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The apolipoprotein E allele ε4 does not correlate with the number of senile plaques or neurofibrillary tangles in patients with Alzheimer's disease

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Abstract

***Background and objectives*—Apolipoprotein E (apoE) has been implicated in regenerative processes in the brain after trauma, as well as in the pathogenesis of Alzheimer's disease. Inheritance of a specific apoε allele (apoε4) determines in part the risk and the mean age at onset of Alzheimer's disease. ApoE has been found to bind isoform specifically to β-amyloid protein, the major component of senile plaques, and to the microtubule associated protein tau, which forms paired helical filaments and neurofibrillary tangles. The aim was to further examine the relation between apoε alleles, especially apoε4, and the development of neuropathological changes associated with Alzheimer's disease.**

***Methods*—Brains of patients with Alzheimer's disease (n = 44) and vascular dementia (n = 11) and of age matched controls (n = 29) were studied. Senile plaques and neurofibrillary tangles in the hippocampus and frontal cortex were quantified.**

***Results*—No correlation was found between the number of apoε4 alleles and the number of senile plaques and neurofibrillary tangles in the hippocampus or the frontal cortex of patients with Alzheimer's disease, or vascular dementia, or control groups. No significant differences in duration or severity of dementia were found between patients with or without the apoε4 allele. No increased frequency of apoε4 was found in vascular dementia.**

***Conclusion and comment*—Although the apoε genotype clearly affects whether Alzheimer's disease will develop or not, the present study suggests that it has no influence on pathology or clinical intellectual status, once the dementia has manifested itself. No increased apoε4 allele frequency was found in neuropathologically diagnosed patients with vascular dementia in whom concomitant Alzheimer's disease can be excluded.**

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Alzheimer's disease is the most common form of dementia. It is a neurodegenerative disease, neuropathologically characterised by the presence of senile plaques and neurofibrillary tangles within neurons of the cerebral cortex and hippocampus, and by neuronal and synaptic degeneration. The major component of senile plaques is β-amyloid protein (β-A4 protein). Neurofibrillary tangles consist of abnormally hyperphosphorylated species of the microtubule associated protein tau, which forms paired helical filaments. Although rare familial forms of Alzheimer's disease exist, most patients have no clear family history, and are classified as having sporadic Alzheimer's disease. The exact aetiology and pathogenesis of sporadic Alzheimer's disease have not been elucidated.

Apolipoprotein E (apoE) is one of the major apolipoproteins which play a part in the transport, metabolism, and cellular recognition of serum lipoproteins.[1] ApoE is also present in the CNS. It is thought to play a vital part in the turnover of lipids or lipid soluble compounds in the CNS under normal conditions, by transporting lipids from the blood stream to brain cells that require them and, conversely, by eliminating excessive lipids from the CNS via the CSF, or through redistributing lipids among the cells in the CNS.[2,3] Immunohistochemical studies in rats have shown that apoE is synthesised within the CNS by astrocytes[2,4,5] and possibly also by oligodendrocytes.[6] ApoE is produced in increased amounts in the CNS after experimental brain injury.[5,7] It has therefore been suggested that apoE may be involved in the pathogenesis of brain disorders.

There is convincing support for apoE playing an important part in the pathogenesis of Alzheimer's disease.[1,8–11] The apoε gene shows polymorphism, with three different alleles (apoε2, ε3, and ε4), giving rise to three different isoforms (apoE2, E3, and E4), and thus six different apoE phenotypes.[12] The apoε3 allele is most common, found in about 78% of the population, whereas apoε4 is found in about 15%, and apoε2 in about 7%.[1] The strong association between the apoε4 allele and both late onset and early onset Alzheimer's disease has been confirmed in many laboratories around the world.[13–19] An increased frequency of the apoε4 allele has also been found in dementia of the Lewy body type,[20–23] whereas studies on vascular dementia have yielded contradictory results, with an increased frequency of apoε4 in some studies,[16,24–26] but not in others.[22,27–29]

The mechanism for the association between Alzheimer's disease and apoε4 is still unclear. One line of evidence suggests a stimulatory role in amyloid deposition and formation of senile plaques,[11,13,20] another line suggests a role in the

development of paired helical filaments and neurofibrillary tangles,[31] and a third line suggests a role in regenerative processes after various forms of brain trauma and degeneration.[32 33]

