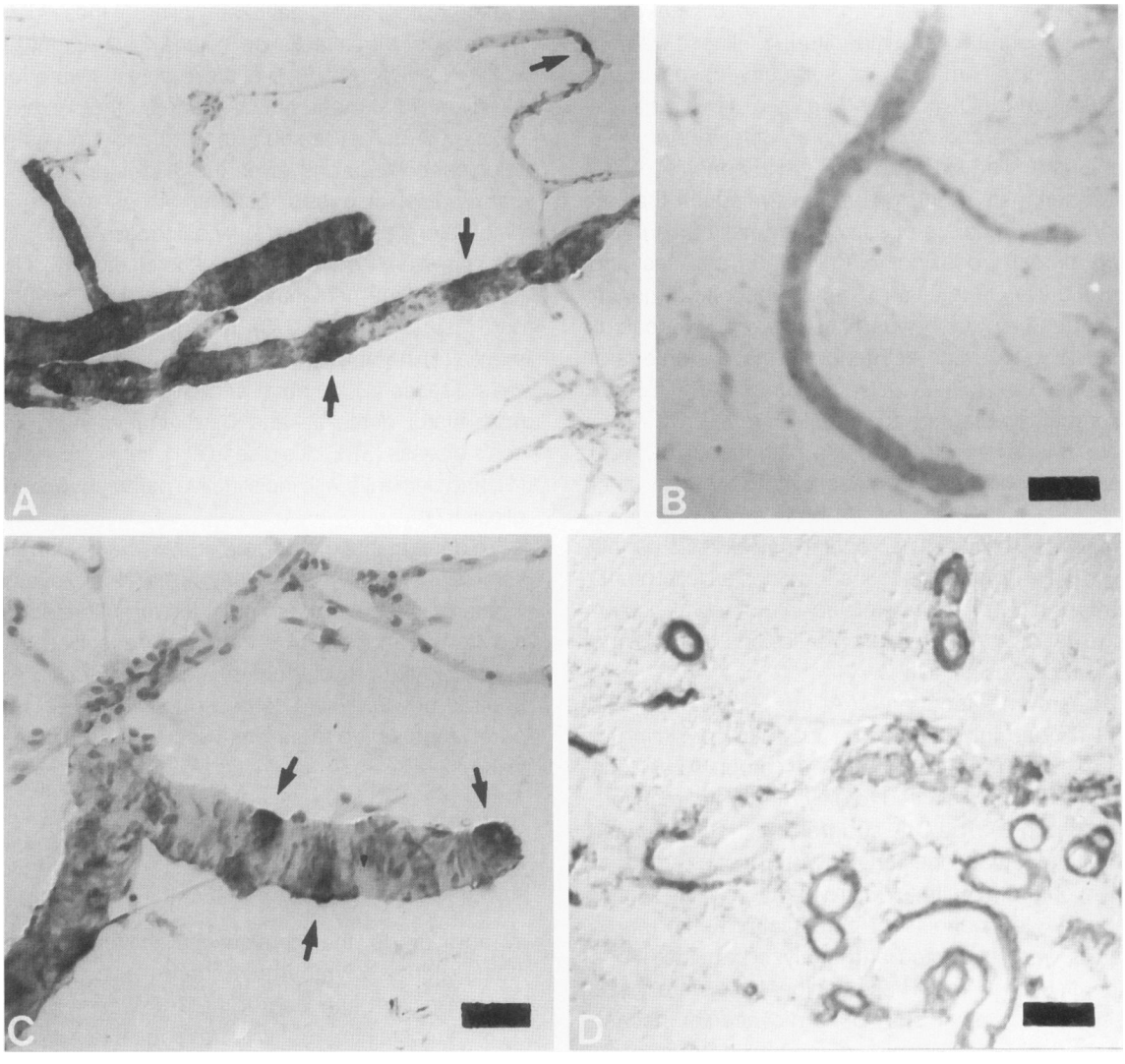

Figure 1. *Immunocytochemical localization of Aβ protein in cerebral vessels of AD subjects matched for age and duration of disease.* **A:** *Intensely staining concentric patterns and focal deposits of Aβ in isolated intracortical microvessels from a 65-year-old AD subject with ε4/ε4 genotype. Note deposition along whole vessel length and fine deposits of Aβ in capillaries* (**arrows**). **B:** *Control subject with ε2/ε3 genotype showing lack of specific immunoreactivity. Preparations immunostained with preimmune serum gave similar background staining.* **C:** *Aβ immunoreactive focal deposits* (**arrows**) *in a microvessel from a 69-year-old AD subject with ε3/ε3 genotype indicating minimal CAA.* **D:** *Severe CAA (grade 3) represented by Aβ immunoreactivity in meningeal and intracortical vessels. Occipital cortex tissue section from an AD subject with ε3/ε4 genotype. Specific staining was not evident either in tissue from age-matched controls with no CAA, when tissue from AD subjects was incubated in the absence of antibody, or in the presence of preimmune serum or antibody (3160) absorbed with Aβ peptide. Nuclei are revealed by hematoxylin counterstain; bars, 50 μm* (**A**, **B**, *and* **C**) *and 20 μm* (**D**).

previously.[35] Cerebral vessels without amyloid staining were graded 0. Those with focal or few streaks of amyloid in media/adventitia as mild or negligible (score = 1), those with greater replacement of media/adventitia with amyloid as moderate (score = 2), and those with complete infiltration of vascular wall[36] and >10 amyloid laden vessels per section as severe (score = 3). An average score for each case was obtained by examination of the four areas in this manner independent of any knowledge of the *APOE* status of the samples.

