

## APOLIPOPROTEIN E, DEMENTIA, AND CORTICAL DEPOSITION OF $\beta$ -AMYLOID PROTEIN

TUOMO POLVIKOSKI, M.D., RAIMO SULKAVA, M.D., MATTI HALTIA, M.D., KATARINA KAINULAINEN, M.D.,  
ALPO VUORIO, M.D., AULI VERKKONIEMI, M.D., LEENA NIINISTÖ, M.D., PIIRJO HALONEN, PH.D.,  
AND KIMMO KONTULA, M.D.

**Abstract** *Background.* The  $\epsilon 4$  allele of apolipoprotein E has been associated with an increased risk of late-onset Alzheimer's disease. In a cohort of elderly subjects we prospectively investigated the relation between the apolipoprotein E genotype, dementia, and the accumulation of  $\beta$ -amyloid protein in the cerebral cortex.

*Methods.* Autopsy involving neuropathological analysis and DNA analysis of frozen blood samples was performed in 92 of 271 persons who were at least 85 years of age, who had been living in Vantaa, Finland, on April 1, 1991, and who had died between that time and the end of 1993. All subjects had been tested for dementia. Apolipoprotein E genotyping was done with a solid-phase minisequencing technique. The percentage of the cortex occupied by methenamine silver-stained plaques was used as an estimate of the extent of  $\beta$ -amyloid protein deposition.

*Results.* The frequency of the  $\epsilon 4$  allele was signifi-

cantly higher in the subjects with Alzheimer's disease than in the subjects without dementia (30 percent vs. 8 percent,  $P < 0.001$ ). There was a greater accumulation of  $\beta$ -amyloid protein in the brain and more neurofibrillary tangles in the subjects with the  $\epsilon 4$  allele than in those without it ( $P < 0.001$ ). The deposition of  $\beta$ -amyloid protein varied according to the genotype in both the subjects with dementia and those without dementia: it was lowest in those with the  $\epsilon 2/\epsilon 3$  genotype, intermediate in those with the  $\epsilon 3/\epsilon 3$  genotype, and highest in those with the  $\epsilon 3/\epsilon 4$  genotype. A single subject had the  $\epsilon 4/\epsilon 4$  genotype and had dementia.

*Conclusions.* The  $\epsilon 4$  allele of apolipoprotein E is significantly associated with Alzheimer's disease. Even in elderly subjects without dementia, the apolipoprotein E genotype is related to the degree of deposition of  $\beta$ -amyloid protein in the cerebral cortex. (N Engl J Med 1995; 333:1242-7.)

**A**POLIPOPROTEIN E, a major component of circulating lipoproteins and an important regulator of lipid metabolism, exists in three major isoforms (E2, E3, and E4) encoded by three alleles ( $\epsilon 2$ ,  $\epsilon 3$ , and  $\epsilon 4$ ) of the apolipoprotein E gene.<sup>1,2</sup> The  $\epsilon 4$  allele has been associated with an increased risk of sporadic and familial late-onset Alzheimer's disease.<sup>3-9</sup> It also modifies the age at onset of familial Alzheimer's disease in a dose-dependent manner<sup>10-13</sup>; in contrast, it seems to have no influence on the risk of the familial form of Alzheimer's disease linked to chromosome 14.<sup>14</sup> The  $\epsilon 2$  allele may have a protective effect against late-onset Alzheimer's disease, independent of the effect of the  $\epsilon 4$  allele.<sup>8,15-17</sup>

Apolipoprotein E has been found in amyloid plaques in the cerebral cortex of patients with Alzheimer's disease as well as in other types of amyloid.<sup>3,18-21</sup> Isoforms E3 and E4 show affinity for the  $\beta$ -amyloid protein, an essential constituent of the senile plaques in Alzheimer's disease.<sup>22</sup> Because apolipoprotein E may also be associated with other types of amyloid proteins, it has been proposed that apolipoprotein E acts as a "pathological chaperone" promoting the tertiary structure needed for the formation of nonsoluble amyloid.<sup>19</sup> Apolipoprotein E may also modify the appearance of neurofibrillary tangles, another neuropathological hallmark of Alzheimer's disease, through interactions with the intraneuronal microtubule-associated  $\tau$  protein.<sup>23,24</sup> The presence of the apolipoprotein E isoform E4 may render

the  $\tau$  protein liable to phosphorylation, a step that favors the formation of neurofibrillary tangles.