Some studies have found apoE4 to have higher avidity than apoE3 to β-A4,[11 34] whereas one study found the opposite.[35] It has also been reported that apoE in general and apoE4 in particular have a stimulatory role in β-A4 fibril formation—that is, they increase the rate of formation and the number of monofibrils being formed.[11 30 34] Moreover, some studies have found that patients with Alzheimer's disease who are homozygous for apoε4 have higher average density of senile plaques than patients with one or no apoε4 allele,[36-38] whereas other studies have found no such relation.[39 40] Based on these results, it has been hypothesised that apoE4 acts as a "pathological chaperone"—that is, by binding to β-A4, it increases the deposition of β-A4 and thus increases the formation of senile plaques.[11 13] In vitro studies have also shown that apoE3 binds to the microtubule associated protein tau with high avidity, whereas apoE4 does not bind to tau.[41] These findings have led to the hypothesis that apoE3 but not apoE4 slows the degree of tau phosphorylation and self assembly into paired helical filaments[31] and thus "protects" against the formation of neurofibrillary tangles. The number of neurofibrillary tangles at the time of death has, however, earlier been shown not to differ between patients with Alzheimer's disease and apoε4/4 and those with apoε3/3 genotypes.[36 37]

The possibility exists that these processes taken together may increase the formation of both senile plaques and neurofibrillary tangles in patients with Alzheimer's disease who possess the apoε4 allele.[37] This induced us to further study the possible involvement of apoE in senile plaque and neurofibrillary tangle formation by examining the extent of histopathological changes in brains from patients with Alzheimer's disease and relate these changes to the apoε4 allele apoE4 isoform. If the numbers of neurofibrillary tangles and senile plaques are dependent on the type of apoε alleles, the amount of these degenerative changes might be augmented in patients with the apoE4 isoform of the apoε4 allele.

Methods

PATIENTS AND CONTROLS

This study comprised the brains of 55 patients with dementia and 29 controls. Of the patients, 44 were clinically diagnosed with Alzheimer's disease (17 men; 27 women) and 11 with vascular dementia (8 men; 3 women).

The mean (SD) age at death was 79·3 (8·7) years in the Alzheimer's disease group, and 79·2 (7·0) years in the vascular dementia group. The duration of the disease was 8·0 (4·0) years in the Alzheimer's disease group, and 6·5 (6·4) years in the vascular dementia group.

All patients had undergone a clinical investigation including medical history, physical, neurological, and psychiatric examinations, screening laboratory tests, ECG, radiograph of the chest, and EEG before death. The age at onset was determined by means of interviewing the closest relatives when they first noticed a significant decline from a previous level of functioning, with reference to memory impairment or other cognitive disturbances.

The patients with Alzheimer's disease fulfilled the clinical NINCDS-ADRDA criteria for probable Alzheimer's disease.[42] They all had dementia with insidious onset and continuous progress, and no patient had signs or symptoms of cerebrovascular disease. No infarcts or other changes that could account for the dementia were present postmortem, and the histopathological score[43] was $\geqslant$ five in patients with Alzheimer's disease. All patients with Alzheimer's disease also fulfilled the National Institute of Aging (NIA) criteria for definite Alzheimer's disease.[44]

The vascular dementia group consisted of patients with history and symptoms of stroke, postmortem findings of brain infarcts, and a histopathological score[43] $\leqslant 4$.

Patients with a dementia of known aetiology, such as major head trauma, were excluded, as were those with manifest or suspected other primary (for example, tumours, infectious disease) or secondary (for example, depression, metabolic disturbances, toxic effects) causes of dementia.

At postmortem examination 29 subjects (19 men and 10 women) were chosen to constitute age matched controls. Their mean (SD) age at death was 74·1 (12·2) years. This group consisted of patients who had died from cardiac or malignant disease. Their medical records disclosed no history of dementia or other psychiatric or neurological disease. There were no macroscopic infarcts on postmortem examination, and the histopathological score[43] was $\leqslant 4$.

During the final stage of dementia, the patients were treated in long term psychiatric wards. In 50 of the demented patients the degree of dementia was rated retrospectively on the day after death. A nurse who was well acquainted with the patient used a dementia rating scale to measure the intellectual impairment included in the dementia syndrome.[45] The scale ranges from 0 (no intellectual impairment) to 58 (maximum intellectual impairment). The patients' condition during the two to three months before death was taken into account, but the agonal phase was disregarded.

For a semiquantitative estimation of senile plaques and neurofibrillary tangles, staining according to Bodian-PAS was performed on buffered formalin fixed, paraffin embedded material from the frontal cortex and the hippocampus. After magnification × 125 the numbers of senile plaques and neurofibrillary tangles were counted in five randomly selected fields. The absolute numbers (the mean count) were rated according to the method described by Alafuzoff *et al.*[43] The degree of arteriosclerosis in the basal arteries was noted[46] and quantified (0–3). The cerebral infarcts were quantified (0–2) by noting the number and locations of infarctions in the brain tissue and evaluating the approximate infarct volume.[47]

The study was approved by the ethics committee of Göteborg University.

