

The Apolipoprotein E $\epsilon 4$ Allele Does Not Influence the Clinical Expression of the Amyloid Precursor Protein Gene Codon 693 or 692 Mutations

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In 31 symptomatic and 5 asymptomatic carriers of the amyloid precursor protein (APP) gene codon 693 mutation, 10 family members without mutation, and 5 carriers of the APP gene codon 692 mutation (3 with early-onset Alzheimer dementia, 2 with cerebral hemorrhage), a high frequency of the apolipoprotein E $\epsilon 4$ allele was found. Age at onset, age at death, occurrence of dementia, and number of strokes did not differ between APP gene mutation carriers with or without $\epsilon 4$ allele, showing that the clinical expression of these APP mutations is not influenced by the apolipoprotein E gene.

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Apolipoprotein E (ApoE) is associated with plasma lipoproteins [1]. In the brain, it is involved in repair, growth, and maintenance of myelin and neuronal membranes. The ApoE gene (APOE) is located at 19q13.2. Three APOE alleles encode for three common ApoE variants, with the following approximate

distribution: $\epsilon 3$ 75%; $\epsilon 4$ (112Cys \rightarrow Arg polymorphism) 15%; and $\epsilon 2$ (158Arg \rightarrow Cys) 8%, resulting in $\epsilon 2/\epsilon 2$ 1%, $\epsilon 3/\epsilon 3$ 60%, $\epsilon 4/\epsilon 4$ 3%, $\epsilon 2/\epsilon 3$ 12%, $\epsilon 2/\epsilon 4$ 2%, and $\epsilon 3/\epsilon 4$ 20% [2].

Recently, a high frequency (40%) of the $\epsilon 4$ allele was found in late-onset familial and sporadic Alzheimer's disease (AD) [3-5]. $\epsilon 4$ Homozygosity was virtually sufficient to cause familial AD by the age of 80 years [5]. Onset of the AD symptoms occurred earlier in patients with an $\epsilon 4$ allele than in those without. The $\epsilon 4$ allele frequency was normal in early-onset familial AD caused by different amyloid precursor protein (APP) gene codon 717 mutations, in AD families linked to chromosome 14 [4, 5], and in other amyloid-forming diseases, such as Creutzfeldt-Jakob disease, familial amyloidotic polyneuropathy, and Down's syndrome [6]. In families with the APP gene 717Val \rightarrow Ile and 670/1Lys/Met \rightarrow Asn/Leu mutations, the presence of an $\epsilon 4$ allele appeared to lower the age at onset [7], suggesting that the $\epsilon 4$ allele can also modify the clinical course of cerebral $\beta/A\beta$ amyloid diseases, even when the disease is not linked to chromosome 19.

In AD, ApoE immunoreactivity was found in senile plaques, amyloid angiopathy, and neurofibrillary tangles [8]. ApoE probably is an integral component of the $\beta/A\beta$ metabolism, because there is high-avidity binding of ApoE to $\beta/A\beta$ amyloid [3] and to soluble $\beta/A\beta$ [9]. Binding properties of ApoE4 (transcript product of the $\epsilon 4$ allele) and ApoE3 (product of $\epsilon 3$) with $\beta/A\beta$ are different [10]. This points at a specific importance of the different alleles in $\beta/A\beta$ amyloidosis, which is underlined by the finding that patients with sporadic AD with an $\epsilon 4$ allele have more vascular amyloid deposits and a higher number of amyloid plaques than do patients without an $\epsilon 4$ allele [11].

We determined APOE genotypes in two other $\beta/A\beta$ amyloid diseases: hereditary cerebral hemorrhage with amyloidosis—Dutch type (HCHWA-D), which is caused by an APP gene codon 693 mutation [12], and early-onset AD-type dementia and cerebral hemorrhages with amyloidosis caused by an APP gene codon 692 mutation in a Dutch family called "Family 1302" [13]. We studied the relationship between the APOE genotype and clinical symptoms, as well as ApoE immunoreactivity in cerebral amyloid deposits in 1 patient with HCHWA-D.

Patients and Methods

Genomic DNA was isolated from whole blood using standard procedures. Part of exon 4 of the APOE was analyzed, as described previously [14], in the following groups of patients:

HCHWA-D patients with the APP gene codon 693 mutation ($n = 31$; 21 men, 10 women). Thirty of them had had one or more cerebral hemorrhages, and 21 were de-

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mented. One female carrier had dementia, starting at the age of 55 years, but without strokes.

Asymptomatic family members ($n = 5$) with HCHWA-D, with codon 693 mutation, not known to have had a stroke, with normal findings at neurological and neuropsychological examinations. Cerebral magnetic resonance imaging (MRI) showed no abnormalities in 4 members (30, 30, 33 and 37 years old), and diffuse white matter hypodensity in 1 (44 years old) [15].