We investigated the possible relation between the genetic variants of apolipoprotein E, cortical accumulation of  $\beta$ -amyloid protein, and the presence of neurofibrillary tangles in a general population of elderly people who had been prospectively evaluated for dementia.

## METHODS

### Subjects

The Vantaa 85+ Study includes all 601 persons born before April 1, 1906, who were living in the city of Vantaa, Finland, on April 1, 1991. A structured clinical examination and interview were carried out by a neurologist and a nurse. The criteria in the *Diagnostic and Statistical Manual of Mental Disorders* (third edition, revised)<sup>25</sup> were used for the diagnosis of dementia, the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association<sup>26</sup> were used for the clinical diagnosis of Alzheimer's disease, and those of the International Work Group of the National Institute of Neurological and Communicative Disorders and Stroke and the Association Internationale pour la Recherche et l'Enseignement en Neurosciences<sup>27</sup> were used for the diagnosis of vascular dementia. Initial blood samples for DNA analysis were obtained from 550 of the 553 subjects examined; 48 subjects could not be evaluated, most because they had died.

Autopsy involving neuropathological analysis was possible in 112 of the 271 subjects who had died by the end of 1993; frozen blood samples for DNA analysis were available from 92 of the 112. These 92 subjects (80 women and 12 men) were the focus of the study, and we evaluated their apolipoprotein E genotypes, the amount of  $\beta$ -amyloid protein deposited in cerebral cortex, and the presence of neurofibrillary tangles. The mean age at death in the subjects who underwent both autopsy and an assay for apolipoprotein E genotypes was similar to that for the entire group of all subjects who died (88.3 years vs. 89.0 years), as was the frequency of clinically diagnosed Alzheimer's disease (39 percent vs. 36 percent). However, there were more women (87 percent vs. 78 percent) and subjects with dementia (65 percent vs. 53 percent) in the group that underwent autopsy.

The average length of time from the clinical examination to death was 362 days (range, 19 to 859). At the time of the clinical examination 52 of the 92 subjects (57 percent) had dementia. The status of the subjects without dementia, of whom there were initially 40, was veri-

From the Departments of Pathology (T.P., M.H.), Medicine (K. Kainulainen, A. Vuorio, K. Kontula), and Neurology (A. Verkkoniemi), University of Helsinki, Helsinki; the Department of Community Health and General Practice, University of Kuopio, Kuopio (R.S., P.H.); and Katarina Geriatric Hospital, Vantaa (L.N.) — all in Finland. Address reprint requests to Dr. Polvikoski at the Department of Pathology, University of Helsinki, P.O. Box 21 (Haartmaninkatu 3), FIN-00014 Helsinki, Finland.

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fied from the written reports maintained by their physicians and the nursing staff. For these subjects the dementia status three months before death was used. The dementia status of 8 subjects changed from negative to positive, so that a total of 60 of the 92 subjects (65 percent) who died and for whom blood samples were available had dementia at the time of their deaths.

### Sampling Procedure and Staining Techniques

The brains were fixed in phosphate-buffered 4 percent formaldehyde solution for at least two weeks before specimens were obtained from the middle frontal, superior temporal, and middle temporal gyri and inferior parietal lobule, according to the protocol for the diagnosis of Alzheimer's disease outlined by the Consortium to Establish a Registry for Alzheimer's Disease (CERAD).<sup>28</sup> Care was exercised during sampling so that the cutting axis was perpendicular to the surface of the gyrus and the full thickness of cortex was present in tissue blocks.

Paraffin sections were cut at a thickness of 8  $\mu$ m for staining with methenamine silver for  $\beta$ -amyloid protein,<sup>29</sup> and 10  $\mu$ m for staining with methenamine silver–Bodian stain for neurofibrillary tangles<sup>30</sup> and with a modified Bielschowsky method for neuritic plaques. Methenamine silver staining has been shown to be as sensitive as  $\beta$ -amyloid protein immunostaining for detecting senile plaques, including diffuse plaques.<sup>29,31</sup>

### Neuropathological Diagnosis of Alzheimer's Disease

For the neuropathological diagnosis of Alzheimer's disease, the density of neuritic plaques was evaluated in the cortical sections stained with the modified Bielschowsky stain according to the CERAD protocol.<sup>28</sup>