Verification of the presence of CAA with Aβ protein was obtained by examination of immunostained isolated vessel preparations and tissue sections from 70 subjects (Figure 1). Briefly, at least two aliquots of isolated vessel fractions from each region per case were immunostained and examined by light microscopy. The average numbers of Aβ-laden vessel profiles in four to five fields per spotted aliquot were determined and assigned a score from 1 to 3. These were evaluated by two independent investigators (D. L. Cohen and R. N. Kalaria), and the information derived from these cases was used to construct the results presented in Figure 2. There was almost complete concordance between the two methods used for evaluation of CAA.[35,36] Attempts to quantify the

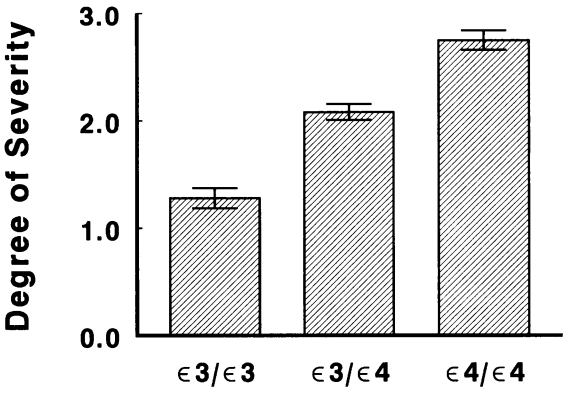

Figure 2. *APOE genotypes in CAA associated with AD. Increased degree of CAA in AD subjects bearing the ε4/ε4 genotype. One-way ANOVA* (P = *0.000) and nonparametric comparisons (Kruskal-Wallis test) showed the degree of CAA severity in subjects with ε3/ε3 genotype was significantly lower* (P = *0.000) compared with the group with ε3/ε4 genotype and that the degree was lower in the ε3/ε4 subjects compared with the ε4/ε4 subjects* (P = *0.001). The degree of CAA (<1.00) in the ε2/ε3 and ε2/ε4 groups was not different from the ε3/ε3 subjects (not shown). Furthermore,* APOE *genotype frequency distributions in the groups with minimal, moderate, and severe CAA were different* (χ^2 = *91.7, P = 0.000, 12 df). The frequencies of the APOE-ε4 allele was also significantly related to the degree of CAA* ($\chi 2$ = *61.1, P = 0.000).*

amounts of Aβ by immunoblotting did not yield reproducible results. This was probably due to frequent aggregation, relative abundance, and variability of solubilized protein in vessel fractions from AD subjects compared with controls. CAA in the cerebral hemorrhage cases was characterized as moderate (grade 2) or severe (grade 3).

The AD cases with CVLs were evaluated by examination of the postmortem records and verified against the original stained tissue sections. The degree of atherosclerosis in the cerebral and communicating arteries forming the circle of Willis at the basal surface was evaluated in each brain at the time of autopsy and graded as none (score = 0), minimal (1), moderate (2) or severe (3). Most of this information was obtained directly from the records (R. N. Kalaria), and only in a few severe cases with atherosclerosis could the grading be verified by examination of available tissue sections.

To assess cortical amyloid load in AD cases with and without CAA, tissue sections from the frontal and occipital cortex immunostained for Aβ (above) were used to determine plaque density by a semi-automated method (developed by P. Hedera). Briefly, immunostained fields containing Aβ plaques were projected onto a video screen and the lesions were counted using computer-aided digitization of the video image.[34] Aβ-positive plaques in five different cortical ribbon fields per section were counted. Immunostained artifacts were avoided by simultaneous manual control of the slide under the microscope.[34] Plaque counts from each field were averaged for each section as well as cortical area per subject.

APOE Genotyping

Genomic DNA was extracted from frozen brain tissue by standard methods.[37] *APOE* genotyping was performed essentially as described previously[38] with slight modification. The *APOE* genotyping was also performed without knowing the pathological classification of the brain samples. For the polymerase chain reaction, 200 ng of genomic DNA was added to a 25-μl reaction mixture containing 25 pmol of sense (5′ TCC AAG GAG CTG CAG GCG GCG CA 3′) and antisense (5′ ACA GAA TTC GCC CCG GCC TGG TAC ACT GCC A 3′) primers, 2.5 μl of dimethylsulfoxide, 10 mmol/L Tris-HCl buffer, pH 8.3, 50 mmol/L KCl, 2 mmol/L $MgCl_2$, and 0.5 U of *Taq* DNA polymerase (Perkin-Elmer-Cetus, Norwalk, CT). The DNA was amplified for 35 cycles as follows: denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 60 seconds. The amplified product was then digested with 20 U of cfo1 (GIBCO BRL, Gaithersburg, MD) for at least 5 hours at 37°C and subjected to electrophoresis on 4% Metaphor (FMC Bioproducts, Rockland, ME) agarose gel against a 10-bp ladder (GIBCO BRL) as marker. The gels were viewed and photographed under ultraviolet light after staining with ethidium bromide. To ensure internal consistency of the genotyping procedure and correct assignment, 30 samples selected at random from the total pool of 240 were reanalyzed on two different occasions. Such analyses revealed 100% internal consistency of the assays.

Statistical Analysis

Comparisons of the distribution of genotypes and allele frequencies in the various groups were made by using the Pearson's χ^2 and Fisher's exact tests. Genotype distributions were computed on the basis that they did not depart from the Hardy-Weinberg equilibrium. Allele frequencies presented in the three tables were determined by direct count from the genotype distribution. Unless otherwise stated, allele frequency comparisons between groups for each of the three alleles were made in the absence and presence of at least one *APOE* allele. The unadjusted odds ratios and 95% confidence intervals were computed from frequency distributions of subjects with at least one *APOE*-ε4 allele and subjects without the allele. One-way analysis of variance (ANOVA) or the Student's two-tailed *t*-test was used to compare the

Table 2. APOE *Allele Frequencies in Control Subgroups*

Category (alleles)	*APOE* allele frequencies						
	ε2	ε3	ε4	χ^2 test* (for ε2)	*P* value	χ^2 test* (for ε4)	*P* value
Normal controls (32)	22%	63%	15%				
Neurological controls (68)	7%†	68%	25%	5.03	0.025	0.75	0.386
All AD (380)	2%†	62%	36%†	31.4	0.000	4.80‡	0.028
				4.89	0.027	2.78	0.096
Controls (70)§	11%	78%	10%	Rebeck et al[8]			
Controls (234)	8%	83%	9%	West et al[39]			
Controls (22)	5%	85%	10%	Hardy et al[10]			
Controls (116)	9%	77%	15%	Harrington et al[16]			