ASSAYS

Apoε genotyping was performed on brain tissue samples by amplification of the fourth exon of the apoε gene by a polymerase chain reaction with biotinylated primers, followed by reverse DNA hybridisation on nitrocellulose strips, using the INNO-LIPA apoE assay (Innogenetics NV, Ghent, Belgium).

STATISTICS

Shapiro-Wilks' W test of normality was computed for the histopathological dementia score that did not meet the assumptions of normality ($P < 0.005$). As the standard tests for comparison of means and for correlation coefficients are based on normal distribution, we used non-parametric methods. Kruskal-Wallis' ANOVA by ranks was used for between group comparisons and Spearman's R for ordinal (rank order) variables. The level of significance chosen for rejection of the null hypothesis was $P \leqslant 0.05$.

Results

COMPARISONS BETWEEN GROUPS

The allelic frequency of the various apoε genotypes differed between the Alzheimer's disease (ε2 4·5%, ε3 52·3%, ε4 43·2%), vascular dementia (ε2 4·5%, ε3 72·7%, ε4 22·7%), and control groups (ε2 8·6%, ε3 70·7%, ε4 20·7%). The apoε4 allelegene was significantly more frequent in the Alzheimer's disease group than in the vascular dementia group and the non-demented controls ($P < 0.01$). The mean age at onset (mean (SD) Alzheimer's disease: 70·8 (9·9) years; vascular dementia: 72·6 (10·9) years), the age at death (mean (SD) Alzheimer's disease: 79·3 (8·7) years; vascular dementia 79·2 (7·0) years), or the duration of illness (mean (SD) Alzheimer's disease: 8·0 (4·0) years; vascular dementia: 6·5 (6·4) years) did not differ significantly between the groups. There was a significantly ($P < 0.001$) decreased brain weight in the Alzheimer's disease group compared with the vascular dementia and the control groups (mean (SD) Alzheimer's disease: 1214 (172)g; vascular dementia: 1283 (129)g; controls: 1362 (148)g).

NUMBER OF APOε4 ALLELES

The table shows comparisons of various variables between patients with and without the apoε4 allele in each of the three diagnostic groups. A semiquantitative estimation of senile plaques and neurofibrillary tangles in the frontal cortex and hippocampus showed that the ε4 allele had no significant influence on the density of senile plaques or neurofibrillary tangles in any of the groups.

There was no effect of the ε4 allele on age at onset of disease or duration of dementia, either in the Alzheimer's disease group or in the vascular dementia group. The brain weight and age at death did not differ significantly between patients with and without the ε4 allele in any of the diagnostic groups. Nor did the assessed degree of dementia—that is, the score on the intellectual impairment—differ between patients with and without the ε4 allele.

CORRELATIONS

The correlations between the assessed degree of dementia and the microscopic and clinical variables were analysed. There were significant correlations between the degree of dementia and the frontal senile plaques ($R = 0.32$, $P = 0.031$), the frontal neurofibrillary tangles ($R = 0.36$, $P = 0.014$), brain weight ($R = -0.36$, $P = 0.010$), duration of disease ($R = 0.54$, $P = 0.00007$), and age at onset of dementia ($R = -0.38$, $P = 0.0084$). No significant correlations were found between the degree of dementia and hippocampal senile plaques, hippocampal neurofibrillary tangles, or age at death.

Discussion

We divided the material into three groups: Alzheimer's disease, vascular dementia, and controls. The groups did not differ in basic variables such as age at onset of disease, duration of disease, and age at death. The brain weight was significantly less in the Alzheimer's disease group than in the control group, probably reflecting that the patients with Alzheimer's disease had a degenerative cerebral disease for on average eight years before death. The groups differed considerably in the distribution of apoε4 alleles, agreeing with earlier findings of increased apoε4 allele frequency in

Clinical variables, dementia ratings, and neuropathological findings in three diagnostic groups, Alzheimer's disease (AD), vascular dementia (VAD), and controls, each group divided into subgroups of patients with and without the apoε allele