### Quantitation of the Deposition of $\beta$ -Amyloid Protein

To estimate the amount of  $\beta$ -amyloid protein deposited in cerebral cortex, we quantified the fraction of the cortical specimens covered by methenamine silver–stained plaques.<sup>32</sup> We examined contiguous cortical microscopical fields, extending from the pial surface to the white matter, at a magnification of 100 $\times$  with a square microscopical graticule, 1.25 mm in width, along a line perpendicular to the pial surface. The graticule consists of 10 horizontal and 10 vertical lines with 100 intersections. All intersections that overlay a methenamine silver–positive plaque were counted. In every specimen at least 7 (maximum, 10) cortical columns (width, 1.25 mm) were examined from the pial surface to the white matter, and the fraction of cortex covered by plaques was determined by dividing the number of intersections overlying a methenamine silver–positive plaque by the total number of cortical intersections.

The average fraction of cortex covered by methenamine silver–positive plaques was calculated for all four specimens (from the middle frontal, superior temporal, and middle temporal gyri and inferior parietal lobule) from each subject to minimize the possible effect of variations in the extent of  $\beta$ -amyloid protein deposition in different regions of the brain. The final value (expressed as a percentage) provided an estimate of the extent of  $\beta$ -amyloid protein deposition in cerebral cortex.

### Counting of Neurofibrillary Tangles

After methenamine silver–Bodian staining, the neurofibrillary tangles were counted in contiguous cortical microscopical fields, extending from the pial surface to the white matter, at a magnification of 200 $\times$  with a square microscopical counting frame, 0.55 mm in width, along a line perpendicular to the pial surface. All neurofibrillary tangles totally inside the frame or hitting the left side of the frame (when examined from the pial side) were counted. In every specimen, 10 cortical columns (width, 0.55 mm) were examined from the pial surface to the white matter. The average number of neurofibrillary tangles counted per cortical specimen was determined by dividing the total number of neurofibrillary tangles in all four cortical specimens by four.

### Apolipoprotein E Genotyping

For apolipoprotein E genotyping a combination of the polymerase chain reaction (PCR) and a solid-phase minisequencing technique was used.<sup>33</sup> In short, the target DNA fragment was first amplified with

primers P1 and P2<sup>34</sup> flanking the two common polymorphic sites present in codons 112 and 158 of the apolipoprotein E gene. The PCR consisted of an initial denaturation at 99°C for 5 minutes, followed by 30 cycles of denaturation at 97°C for 1 minute, annealing at 65°C for 2 minutes, and a final extension for 10 minutes at 72°C. The amplified DNA was separated into two strands by alkali treatment, and the variable nucleotides in codons 112 and 158 were identified by two different primer-extension reactions.<sup>34</sup>

### Statistical Analysis

The data were analyzed with the SPSS/PC+ program. The non-parametric Mann–Whitney rank-sum test was used to compare the fraction of the cortex covered by methenamine silver–positive plaques in subjects with dementia and subjects without dementia, according to the genotype; to compare the amount of  $\beta$ -amyloid protein in subjects with cerebrovascular events and in those without them; to compare the number of neurofibrillary tangles in subjects with the  $\epsilon$ 4 allele of apolipoprotein E and in those without it; and to determine whether there was an association between dementia and the number of neurofibrillary tangles in subjects with the apolipoprotein E  $\epsilon$ 2/ $\epsilon$ 3,  $\epsilon$ 3/ $\epsilon$ 3, or  $\epsilon$ 3/ $\epsilon$ 4 genotype.

The chi-square test of independence was used to compare the frequency of the occurrence of  $\beta$ -amyloid protein deposition in subjects with dementia and in those without dementia and in subjects with the  $\epsilon$ 4 allele and in those without it. The Spearman nonparametric test was used to determine whether there was a correlation between the deposition of  $\beta$ -amyloid protein and the number of neurofibrillary tangles and between age and the amount of  $\beta$ -amyloid protein deposited or the number of neurofibrillary tangles. The distributions of the genotype across the diagnostic groups were compared by Fisher's exact test with a two-by-six table, and differences in the frequencies of specific apolipoprotein E genotypes or alleles were compared with Fisher's exact test with two-by-two tables.

### Approval for the Study

The study was approved by the Ethics Committee of the Health Center of the city of Vantaa. Permission for autopsy was obtained from the closest relative in all cases.