*χ^2 values (with continuity correction) for frequencies *versus* normal controls (upper figures) or *versus* neurological controls (lower numbers).
†Statistically different frequencies compared with normal controls.
‡The odds ratio for this group with one *APOE*-ε4 allele was 3.23 (95% confidence interval, 1.07 to 9.66).
§*APOE* allele frequencies in autopsy controls with and without cardiovascular disease were taken from previous reports for comparison. For neurological controls, see Refs. 12 and 13.

degree of pathological lesions, ie, severity of CAA and atherosclerosis as numerical variables, between each of the allele subgroups. Significance values of non-normal variables was verified using the non-parametric Kruskal-Wallis test. All of the data were analyzed using SPSS (Chicago, IL) software package for Windows. For comparison and further consistency, *APOE* allele frequencies of non-AD control subjects from four different previous autopsy studies[8,11,15,39] were calculated and also presented (Table 2).

Results

APOE Genotypes in Control and AD Subjects

We assessed the *APOE* genotypes and allele frequencies in 240 age-matched subjects who had come to autopsy through our AD research program; 50 of the subjects were diagnosed as having no AD and categorized as controls including normal and neurological controls (Table 1). There were no subjects with the rare ε2/ε2 genotype. The ε3/ε3 genotype was most common in the control groups whereas ε3/ε4 was common among AD subjects. We did not find differences in the *APOE* genotype frequencies between men and women in either the controls or the AD group ($P > 0.80$). Although we noted that frequencies of the *APOE* genotypes were not significantly different between the two control subgroups (Table 1), they were always compared separately with the various AD subgroups (see Tables 3 to 5). *APOE* genotype frequencies in the 190 AD subjects were distinctly different from those in normal controls and when compared with the neurological controls (Table 1). Although there were no subjects homozygous for the *APOE*-ε4 allele in the normal control group, there were, however, 2 subjects among the neurological controls diagnosed with Creutzfeldt-Jakob disease and Pick's disease (Table 1). Both of these exhibited severe CAA and had only sparse diffuse plaques, which was not sufficient to warrant a diagnosis of AD.[29] The frequencies of *APOE* genotypes in all of the controls compared as a single group were also significantly different from that of the 190 AD subjects (not shown).

Table 2 gives the three *APOE* allele frequencies in the control and AD groups. We found that the *APOE*-ε4 allele frequency (15%) in the normal controls was comparable to previously published studies.[8,10,16,39] In these studies, autopsy-confirmed AD subjects were assessed and the reported frequencies for normal controls were between 9 and 15% (Table 2). However, this frequency was not significantly different from the allele frequency (25%) in neurological controls (Table 2). The frequency of the *APOE*-ε4 allele in the neurological controls was also comparable to previously reported high frequencies in such controls.[16,17,40] As expected, the frequency of the *APOE*-ε4 allele was appropriately over-represented in the AD subjects compared with normal controls (Table 2). Whereas the allele frequencies in the neurological controls and the AD subjects were not statistically different by χ^2 analyses (Table 2), comparison of the three groups by Kruskal-Wallis one-way ANOVA suggested differences in the gene occurrence ($P < 0.02$). The relatively high frequency of the *APOE*-ε4 allele among the neurological controls reflected the large number of subjects that carried the ε3/ε4 genotype but had insufficient evidence to be diagnosed with AD. In contrast, the *APOE*-ε2 allele frequencies in the AD subjects and in the neurological controls were profoundly lower (more than threefold) than those in normal controls. The frequency of this allele, however, was comparably

Table 3. *Distribution of* APOE *Genotypes in CAA*

Groups	n	*APOE* genotypes					χ^2 statistics*	
		ε2/ε3	ε2/ε4	ε3/ε3	ε3/ε4	ε4/ε4	χ^2	*P* value
Normal controls	16	5 (31%)	2 (31%)	6 (38%)	3 (19%)	0 (0%)		
AD with CAA	182	7 (4%)	0 (0%)	63 (35%)	88 (48%)	24 (13%)	45.9	0.000
CAA group A (no to minimal CAA)†	55	6 (11%)	0 (0%)	40 (73%)	9 (16%)	0 (0%)	12.9	0.005
CAA group B (moderate to severe)†	135	3 (2%)	0 (0%)	28 (21%)	80 (59%)	24 (18%)	48.5 60.9	0.000 0.000
AD with cerebral hemorrhage (CH)	13	0 (0%)	0 (0%)	1 (7%)	8 (62%)	4 (31%)	16.7 8.00	0.002 0.046
Neurological controls with no CAA	23	3 (13%)	1 (4%)	14 (61%)	5 (22%)	0 (0%)		
Neurological controls with CAA	11	0 (0%)	1 (9%)	2 (18%)	6 (55%)	2 (18%)	11.3	0.024

Normal controls are from Table 1.
*χ^2 values *versus* normal or neurological controls without CAA (single or upper numbers) and *versus* group A or AD group without CH (lower numbers).
†Density of amyloid β plaques were similar between CAA group A and group B.

greater in our normal controls than in those reported previously (Table 2).

CAA in AD and the APOE *Genotypes*

Of all the AD subjects, 182 (96% of cases) exhibited CAA to some degree (Table 3). The degree of CAA varied from single or focal deposits in the meningeal vessels to profound infiltration of Aβ amyloid and accompanying fibrosis in walls of meningeal vessels as well as intracortical arterioles and precapillaries. We also noted many cases with dyshoric angiopathy, which was characterized by diffuse Aβ deposits around intraparenchymal microvessels including arterioles and capillaries. CAA was generally evident in all four lobes but particularly prominent in the occipital cortex. Cortical vessels in the sulcal regions were more often involved than those in the gyral regions. The surface vessels of cerebellum were often involved in the severe cases. We also used isolated vessel preparations to corroborate and correlate *APOE* genotype with the degree of CAA in tissue sections (Figure 1). These assessments provided a method to better define CAA in intracortical vessels as the isolation procedure largely excluded the meningeal and surface vessels that were stripped away before fractionation.[31] Intense Aβ immunoreactivity in isolated microvessel preparations from *APOE*-ε4 allele bearers was often observed along the vessel length (Figure 1A) and could be distinguished from focal sporadic deposits (Figure 1C). Focal deposits observed in isolated preparations (Figure 1C) usually could not be readily discerned in tissue sections. However, these observations in isolated preparations from 70 individual subjects were compatible with and substantiated the observations made in tissue sections (Figure 1D). We also found that apoE protein immunoreactivity in isolated microvessel preparations correlated with Aβ deposits (not shown).