Asymptomatic first-degree relatives of HCHWA-D patients ($n = 10$; 30–80 years old) without mutation (“escapees”). Members of Family 1302 ($n = 5$), all with APP gene codon 692 mutation [13]. Two patients had had a cerebral hemorrhage due to amyloid angiopathy (proved by biopsy in one of them), and 3 had early-onset AD dementia. One of the demented patients died, and histological examination showed AD pathology (amyloid angiopathy, plaques, and tangles).

Clinical features in $\epsilon 4$ carriers and noncarriers were compared using Student’s t and χ^2 tests.

The brain of 1 woman with HCHWA-D (age at death, 58 years, due to a cerebral hemorrhage), and the brain of 1 control patient without cerebral disease (age at death, 36 years, due to myocardial infarction) were studied immunohistochemically. Pieces of frontal cerebral cortex were frozen in liquid nitrogen. First, 8- μ m-thick cryostat sections were incubated with ApoE antiserum, and then the sections were incubated with peroxidase-labeled rabbit anti-goat antiserum (Nordic Immunology). Peroxidase activity was visualized with 3,3'-diaminobenzidine. The use of Tris-buffered saline solution as the first-step reagent served as negative control. Adjacent sections were stained with alkaline Congo red or anti- β /A4.

Results

The APOE $\epsilon 4$ allele frequency in HCHWA-D (52% of the subjects had at least one $\epsilon 4$ allele) and in Family 1302 (80%) was higher than the normal Dutch $\epsilon 4$ allele frequency (30%) [16] (Table). The separate groups showed the following distribution. Of the 31 symptomatic HCHWA-D patients (see Table), 16 had one or two $\epsilon 4$ alleles (5 $\epsilon 4$ homozygotes, 11 heterozy-

gotes). There were no statistically significant differences in age at onset (in 30 patients a cerebral hemorrhage was the first sign of the disease), age at death (15 of the patients died), occurrence of dementia ($n = 21$), and number of strokes between $\epsilon 4$ homozygotes, heterozygotes, and patients without the $\epsilon 4$ allele. The demented patient without stroke was an $\epsilon 3$ homozygote. Four of the 5 asymptomatic carriers of the codon 693 mutation ($n = 5$) were $\epsilon 3/\epsilon 3$; the 44-year-old woman with MRI abnormalities was $\epsilon 3/\epsilon 4$. Of the 10 HCHWA-D escapees, 7 (70%) were $\epsilon 4$ heterozygotes. Three subjects had no $\epsilon 4$ allele. There were no $\epsilon 4$ homozygotes in this group. In Family 1302, 4 patients (80%) were $\epsilon 4/\epsilon 3$ (age at onset, 41, 42, 47, and 49 years), and 1 patient was $\epsilon 3$ homozygote (age at onset, 45 years). The $\epsilon 3/\epsilon 3$ patient had early-onset AD, as did 2 $\epsilon 3/\epsilon 4$ patients. The other 2 $\epsilon 3/\epsilon 4$ patients had cerebral hemorrhages.

The brain of the HCHWA-D patient (genotype $\epsilon 2/\epsilon 3$) showed strong staining with ApoE antibodies in the wall of small blood vessels and in the parenchyma (Fig). Adjacent sections (not shown) showed strong staining with Congo red or anti- β /A4 in the same structures, proving the colocalization of β /A4 amyloid and ApoE. No ApoE or β /A4 immunoreactivity was found in the control brain.