## RESULTS

According to the neuropathological criteria of the CERAD protocol,<sup>28</sup> 46 of the 60 subjects with dementia had definite or probable Alzheimer's disease. Of the remaining 14 subjects with dementia, 9 were judged to have vascular dementia on the basis of clinical findings, 1 had a malignant lymphoma infiltrating her brain, and 4 had dementia that could not be classified (Table 1).

There were significant differences in the distributions of the apolipoprotein E genotypes between the subjects with dementia and those without it ( $P=0.04$ ) and between the subjects with Alzheimer's disease and the subjects without dementia ( $P=0.002$ ) (Table 1). The apolipoprotein E  $\epsilon$ 3/ $\epsilon$ 4 genotype occurred more often in subjects with dementia than in those without it (40 percent vs. 16 percent,  $P=0.02$ ). The prevalence of this genotype was 50 percent in subjects with Alzheimer's disease ( $P=0.002$  for the comparison with the subjects without dementia who had this genotype). The frequency of the  $\epsilon$ 4 allele was significantly higher in the subjects with dementia (24 percent,  $P=0.009$ ) and in the group with Alzheimer's disease (30 percent,  $P<0.001$ ) than in the subjects without dementia (8 percent) (Table 1). There was only one carrier of the  $\epsilon$ 4 allele among the subjects with non-Alzheimer's dementia (Table 1).

Only 17 of the 92 subjects (18 percent) had no detectable  $\beta$ -amyloid protein in cortical specimens (Fig. 1).

The presence of  $\beta$ -amyloid protein was strongly associated with the presence of the  $\epsilon 3$  and  $\epsilon 4$  alleles: staining was positive in 31 of 33 subjects (94 percent) with at least one  $\epsilon 4$  allele and in 39 of 47 (83 percent) subjects who were homozygous for the  $\epsilon 3$  allele, but in only 5 of 12 (42 percent) with the  $\epsilon 2/\epsilon 2$  or  $\epsilon 2/\epsilon 3$  genotype (Fig. 1).

The mean fraction of the cortex occupied by methenamine silver–positive plaques was larger in the subjects with dementia than in those without it, but it varied in both groups in a similar way according to the apolipoprotein E genotype (Fig. 1). The lowest mean values for  $\beta$ -amyloid protein were associated with the  $\epsilon 2/\epsilon 2$  and  $\epsilon 2/\epsilon 3$  genotypes, whereas the highest mean values were associated with the  $\epsilon 2/\epsilon 4$ ,  $\epsilon 3/\epsilon 4$ , and  $\epsilon 4/\epsilon 4$  genotypes. When all 92 subjects were analyzed together, the mean value for  $\beta$ -amyloid protein was significantly higher ( $P < 0.001$ ) in the 33 with at least one  $\epsilon 4$  allele than in the 59 subjects without an  $\epsilon 4$  allele. When the  $\epsilon 2$  allele occurred together with the  $\epsilon 3$  allele (genotype,  $\epsilon 2/\epsilon 3$ ), the extent of  $\beta$ -amyloid protein deposition was significantly less ( $P < 0.02$ ) than that found in subjects with the  $\epsilon 3/\epsilon 3$  genotype.

In each of the three genotypes with the largest numbers of subjects (11 subjects with the  $\epsilon 2/\epsilon 3$  genotype, 47 with  $\epsilon 3/\epsilon 3$ , and 29 with  $\epsilon 3/\epsilon 4$ ), the mean values for  $\beta$ -amyloid protein were higher in the subjects with dementia than in those without it (Fig. 1). This difference was statistically significant in the subjects with the  $\epsilon 2/\epsilon 3$  genotype ( $P = 0.01$ ) or the  $\epsilon 3/\epsilon 4$  genotype ( $P = 0.03$ ), but not in those with the  $\epsilon 3/\epsilon 3$  genotype ( $P = 0.29$ ). However, the mean values for  $\beta$ -amyloid protein deposition in subjects with dementia and the  $\epsilon 2/\epsilon 3$  genotype were lower than those in the subjects with the