The frequencies of *APOE* genotypes and alleles in the 182 AD subjects with CAA were significantly greater than that in normal controls (Tables 3 and 4) or neurological controls (Table 5). The genotype distributions readily indicated that CAA was common in AD subjects who were homozygous or heterozygous for the *APOE*-ε4 allele (Table 4). Indeed, when we assessed the severity of CAA by the *APOE* genotypes, we found that the ε4/ε4-bearing subjects exhibited the most severe CAA (Figure 2). Conversely, significantly higher frequency of the *APOE*-ε4 allele was associated with an increasing degree of CAA (Figure 2 legend). This observation was also supported by a gene dose effect. The proportion of AD subjects with advanced CAA increased with the number of *APOE*-ε4 alleles from 40% for those without an allele to 90% for those with one allele and to 100% for *APOE*-ε4 homozygotes (χ^2 = 53.4, *P* = 0.000). These indications were strengthened by the fact that the 2 non-AD cases among neurological controls homozygous for ε4 both exhibited severe CAA.

To further define that the *APOE*-ε4 allele was a factor for predisposition to CAA, we divided the 190 AD cases into two subgroups. One exhibited minimal or no apparent CAA (group A) and the other exhibited moderate to severe or advanced CAA (group B). The *APOE*-ε4 allele frequency was nearly 50% and increased sixfold in group B with advanced CAA compared with group A (Table 4). The occurrence of this allele in group B was increased by a factor of 17

Table 4. APOE *Allele Frequencies in CAA*

Category (alleles)	*APOE* allele frequencies*						
	ε2	ε3	ε4	χ^2 (for ε2)	*P* value	χ^2 (for ε4)	*P* value
Normal controls (32)	22%	63%	15%				
AD with CAA (364)	2%†	61%	37%†	35.6	0.000	5.58[a]	0.018
CAA group A (110)	6%†	86%‡	8%	9.18	0.003	1.83	0.176
CAA group B (270)	1%†	51%	48%†	39.9	0.000	14.9[b]	0.000
				6.54	0.011	59.7[c]	0.000
AD with CH (26)	0%	38%	62%†	7.50	0.006	11.0[d]	0.001
				0.56	0.456	6.17[e]	0.013
Neurological controls with no CAA (46)	9%	78%	13%				
Neurological controls with CAA (22)	4%	46%	50%†	0.41	0.523	9.38[f]	0.002

*χ^2 values *versus* normal or neurological controls without CAA (single or upper numbers) and *versus* group A or AD group without CH (lower numbers).
†Statistically different frequencies compared with normal or neurological controls.
‡*APOE*-ε3 allele *versus* normal controls (χ^2 = 7.2, *P* = 0.007).
The odds ratios for tested groups for the presence of one *APOE*-ε4 allele were as follows (95% confidence interval in parentheses): a, 3.52 (1.07 to 9.66); b, 7.38 (2.38 to 22.9); c, 17.2 (7.56 to 38.9); d, 26.4 (2.65 to 26.2); e, 8.94 (1.14 to 70.3); and f, 12.8 (2.12 to 76.6).

(95% confidence interval, 7.56 to 38.9). In addition to neuropathological assessments[29] for AD, we determined Aβ plaque densities in the frontal and occipital cortex in a sample of the AD subjects with advanced CAA and those with minimal CAA. The average plaque densities for both areas (mean ± SEM) were determined to be 29 ± 2 and 27 ± 2 per cm^2 for AD subjects with advanced CAA (n = 35) and those with minimal CAA (n = 15), respectively. This indicated that cortical amyloid deposits *per se* were similar in the two AD groups A and B and argued against the fact that parenchymal amyloid deposition influenced the observed results (also S. Greenberg and B. Hyman, personal communication). In related observations, we noted that, although the *APOE*-ε4 allele frequency was lower, the *APOE*-ε3 allele was higher in group A with minimal CAA than in the controls (Table 4).

To determine the association between CH and the presence of the *APOE*-ε4 allele, we computed *APOE* genotypes as well as allele frequencies in 13 CAA cases exhibiting CH that varied from circumscribed hemorrhagic bleeds to massive lobar hemorrhages (Tables 3 and 4). Such analyses showed that the *APOE*-ε4 allele was four times more common (62%) in the CAA-related CH cases than in normal controls (15%, $P < 0.001$). The allele was also significantly more common in the hemorrhage cases than in those AD cases without the lesion (62% *versus* 45%, $P < 0.02$). The odds ratio for the presence of one ε4 allele in this subgroup was 8.20 (95% confidence interval, 1.04 to 64.5).

We also found that the frequency of the *APOE*-ε4 allele was significantly increased in neurological controls with CAA (Table 4) compared with those without CAA (odds ratio = 12.8; 95% confidence interval, 2.12 to 76.6). This finding in non-AD controls, where parenchymal Aβ deposition was not involved, also clearly supports the above finding of a relationship between CAA and the *APOE*-ε4 genotype. *APOE*-ε4 allele frequencies in the AD subgroups with minimal CAA, advanced CAA, or cerebral hemorrhage were also different from the neurological controls (Table 5).