Discussion

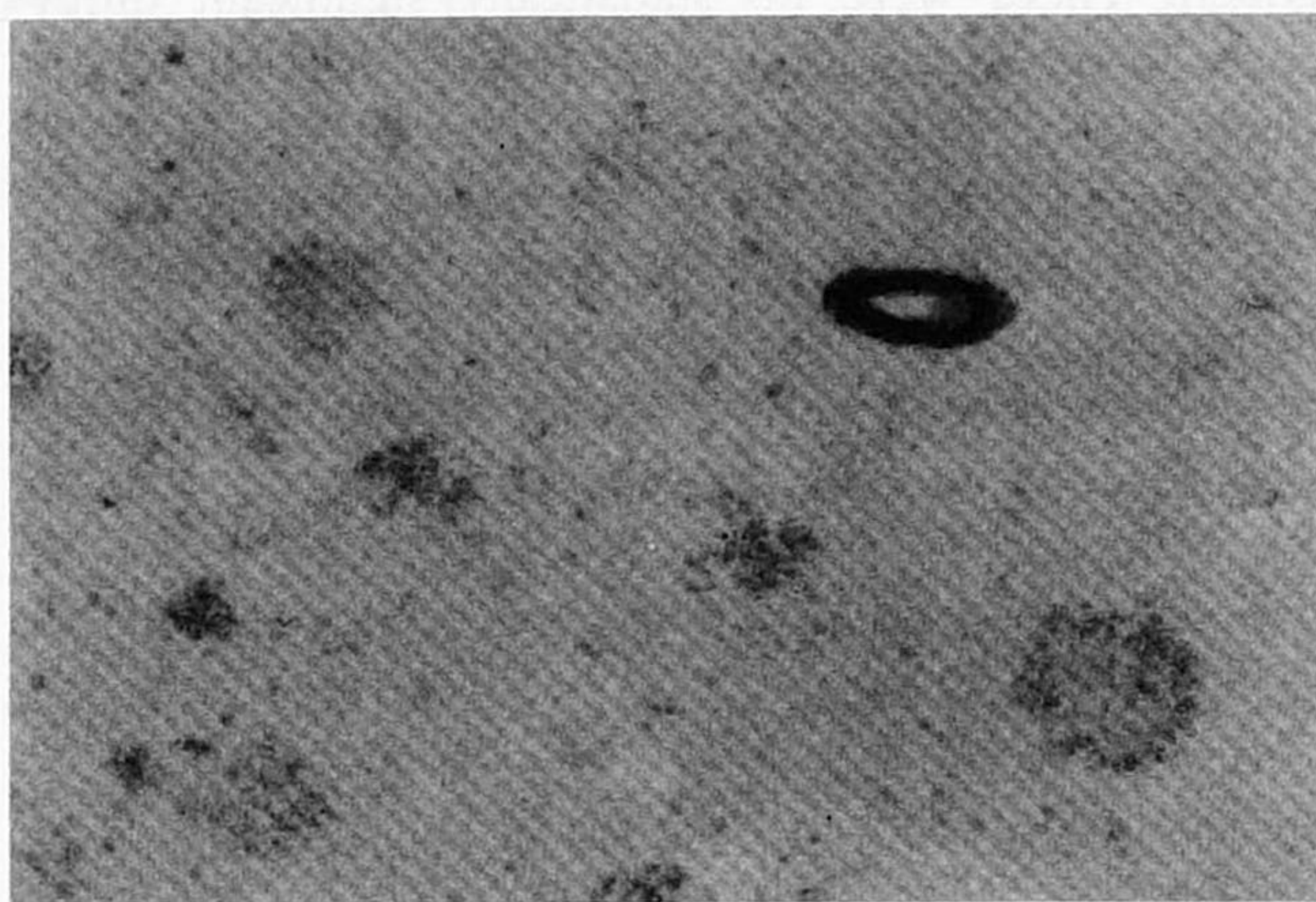
In this study, the $\epsilon 4$ allele frequency in patients with the APP gene codon 693 and 692 mutations was higher than that in the Dutch population. This resembles the high $\epsilon 4$ allele frequency found in AD families [3–5], but the high $\epsilon 4$ allele frequency found in this study probably has a different cause. There are several large HCHWA-D families, and although our genealogical data go back to the eighteenth century, we are still unable to prove that there is one common founder [17]. However, it is very likely that we are dealing with one pedigree, because the clinical features of the separate families are very similar, and HCHWA-D seg-

Gene Frequency and Comparison of Clinical Characteristics of HCHWA-D Patients With and Those Without the $\epsilon 4$ Allele

	Dutch Population Study [16]	HCHWA-D ($n = 31$)	Age (yr) at Onset ^a	Age (yr) at Death ($n = 15$) ^a	Dementia	No. of Strokes ^a
$\epsilon 4$ Homozygotes	3%	16% ($n = 5$)	49.8 (7.5)	62.7 (6.4) ($n = 3$)	100% ($n = 5$)	3.0 (1.0)
$\epsilon 4$ Heterozygotes	25%	35% ($n = 11$)	48.9 (4.9)	54.8 (13.0) ($n = 6$)	55% ($n = 6$)	2.5 (1.2)
$\epsilon 4$ Homozygotes + heterozygotes	30%	50% ($n = 16$)	49.2 (5.6)	57.4 (11.5) ($n = 9$)	70% ($n = 11$)	2.6 (1.2)
No $\epsilon 4$	70%	50% ($n = 15$)	51.5 (7.4)	59.3 (9.3) ($n = 6$)	67% ($n = 10$)	3.0 (1.9)

^aMean (standard deviation).

HCHWA-D = hereditary cerebral hemorrhages with amyloidosis (Dutch).



Immunohistochemical study of the brain of a patient with hereditary cerebral hemorrhages with amyloidosis (Dutch) showing apolipoprotein E (ApoE) antibody staining in a cerebral blood vessel and in parenchymal plaque-like lesions. In adjacent sections (not shown) the same structures stained with anti- β /A4 and Congo red. (ApoE 1:100 cryostat section; $\times 10$ before 46% reduction.)

regates with the APP gene codon 693 mutation in all pedigrees [12]. The high frequency of the APOE $\epsilon 4$ allele in HCHWA-D—but also in Family 1302—probably reflects the fact that we are dealing with members of one family, whereas in AD the high $\epsilon 4$ allele frequency was found in individuals from a large number of different nonrelated pedigrees.