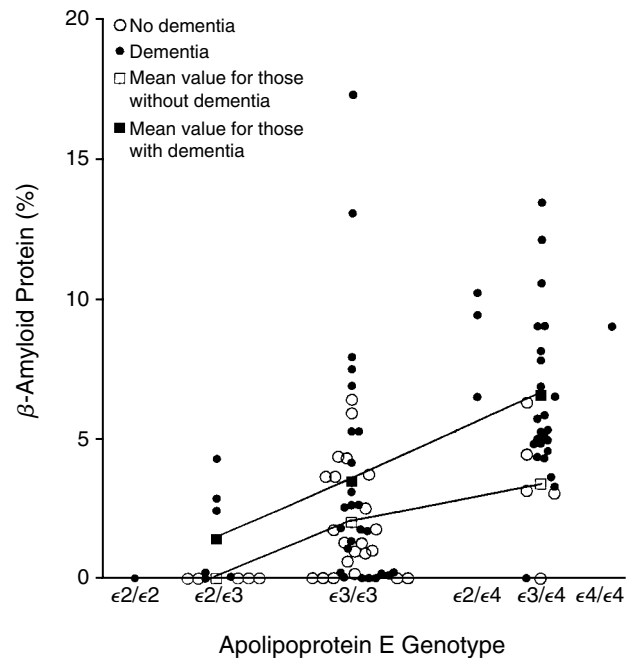


Figure 1. The Extent of Cortical Deposition of  $\beta$ -Amyloid Protein in the Brains of Subjects with Dementia and Subjects without Dementia, According to the Apolipoprotein E Genotype.

The extent of  $\beta$ -amyloid protein deposition was determined by calculating the percentage of cortex covered by methenamine silver–stained plaques. In each of the three largest genotype groups ( $\epsilon 2/\epsilon 3$ ,  $\epsilon 3/\epsilon 3$ , and  $\epsilon 3/\epsilon 4$ ), subjects with dementia had higher mean values for  $\beta$ -amyloid protein than subjects without dementia. The  $\epsilon 3$  and  $\epsilon 4$  alleles were strongly associated with the accumulation of  $\beta$ -amyloid protein in the cerebral cortex of both subjects with dementia and those without it.

other two genotypes, regardless of whether they had dementia.

There was a significant correlation between the number of neurofibrillary tangles and the presence of dementia in subjects with the  $\epsilon 3/\epsilon 4$  genotype ( $P = 0.01$ ), but not in subjects with the  $\epsilon 2/\epsilon 3$  or  $\epsilon 3/\epsilon 3$  genotype. The mean ( $\pm$ SD) number of neurofibrillary tangles was significantly higher in subjects with at least one  $\epsilon 4$  allele than in the subjects with no  $\epsilon 4$  allele ( $38.4 \pm 49.4$  vs.  $10.1 \pm 36.3$ ,  $P < 0.001$ ). There was a correlation between the extent of  $\beta$ -amyloid protein deposition and the number of neurofibrillary tangles ( $r = 0.74$ ,  $P < 0.001$ ) (Fig. 2).

There was no significant correlation between the age of the subjects and the accumulation of  $\beta$ -amyloid protein or the number of neurofibrillary tangles, as analyzed in the genotype groups with the largest numbers of subjects ( $\epsilon 2/\epsilon 3$ ,  $\epsilon 3/\epsilon 3$ , and  $\epsilon 3/\epsilon 4$ ). The only exception was the correlation between age and the number of neurofibrillary tangles in subjects with the  $\epsilon 2/\epsilon 3$  genotype ( $r = 0.71$ ,  $P = 0.01$ ). The lack of correlation may be due to the fact that only subjects who were at least 85 years old were enrolled in the study and to the relatively small number of the subjects in each group. Neither the amount of  $\beta$ -amyloid protein nor the number of neurofibrillary tangles differed significantly between the sexes within these genotype groups.

Seven of the 32 subjects without dementia and 17 of

Table 1. Clinical and Neuropathological Diagnosis of Dementia According to the Apolipoprotein E Genotype and Alleles.\*

GENETIC VARIABLE	DEMENTIA			No DEMENTIA
	ALZHEIMER'S DISEASE†	OTHER TYPES OF DEMENTIA	TOTAL	
	<i>number of patients (%)</i>			
Genotype				
$\epsilon 2/\epsilon 2$	0	1 (7)	1 (2)	0
$\epsilon 2/\epsilon 3$	3 (6)	3 (21)	6 (10)	5 (16)
$\epsilon 2/\epsilon 4$	3 (6)	0	3 (5)	0
$\epsilon 3/\epsilon 3$	16 (35)	9 (64)	25 (42)	22 (69)
$\epsilon 3/\epsilon 4$	23 (50)‡	1 (7)	24 (40)	5 (16)
$\epsilon 4/\epsilon 4$	1 (2)	0	1 (2)	0
All	46 (100)	14 (100)	60 (100)	32 (100)
Allele				
$\epsilon 2$	6 (7)	5 (18)	11 (9)	5 (8)
$\epsilon 3$	58 (63)	22 (79)	80 (67)	54 (84)
$\epsilon 4$	28 (30)§	1 (4)	29 (24)¶	5 (8)
All	92 (100)	28 (100)	120 (100)	64 (100)