APOE Genotypes and Cerebrovascular Lesions Associated with AD

Upon further examination, we noted that previously defined vascular lesions co-existed in 36% of the AD

Table 5. APOE-ε4 *Allele Frequency Comparisons between Neurological Controls and AD Subgroups*

Category (Alleles)	*APOE*-ε allele frequencies				
	ε2	ε3	ε4	χ^2 (ε4 only)*	*P* value
Neurological controls (68)	7%	68%	25%		
All AD (380)	2%	62%	36%	2.78	0.096
AD with CAA (364)	2%	61%	37%†	3.59	0.050
CAA group A (110)	6%	86%	8%†	8.22	0.004
CAA group B (270)	1%	51%	48%†	14.1	0.000
AD with CH (26)	0%	38%	62%†	13.4	0.009

*χ^2 values *versus* neurological controls.
†Significant frequencies *versus* controls.

Table 6. *Distribution of* APOE *Genotypes in AD Subjects with CVL*

Groups	n	*APOE* genotypes*					*APOE* alleles		
		ε2/ε3	ε2/ε4	ε3/ε3	ε3/ε4	ε4/ε4	ε2	ε3	ε4
Normal controls	16	5 (31%)	2 (12%)	6 (38%)	3 (19%)	0 (0%)	22%	63%	15%
AD without CVL	121	6 (5%)	0 (0%)	51 (42%)	50 (41%)	14 (12%)	3%	65%	32%
AD with CVL	69	3 (4%)	0 (0%)	17 (25%)	39 (56%)	10 (15%)	2%†	55%	43%‡

Normal controls are the same as in Tables 1 and 2.
*χ^2 value for genotype distribution comparisons between normal controls and AD subjects with CVL was 25.5 (P = 0.000) and between AD subjects with and without CVL was 6.26 (P = 0.095).
†χ^2 = 19.4 and P = 0.000 *versus* normal controls.
‡χ^2 = 8.86 and $P < 0.003$ *versus* normal controls and χ^2 = 6.00 and P = 0.014 *versus* AD subjects without CVL.

cases. These lesions were too varied in size and origin to be assigned into further subgroups. Expectedly, the frequencies of *APOE* genotypes in the CVL group was different from those in normal controls but not significantly so from those in AD subjects without such lesions (Table 6). However, the ε4 allele frequency in the CVL subjects was apparently greater (43%) than in AD subjects without the lesions (32%, $P < 0.02$; Table 6). Examination of available clinical records showed that only four subjects had had a history of hypertension, suggesting that this was unlikely to be the contributing factor. We also examined the distribution of *APOE* genotypes in all AD subjects in relation to the severity of atherosclerosis of the basal surface arteries (Figure 3). The acquisition of a particular genotype was not associated with severity of atherosclerosis and there were no apparent differences in the frequencies of the *APOE* genotypes or the ε4 alleles in the minimal, moderate, and severe groups (Figure 3 legend).

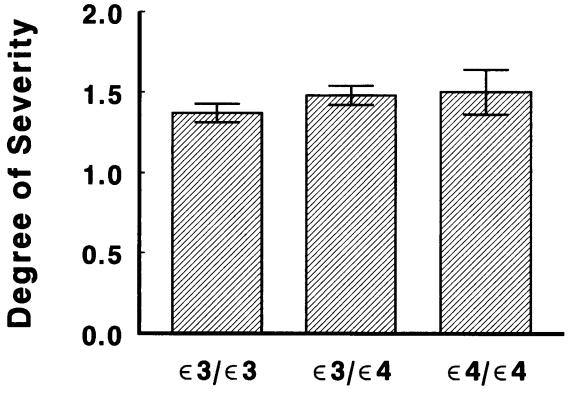

Figure 3. *APOE genotypes and the degree of cerebral atherosclerosis in AD. There was no apparent relationship between degree of atherosclerosis and* APOE *genotype. One-way ANOVA and nonparametric comparison tests (Kruskal-Wallis) showed the degree of atherosclerosis in subjects with ε3/ε3 genotype was not significant* (P = *0.696) from the ε3/ε4 group and similarly that of ε3/ε4 from the ε4/ε4 subjects* (P = *0.861). The degree of atherosclerosis (approximately 1.00) in the ε2/ε3 and ε2/ε4 groups were also not different from the ε3/ε3 subjects (not shown). The* APOE *genotype frequencies were similar in the minimal, moderate, and severe atherosclerosis groups (χ^2 = 11.6, P = 0.169, 8 df). Conversely, the frequencies of the APOE-ε4 allele were also not related to the degree of atherosclerosis ($\chi 2$ = 3.67, P = 0.153).*

We also examined frequencies of *APOE* genotypes and the three alleles in AD subjects with accompanying DLBD, some of whom exhibited CAA (Table 7). First, we observed that neither the *APOE* genotype nor the ε4 allele frequencies were significantly different in the AD subjects with concomitant DLBD compared with those in controls or with those in AD subjects without DLBD (Table 7). In fact, the *APOE*-ε4 allele frequency was intermediate to that in controls and the rest of the AD subjects (Table 2). As we did not encounter any cases of pure DLBD, we could not solely determine allele frequencies in disease cases without accompanying AD. Second, we noted that the *APOE*-ε4 allele frequency in the DLBD group with advanced CAA (45%) was significantly greater than in the AD subgroup without CAA (4%, P = 0.000; Table 7). This observation again supported the principal finding (Table 4) implicating the influ-

Table 7. *Distribution of* APOE *Genotypes in AD Subjects with DLBD*

Groups	n	*APOE* genotypes†					*APOE* alleles		
		ε2/ε3	ε2/ε4	ε3/ε3	ε3/ε4	ε4/ε4	ε2	ε3	ε4
Normal controls	16	5 (31%)	2 (12%)	6 (38%)	3 (19%)	0 (0%)	22%	63%	15%
AD subjects with DLBD	33	1 (3%)	0 (0%)	16 (49%)	14 (42%)	2 (6%)	2%*	71%	27%
DLBD with no CAA	14	0 (0%)	0 (0%)	13 (93%)	1 (7%)	0 (0%)	0%	96%	4%
DLBD with CAA‡	19	1 (5%)	0 (0%)	3 (16%)	13 (68%)	2 (11%)	3%	52%	45%**