An influence of the $\epsilon 4$ allele on age at onset in AD is suggested by several studies [5, 7]. The high number of individuals with $\epsilon 4$ allele in both β /A4 amyloid diseases studied here allowed for a comparison of the clinical features of patients with and those without an $\epsilon 4$ allele. In patients with the APP gene codon 692 mutation, no difference in age at onset or clinical presentation appeared to be present between the $\epsilon 3/\epsilon 3$ patient and the 4 $\epsilon 3/\epsilon 4$ patients, making it unlikely that the $\epsilon 4$ allele influences the clinical manifestations in this disease. However, the number of patients is too small to allow a definite conclusion. In patients with an APP gene codon 693 mutation, age at onset, age at death, occurrence of dementia, and number of strokes were not influenced by the presence or absence of an $\epsilon 4$ allele (see Table). The finding that 100% of the $\epsilon 4$ homozygotes were demented is probably caused by coincidence due to the limited number ($n = 5$). This is supported by the finding that dementia was more often present in patients without $\epsilon 4$ allele (67%) than in $\epsilon 3/\epsilon 4$ heterozygotes (55%).

There are several possible explanations for the lack of relation between APOE genotype and clinical characteristics. First, it is possible that cerebral hemorrhages in patients with chronic genetically defined β /

A4 amyloid angiopathy result from superimposed environmental factors such as head trauma or sudden increase in blood pressure [17]. As a result, it is problematic to study the relationship between APOE and the age at onset of the disease (which is most often a hemorrhage), because data on the external factors related to the hemorrhages are not available and cannot be adjusted for. Consequently, the number of strokes and the age at death (mostly due to a hemorrhage) also are not true “intrinsic” indicators for the severity of the pathology underlying the disease process. Dementia in HCHWA-D appears to be at least partially related to the number of strokes [18], and therefore, also the occurrence of dementia cannot be regarded as an indicator of severity. The most valid measurements of disease severity are the occurrence and severity of β /A4 amyloid angiopathy itself, which are impossible to determine. Our prospective study of presymptomatic APP gene codon 693 mutation carriers shows that diffuse leukoencephalopathy is the first clinical indicator of damage caused by β /A4 amyloid angiopathy [15]. In the present study, one asymptomatic carrier of the mutation showed leukoencephalopathy on MRI, and this patient was the only asymptomatic carrier with an $\epsilon 4$ allele (the other 4 subjects had $\epsilon 3/\epsilon 3$). However, the $\epsilon 4/\epsilon 3$ patient was the oldest subject in this group, which might also be an explanation for the MRI changes.

A second possible explanation for the lack of relation between the presence of $\epsilon 4$ alleles and the clinical expression of the APP gene codon 693 and 692 mutations is as follows: The binding of ApoE to β /A4 requires residues 12 to 28 of β /A4 protein [9, 10]. The APP gene codon 693 and 692 mutations cause a base change in this particular region of β /A4, which may result in a lower binding affinity of ApoE to β /A4, and consequently to a lack of influence of APOE on the clinical characteristics. The APP gene codon 717 and 670/1 mutations are outside this binding region, and therefore the binding of ApoE to β /A4 will not be influenced. This may explain the influence of ApoE on clinical features in patients with these mutations [7]. To prove these possibilities, binding properties of the various aberrant β /A4 proteins to ApoE isoforms must be compared with each other and with the binding properties of normal β /A4.

Our findings do not affect the concepts about an association between APOE and AD. However, they illustrate the multifactorial etiology of β /A4 amyloid diseases, in which several genes relate to susceptibility or etiology.

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Immune Complex Deposition in the Choroid Plexus of Patients with Acquired Immunodeficiency Syndrome

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We identified immune complex deposits in the choroid plexus of approximately 75% of patients with acquired immunodeficiency syndrome (AIDS) who had either normal brains or human immunodeficiency virus encephalitis. Since circulating immune complexes are common in AIDS patients, and since local choroid plexus pathology usually was absent, their likely origin is from the bloodstream. Choroid plexus deposits of immune complexes have been implicated in altering the function of this structure or in enhancing its vulnerability to infection. Therefore, immune complex deposition in the choroid plexus of AIDS patients may be responsible for some of the common alterations in the cerebrospinal fluid and for the frequency of opportunistic infections in this region.

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Circulating immune complexes (ICs) are found in more than 80% of both adults and children with the acquired immunodeficiency syndrome (AIDS) [1, 2] and in chil-

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