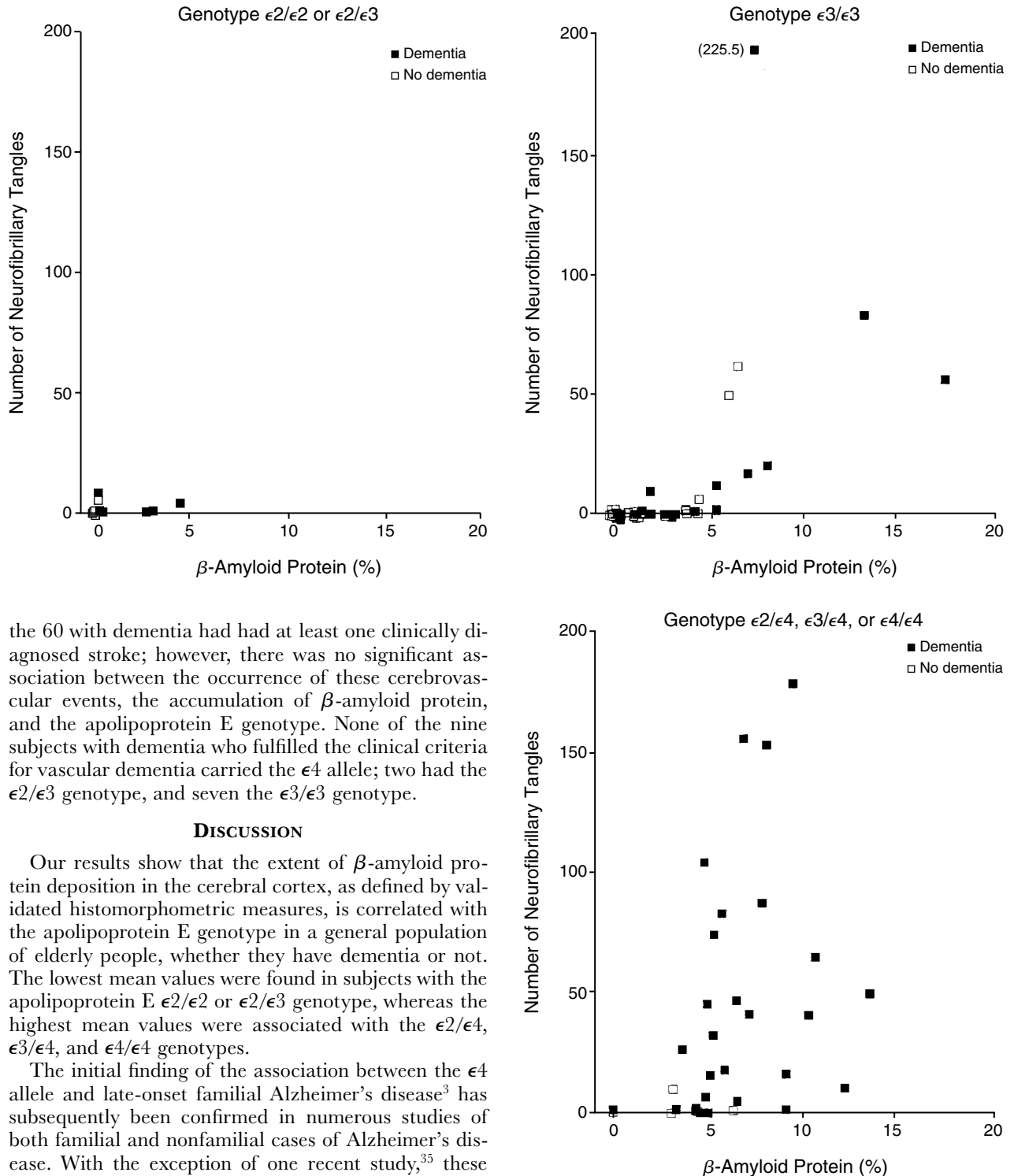
\*Because of rounding not all columns total 100 percent.