Normal controls are the same as in Tables 1 and 2. †Genotype frequencies in normal controls were different (χ^2 = 14.1, $P < 0.01$). The genotype frequencies in AD subjects without DLBD were not different (χ^2 = 3.58, P = 0.311). ‡Similarly, χ^2 value for frequency comparisons in DLBD with no CAA was χ^2 = 19.2, $P < 0.001$.
*χ^2 = 13.1 and P = 0.000 *versus* normal controls.
**χ^2 = 16.6 and P = 0.000 (odds ratio, 48.8; 95% confidence interval, 4.82 to 493) *versus* DLBD without CAA.

ence of the *APOE*-ε4 allele on manifestation of CAA in AD. On the other hand, these observations indicated that *APOE*-ε4 was not a dominant factor in AD subjects with DLBD.[10,11]

Discussion

Our observations provide evidence and confirm previous reports[6–16] that the *APOE*-ε4 allele is a factor in the pathogenesis of AD. We demonstrate this by comparison of primarily late-onset sporadic AD subjects with appropriate normal controls and a group of neurological control subjects exhibiting various disorders. The frequency of the *APOE*-ε4 allele in these AD-confirmed subjects we report is within range of those described previously.[6–8] Our observations also support the notion that inheritance of the *APOE*-ε2 allele is protective against developing AD.[41,42] Nevertheless, the primary objective of our studies was to examine whether the inheritance of the *APOE*-ε4 allele predisposes one to CAA. As indicated by initial studies of Roses and colleagues,[21] our observations strongly imply that the acquisition of the *APOE*-ε4 allele is an important factor in the development of CAA. First, we found that the frequency of the ε4 allele was significantly higher in AD subjects with CAA. Second, the frequency was even greater in those subjects exhibiting advanced CAA than in those with negligible CAA, where both groups had similar cortical Aβ deposition. Third, the *APOE*-ε4 allele frequency in neurological controls with CAA or in the DLBD subjects with CAA[30] was greater than in those with no apparent CAA. Fourth, we noted two subjects diagnosed with Creutzfeldt-Jakob disease and Pick's disease, both of whom exhibited severe CAA and were carriers of the ε4/ε4 genotype. These indications are also consistent with increased incidence of CAA during aging,[24,25] along with increased frequency of the ε4 allele in older subjects during the seventh and eighth decades, compared with all of the AD subjects.[43] Thus, the various comparisons between groups controlled for other factors including age would suggest that inheritance of the *APOE*-ε4 is a likely factor for CAA. This fact negates the idea that the association between the *APOE*-ε4 and CAA found here was merely a consequence of parenchymal Aβ deposition.

Additional support of the association of the frequency of the *APOE*-ε4 allele with CAA in sporadic AD could be found by examination of subjects with sporadic aging-associated CAA as well as CAA cases in the absence of AD. We could not locate such cases readily. However, the association appeared different from that in the Dutch subjects with hereditary cerebral hemorrhage with amyloidosis and profound CAA.[44] These previous studies in the Dutch subjects with up to 50% of patients without an *APOE*-ε4 allele suggested a lack of association of the *APOE*-ε4 allele as a factor in the development of the disease.[45] The mutations involved in the early-onset Dutch cases presumably override other factors such as the *APOE* genotype. Regardless, our studies imply that the *APOE*-ε4 allele is a strong factor in the development of CAA in sporadic and possibly late-onset familial forms of AD.

Our findings on CAA were also strengthened by the fact that the frequency of the *APOE*-ε4 allele in the AD subjects with cerebral hemorrhage exhibiting CAA was significantly greater than in those AD cases without such lesions or in the controls. Similar results have been recently observed by others (S. Greenberg and B. Hyman, personal communication). This fact is particularly important if the CAA or related vascular abnormalities are a cause of the hemorrhages.[24] Alternatively, cerebral hemorrhages could be precipitated by head trauma,[20] a risk factor for AD that has recently been found to be highly associated with the *APOE*-ε4 allele. The mechanism we suggest could be that the ε4 allele causes a phenotypic expression of an apoE that plays a role in the sequestration and aggregation[46,47] of the normally secreted Aβ in the early stages of the development of CAA in the vessel wall.[48]

We were also intrigued to find nearly 40% of the subjects with a pathological diagnosis of AD who exhibited some cerebrovascular pathology[49] including multiple infarcts, lacunes, Binswanger lesions, and hemorrhagic infarcts. They revealed a higher frequency of the *APOE*-ε4 allele compared with those without such lesions or controls. These results are not inconsistent with the slightly greater frequency of the *APOE*-ε4 allele in vascular conditions associated with dementia.[18,19] It is possible that vascular lesions associated with AD are responsible for the observed high frequencies of the *APOE*-ε4 allele in subjects with probable AD.[50,51] We have previously shown that a variety of vascular lesions were associated with AD that may in fact be related to Aβ deposition.[25–27] Although the CVLs could be a consequence of Aβ deposition, the presence of an *APOE*-ε4 allele may be a factor in the occurrence of the associated vascular pathology.[40] It is unlikely that the increase in the ε4 allele frequency in this subgroup of subjects was related to cardiovascular abnormalities, as in a previous study[15] there were no apparent differences in the *APOE* genotypes and

allele frequencies between control subjects with and without coronary disease.

We also confirmed that the frequency of the *APOE*-ε4 allele in AD subjects with DLBD[9–14] was as common as that in other AD subjects. This finding is consistent with previous reports showing that the frequency of the *APOE*-ε4 allele in AD subjects with DLBD was intermediate between that in AD subjects and the normal controls.[9–11] We found some evidence to suggest that the presence of advanced CAA was associated with high ε4 allele frequency even in the DLBD cases. Although previous studies[10–17] did not specifically address the degree of CAA in the DLBD cases,[30] our findings are consistent with a recent report suggesting that the ε4 allele frequency is higher in DLBD subjects with CAA when compared with those without.[52]

In summary, our analyses of a relatively small sample suggests the development of CAA in AD is increased by inheritance of at least one ε4 allele. We provide additional evidence for an association between *APOE*-ε4 allele frequency and CAA-related cerebral hemorrhage. The possibility exists that apoE is an independent vascular factor and that specified *APOE* gene products may be temporally involved in promoting pathogenetic processes within the cerebral vasculature in concert with aging.