†Cases of definite or probable Alzheimer's disease according to the CERAD protocol.

‡ $P = 0.002$  for the comparison with the value for subjects with the  $\epsilon 3/\epsilon 4$  genotype who did not have dementia.

§ $P < 0.001$  for the comparison with the value for subjects with the  $\epsilon 4$  allele who did not have dementia.

¶ $P = 0.009$  for the comparison with the value for subjects with the  $\epsilon 4$  allele who did not have dementia.



the 60 with dementia had had at least one clinically diagnosed stroke; however, there was no significant association between the occurrence of these cerebrovascular events, the accumulation of  $\beta$ -amyloid protein, and the apolipoprotein E genotype. None of the nine subjects with dementia who fulfilled the clinical criteria for vascular dementia carried the  $\epsilon 4$  allele; two had the  $\epsilon 2/\epsilon 3$  genotype, and seven the  $\epsilon 3/\epsilon 3$  genotype.

**DISCUSSION**

Our results show that the extent of  $\beta$ -amyloid protein deposition in the cerebral cortex, as defined by validated histomorphometric measures, is correlated with the apolipoprotein E genotype in a general population of elderly people, whether they have dementia or not. The lowest mean values were found in subjects with the apolipoprotein E  $\epsilon 2/\epsilon 2$  or  $\epsilon 2/\epsilon 3$  genotype, whereas the highest mean values were associated with the  $\epsilon 2/\epsilon 4$ ,  $\epsilon 3/\epsilon 4$ , and  $\epsilon 4/\epsilon 4$  genotypes.

The initial finding of the association between the  $\epsilon 4$  allele and late-onset familial Alzheimer’s disease<sup>3</sup> has subsequently been confirmed in numerous studies of both familial and nonfamilial cases of Alzheimer’s disease. With the exception of one recent study,<sup>35</sup> these investigations have had a case-control design, rendering the data vulnerable to selection bias. Our study involved a randomly selected elderly population, was prospective in nature, and included a neuropathological examination of every subject. An important limitation of our study design is that the age of the subjects at enrollment ( $\geq 85$  years) was relatively high. This may explain the fact that in previous reports<sup>3-6,8,10</sup> the frequency of the  $\epsilon 4$  allele ranged from 0.36 to 0.52 in subjects with dementia and from 0.10 to 0.17 in subjects without

Figure 2. The Number of Neurofibrillary Tangles and the Extent of  $\beta$ -Amyloid Protein Deposition in the Brains of Subjects with Dementia and Subjects without Dementia, According to the Apolipoprotein E Genotype.

The number of neurofibrillary tangles was counted in paraffin sections from four cortical areas after staining with methenamine silver-Bodian stain. There was a positive correlation between the extent of  $\beta$ -amyloid protein deposition and the number of neurofibrillary tangles. The mean number of neurofibrillary tangles was significantly higher in subjects with an  $\epsilon 4$  allele than in those without it.

dementia, whereas the corresponding figures in our study were 0.30 and 0.08, respectively. In fact, we found recently that the frequency of the  $\epsilon 4$  allele in all living Finnish centenarians was only 0.08,<sup>36</sup> and there was no statistically significant association between the apolipoprotein E genotype and dementia in this very old group.<sup>37</sup> Thus, the  $\epsilon 4$  allele may adversely affect longevity, perhaps through its role as a risk factor for Alzheimer's disease or coronary artery disease<sup>1,2</sup> or some other mechanism.

In confirmation of earlier studies, our data indicate an association between the  $\epsilon 4$  allele and Alzheimer's disease. Twenty-eight of the 33 subjects (85 percent) carrying the  $\epsilon 4$  allele had dementia, and neuropathological examination disclosed Alzheimer's disease in 27 of them. However, since 5 carriers of the  $\epsilon 4$  allele did not have dementia and 19 of the 59 noncarriers of the allele (32 percent) had Alzheimer's disease confirmed by neuropathological examination, other factors, genetic or nongenetic, must also be important determinants of the risk of Alzheimer's disease.