Acknowledgments

We very much appreciate the cooperation of Dr. P. Gambetti and colleagues in the Neuropathology Division in helping us to readily obtain postmortem human brain tissues and the neuropathological records. We thank Drs. K. S. Kim and S. Younkin for provision of antibodies and S. Foss for secretarial help. We also thank Dr. S. Amini, Department of Biostatistics and Epidemiology, for critically reading the manuscript and suggesting statistical applications. We are grateful to Steve Greenberg and Bradley Hyman (Massachusetts General Hospital) for discussions and sharing their results before submission.

References

1. Namba Y, Tomonaga M, Kawasaki H, Otomo E, Ikeda K: Apolipoprotein E immunoreactivity in cerebral amyloid deposits and neurofibrillary tangles in Alzheimer's disease and kuru plaque amyloid in Creutzfeldt-Jakob disease. Brain Res 1991, 541:163–166
2. Wisniewski T, Frangione B: Apolipoprotein E: a pathological chaperone protein in patients with cerebral and systemic amyloid. Neurosci Lett 1992, 135:235–238
3. Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, Roses AD: Apolipoprotein E: high-avidity binding to β-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci USA 1993, 90: 1977–1981
4. Mahley RW: Apolipoprotein E: cholesterol transport protein with an expanding role in cell biology. Science 1989, 240:622–630
5. Davignon J, Gregg RE, Sing CF: Apolipoprotein E polymorphism and atherosclerosis. Arterioscler Thromb 1988, 8:1–21
6. Roses AD: Apolipoprotein E affects the rate of Alzheimer disease expression: β-amyloid burden is a secondary consequence dependent on *APOE* genotype and duration of disease. J Neuropathol Exp Neurol 1994, 53:429–437
7. Poirier J, Davignon J, Bouthillier D, Kogan S, Bertrand P, Gauthier S: Apolipoprotein E polymorphism and Alzheimer's disease. Lancet 1993, 342:697–699
8. Rebeck GW, Reiter JS, Strickland DK, Hyman BT: Apolipoprotein E in sporadic Alzheimer's disease: allelic variation and receptor interactions. Neuron 1993, 11: 575–580
9. Saunders AM, Schmader K, Breitner JCS, Benson D, Brown WT, Goldfarb L, Goldgaber D, Manwaring MG, Szymanski, McCown N, Dole KC, Schmechel DE, Strittmatter WJ, Pericak-Vance MA, Roses AD: Apolipoprotein E ε4 allele distribution in late-onset Alzheimer's disease and in other amyloid-forming diseases. Lancet 1993, 342:710–711
10. Hardy J, Crook R, Prihar G, Roberts G, Raghavan R, Perry R: Senile dementia of the Lewy body type has an apolipoprotein E ε4 allele frequency intermediate between controls and Alzheimer's disease. Neurosci Lett 1994, 182:1–2
11. Pickering-Brown SM, Siddons M, Owen F, Neary D, Snowden JS: Apolipoprotein E allelic frequencies in patients with lobar atrophy. Neurosci Lett 1995, 188: 205–207
12. Zerr I, Helmhold M, Weber T: Apolipoprotein E in Creutzfeldt-Jakob disease. Lancet 1994, 345:68–69
13. Pickering-Brown SM, Mann DMA, Bourke JP, Roberts DA, Balderson D, Burns A, Byrne J, Owen F: Apolipoprotein E4 and Alzheimer's disease pathology in Lewy body disease and in other β-amyloid-forming diseases. Lancet 1994, 343:1155
14. Hansen LA, Galasko D, Samuel W, Xia Y, Chen X, Saitoh T: Apolipoprotein-E ε-4 is associated with increased neurofibrillary pathology in the Lewy body variant of Alzheimer's disease. Neurosci Lett 1994, 182:63–65
15. Arai H, Higuchi S, Muramatsu T, Iwatsubo T, Sasaki H, Trojanowski JQ: Apolipoprotein E gene in diffuse Lewy body disease with or without co-existing Alzheimer's disease. Lancet 1994, 344:1307