Our neuropathological findings show that plaques containing  $\beta$ -amyloid protein in cerebral cortex are common in elderly people: 75 of the 92 subjects (82 percent) had detectable levels of  $\beta$ -amyloid protein. The presence of  $\beta$ -amyloid protein was significantly associated with the apolipoprotein E genotype: the percentages of subjects with detectable cortical accumulation of  $\beta$ -amyloid protein in the three most common genotypes —  $\epsilon 2/\epsilon 3$ ,  $\epsilon 3/\epsilon 3$ , and  $\epsilon 3/\epsilon 4$  — were 45 percent, 83 percent, and 93 percent, respectively. In addition, the extent of  $\beta$ -amyloid protein deposition varied in a similar way according to the apolipoprotein E genotype. Whereas all the subjects without dementia who had the  $\epsilon 2/\epsilon 3$  genotype were negative for cortical  $\beta$ -amyloid protein, most of the subjects without dementia who had the  $\epsilon 3/\epsilon 3$  or  $\epsilon 3/\epsilon 4$  genotype had cortical accumulation of  $\beta$ -amyloid protein (Fig. 1).

Although the older age of our subjects and the small size of the genotype groups preclude us from drawing definitive conclusions, our data suggest that whereas during normal aging cortical accumulation of  $\beta$ -amyloid protein is uncommon in persons with the  $\epsilon 2/\epsilon 3$  genotype, it often takes place in persons with the  $\epsilon 3/\epsilon 3$  or  $\epsilon 3/\epsilon 4$  genotype. It is also interesting that several carriers of the  $\epsilon 4$  allele had a substantial deposition of  $\beta$ -amyloid protein in their brains and yet did not have dementia. Our results are in agreement with previous studies. Schmechel et al.<sup>18</sup> and Rebeck et al.<sup>38</sup> reported that the density of cortical plaques in patients with Alzheimer's disease is associated with the type of apolipoprotein E4 genotype present: the highest density is associated with the  $\epsilon 4/\epsilon 4$  genotype, followed by  $\epsilon 3/\epsilon 4$  and then by  $\epsilon 3/\epsilon 3$ . There are contradictory data on the relative in vitro binding affinities of apolipoprotein isoforms E3 and E4 for the  $\beta$ -amyloid peptide.<sup>22,39</sup> Similar experiments with the E2 isoform have not been reported.

In addition to amyloid plaques, apolipoprotein E is also deposited in neurofibrillary tangles,<sup>19,20</sup> another neuropathological hallmark of Alzheimer's disease. The average number of neurofibrillary tangles was previously

reported to be greater in patients with Alzheimer's disease and the  $\epsilon 4/\epsilon 4$  genotype than in those with the  $\epsilon 3/\epsilon 3$  genotype, although this difference was at least partially attributed to a longer duration of illness in the former group.<sup>18</sup> A major component of the neurofibrillary tangles is the  $\tau$  protein, the phosphorylation and function of which may be regulated by apolipoprotein E in an isoform-dependent manner.<sup>23</sup> In our study, subjects with the  $\epsilon 4$  allele had significantly more neurofibrillary tangles than those without this allele (Fig. 2). The number of tangles was correlated with the extent of  $\beta$ -amyloid protein deposition.

Data on genetic variation of apolipoprotein E in vascular dementia seem to be scanty, possibly reflecting the lack of unequivocal diagnostic criteria for this form of dementia. A Japanese case-control study<sup>40</sup> reported that the frequency of the  $\epsilon 4$  allele in patients with vascular dementia was 21 percent, a figure close to that in patients with Alzheimer's disease (28 percent) and significantly higher than that in controls (9 percent). In our study none of the nine subjects judged to have pure vascular dementia had the  $\epsilon 4$  allele. Additional studies are required to clarify whether a common genetic variant of apolipoprotein E is associated with an increased risk of vascular dementia. Such studies must include autopsies involving neuropathological analysis, since it is not possible to exclude Alzheimer's disease on the basis of clinical criteria alone.

In conclusion, our results show that the common genetic variants of apolipoprotein E strongly modulate the accumulation of  $\beta$ -amyloid protein in the cerebral cortex of elderly people, regardless of whether they have dementia, with the most extensive deposition in persons with the  $\epsilon 4$  allele and the lowest in those with the  $\epsilon 2$  allele. Even in this very elderly population, the  $\epsilon 4$  allele was associated with Alzheimer's disease, although the association seemed to be weaker than in younger age groups. However,  $\beta$ -amyloid protein did not accumulate with advancing age in all carriers of the  $\epsilon 4$  allele, indicating that other genetic or nongenetic mechanisms must also have a role in the causation and pathogenesis of late-onset Alzheimer's disease.

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