16. Harrington CR, Louwagie J, Rossau R, Vanmechelen E, Perry RH, Perry EK, Xuereb JH, Roth M, Wischik CM: Influence of apolipoprotein E genotype on senile dementia of the Alzheimer and Lewy body types. Am J Pathol 1994, 145:1472–1484
17. Amouyel P, Vidal O, Launay JM, Laplanche JL for the French Group: The apolipoprotein E alleles as major susceptibility factors for Creutzfeldt-Jakob disease. Lancet 1994, 344:1315–1318
18. Noguchi S, Murakami K, Yamada N: Apolipoprotein E genotype and Alzheimer's disease. Lancet 1993, 342: 737
19. Frisoni GB, Calabresi O, Geroldi C, Bianchetti A, D'Acquarica AL, Govoni S, Sirtori CR, Trabucchi M, Franceschini G: Apolipoprotein E+4 allele in Alzheimer's disease and vascular dementia. Dementia 1994, 5:240–242
20. Nicoll JAR, Roberts GW, Graham DI: Apolipoprotein E $\epsilon 4$ allele is associated with deposition of amyloid β-protein following head injury. Nat Med 1994, 1:135–137
21. Schmechel DE, Saunders AM, Strittmatter WJ, Crain BJ, Hulette CM, Joo SH, Pericak-Vance MA, Goldgaber D, Roses AD: Increased amyloid β-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. Proc Natl Acad Sci USA 1993, 90:9649–9653
22. Regli F: Cerebral amyloid angiopathy. Handbook of Clinical Neurology. Edited by PJ Vinken, GW Bruyn, HL Klawans. Amsterdam, Elsevier, 1989, pp 333–344
23. Vinters HV: Cerebral amyloid angiopathy: a critical review. Stroke 1987, 18:311–324
24. Kase CS: Cerebral amyloid angiopathy. Intracerebral Hemorrhage. Edited by CS Kase, LR Caplan. Boston, Butterworth-Heinemann, 1994, pp 179–200
25. Kalaria RN: Cerebral vessels in ageing and Alzheimer's disease. Pharmacol Ther 1996 (in press)
26. Kalaria RN: The blood-brain barrier and cerebral microcirculation in Alzheimer's disease. Cerebrovasc Brain Metab Rev 1992, 4:226–260
27. Kawai M, Kalaria RN, Cras P, Siedlak SL, Velasco ME, Shelton ER, Chan HW, Greenberg BD, Perry G: Degeneration of amyloid precursor protein-containing smooth muscle cells in cerebral amyloid angiopathy. Brain Res 1993, 623:142–146
28. Khachaturian ZS: Diagnosis of Alzheimer's disease. Arch Neurol 1985, 42:1097–1105
29. Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, Vogel FS, Hughes JP, van-Belle G, Berg L: The consortium to establish a registry for Alzheimer's disease (CERAD). II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology 1991, 41:479–486
30. Wu E, Lipton RB, Dickson DW: Amyloid angiopathy in diffuse Lewy body disease. Neurology 1992, 42:2131–2135
31. Kalaria RN, Sromek S, Grahovac IG, Harik SI: Transferrin receptors of human and rat cerebral microvessels and brain: assessment in Alzheimer's Disease. Brain Res 1992, 585:87–93
32. Premkumar DRD, Kalaria RN: Altered expression of amyloid β precursor mRNAs in cerebral vessels, meninges and choroid plexus in Alzheimer's disease. Ann NY Acad Sci 1996, 777:288–292
33. Kalaria RN, Golde TE, Kroon SN, Perry G: The serine protease inhibitor antithrombin III and its mRNA in the pathogenesis of Alzheimer's disease. Am J Pathol 1993, 143:886–893
34. Kalaria RN, Hedera P: Evidence for differential degeneration of the endothelium and basement membrane of capillaries in Alzheimer's disease. Neuroreport 1995, 6:477–480
35. Vonsattel JP, Myers RH, Hedley-Whyte ET, Ropper AH, Bird ED, Richardson EP Jr: Cerebral amyloid angiopathy without and with cerebral hemorrhages: a comparative histological study. Ann Neurol 1991, 30:637–649
36. Mandybur TI: Cerebral amyloid angiopathy: the vascular pathology and complications. J Neuropathol Exp Neurol 1986, 45:79–90
37. Sambrook J, Fritsch EF, Maniatis T: Molecular Cloning: A Laboratory Manual, vols 1–3. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press, 1989.
38. Wenham PR, Price WH, Blundell D: Apolipoprotein E genotyping by one stage PCR. Lancet 1991, 337: 1158–1159
39. West HL, Rebeck WG, Hyman BT: Frequency of the apolipoprotein E $\epsilon 2$ allele is diminished in sporadic Alzheimer disease. Neurosci Lett 1994, 175:46–48
40. Schneider JA, Gearing M, Mirra SS: Apolipoprotein E (ApoE) genotype in diverse neurodegenerative diseases (ND). Brain Pathol 1994, 4:538
41. Corder EH, Saunders AM, Risch NJ, Strittmatter WJ, Schmechel DE, Gaskell PC, Rimmler JB, Locke PA, Conneally PM, Schmader KE, Small GW, Roses AD, Haimes JL, Pericak-Vance MA: Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. Nat Genet 1994, 7:180–184
42. Talbot C, Lendon C, Craddock N, Shears S, Morris JC, Goate A: Protection against Alzheimer's disease with apoE $\epsilon 2$. Lancet 1994, 343:1432–1433
43. Kalaria RN, Cohen DL, Premkumar DRD: Apolipoprotein E alleles and brain vascular pathology in Alzheimer's disease. Ann NY Acad Sci 1996, 777:266–270
44. Haan J, Hardy JA, Roos RAC: Hereditary cerebral hemorrhage with amyloidosis - Dutch type: its importance for Alzheimer research. Trends Neurosci 1991, 14:231–234
45. Haan J, Van Broeckhoven C, van Duijn CM, Voorhoeven E, van Harskamp F, van Swieten JC, Maat-Schieman MLC, Roos RAC, Baker E: Apolipoprotein E $\epsilon 4$ allele does not influence the clinical expression of the amyloid precursor protein gene codon 693 or 692 mutations. Ann Neurol 1994, 36:434–437
46. Wisniewski T, Castano EM, Golabek A, Vogel T, Frangione B: Acceleration of Alzheimer's fibril formation by

apolipoprotein E *in vitro*. Am J Pathol 1994, 145:1030–1035

47. Ma J, Yee A, Brewer HB, Das S, Potter H: Amyloid-associated proteins α_1-antichymotrypsin and apolipoprotein E promote assembly of Alzheimer β-protein into filaments. Nature 1994, 372:92–94
48. Yamaguchi H, Ishiguro K, Sugihara S, Nakazato Y, Kawarabayashi T, Sun X, Hirai S: Presence of apolipoprotein E on extracellular neurofibrillary tangles and on meningeal blood vessels precedes the Alzheimer β-amyloid deposition. Acta Neuropathol 1994, 88:413–419
49. Englund E, Brun A: White matter changes in dementia of the Alzheimer's type: the difference in vulnerability between cell compartments. Histopathology 1990, 16: 433–439
50. Pirttila T, Lehtimaki T, Nikkari T, Frey H, Mattila K: The apolipoprotein E *E4* alleles and sex-specific risk of Alzheimer's disease. JAMA 1995, 273:375
51. Kosunen O, Talasniemi S, Lehtovirta M, Heinonen O, Helisalmi S, Mannermaa A, Paljarvi L, Ryynanen M, Riekkinen PJ, Soininen S: Relation of coronary atherosclerosis and apolipoprotein genotypes in Alzheimer patients. Stroke 1995, 26:743–748
52. Lippa CF, Smith TW, Saunders AM, Crook R, Pulaski-Salo D, Davies P, Hardy J, Roses AD, Dickson DW: Apolipoprotein E genotype and Lewy body disease. Neurology 1995, 45:97–103