	No of apoε4 alleles					
	AD		*VAD*		*Controls*	
	0 (n = 12)	*1–2 (n = 32)*	*0 (n = 8)*	*1–2 (n = 3)*	*0 (n =19)*	*1–2 (n = 10)*
Age at death (y)	82·5 (6·0)	78·1 (9·3)	80·1 (6·3)	76·7 (9·7)	75·7 (11·4)	71·0 (13·6)
Age at onset (y)	75·1 (8·9)	69·3 (10·0)	75·9 (6·7)	64·0 (16·8)	—	—
Duration (y)	7·0 (3·9)	8·4 (4·0)	4·2 (3·1)	12·7 (9·7)	—	—
Brain weight (g)	1189 (170)	1224 (174)	1298 (142)	1242 (94)	1345 (165)	1395 (108)
GGS scores	56·1 (2·1)	50·0 (9·4)	42·5 (9·7)	47·0 (13·5)	—	—
Frontal SP	1·8 (0·8)	1·8 (0·7)	0·62 (0·5)	0·7 (0·6)	0·6 (0·5)	0·4 (0·5)
Frontal NFT	1·4 (0·9)	1·3 (0·7)	0·25 (0·5)	0·3 (0·6)	0·16 (0·4)	0·0 (0·0)
Hippocampal SP	2·1 (0·8)	1·9 (0·8)	0·75 (0·5)	0·3 (0·6)	0·68 (0·5)	0·5 (0·5)
Hippocampal NFT	1·8 (0·7)	2·0 (0·9)	1·1 (1·0)	0·7 (0·6)	0·47 (0·6)	0·1 (0·3)
Arteriosclerosis	1·3 (0·7)	1·5 (0·8)	1·9 (0·8)	1·3 (0·6)	1·3 (0·8)	0·9 (0·7)
Cerebral infarcts	0·4 (0·8)	0·25 (0·6)	1·4 (0·9)	0·7 (0·6)	0·05 (0·2)	0·0 (0·0)

Values are means (SD).
No significant differences were found within the diagnostic groups between subjects with and without the apoε4 allele using Kruskal-Wallis' ANOVA by ranks. SP = Senile Plaques; NFT = neurofibrillary tangles; GGS = Gottfries-Gottfries scale: a rating scale measuring intellectual impairment of the dementia syndrome, range 0–58. Celebral infarcts were quantified using a semiquantitative measure (0–2) of the number, locations, and approximate volume of infarcts in the brain.

Alzheimer's disease. We found no association between vascular dementia and the apoε4 allele frequency, but several other clinical studies have found this association.[16 24-26] These findings may be explained by possible inclusion of cases with mixed Alzheimer's disease and vascular dementia pathology. Such mixed pathology is not likely in the present study, as the diagnoses were neuropathologically confirmed.

In some studies, patients with Alzheimer's disease homozygous for apoε4 have been shown to have higher average senile plaque density than patients with only one apoε4 allele,[36-38] whereas other studies have not found such a relation.[39 40] In the present study, we found no difference in senile plaque counts either in the frontal cortex or in the hippocampus, between patients with Alzheimer's disease with and without the apoε4 allele. Thus there are contradictory results concerning the relation between senile plaque load and the apoε4 allele in Alzheimer's disease.

The basis for the notion of a relation between the formation of senile plaques and the apoε4 allele is the finding that apoE has been shown to bind to β-A4 in vitro.[10 13] The results are contradictory, however. One study, using apoE purified from human plasma, found apoE4 to have higher avidity than apoE3 to β-A4,[11] whereas another study using apoE from transfected cell lines, found apoE3 to have higher avidity than apoE4.[35] Using in situ binding techniques, apoE has also been found to bind to senile plaques, probably by conformation dependent binding to β-A4, but this binding did not differ between brains with different apoE alleles.[48] The number of β-A4 positive plaques is more abundant than apoE positive plaques[49] and the apoE immunoreactivity is preferentially found in the core of classic senile plaques; many diffuse plaques do not immunostain with an anti-apoE antibody.[49 50] Taken together, these findings do not support the idea that apoE is involved in the early stages of fibrillary amyloid formation. Instead, the binding between β-A4 and apoE may be a secondary absorption by existing senile plaques, which would explain the failure to find any relation between the ε4 allele and senile plaque load.

In the present study, we found no differences in neurofibrillary tangle counts either in the frontal cortex or in the hippocampus between patients with Alzheimer's disease with and without the apoε4 allele. In agreement with our results, most studies have found that the number of neurofibrillary tangles does not differ between patients with Alzheimer's disease with and without the apoε4 allele.[37 39 51 52] However, Morris and coworkers found a higher density of neurofibrillary tangles in the frontal cortex of patients with Alzheimer's disease who were homozygous for the apoε4 allele than in those patients with one or no apoε4 allele. This difference was, however, not found in the parietal or temporal cortex.[40]

The hypothesis of a relation between the formation of neurofibrillary tangles and a specific apoε allele is based on studies showing that apoE3 binds to the microtubule associated protein tau with high avidity, whereas apoE4 does not bind to tau.[41] However, apoE has been found to be present in some neurons that do not contain neurofibrillary tangles both in patients with Alzheimer's disease and in non-demented aged subjects.[53] These findings suggest that intraneuronal apoE3, but not apoE4, slows the degree of tau phosphorylation and self assembly into paired helical filaments and neurofibrillary tangles by binding to tau, and may thus prevent the development of the neuronal pathology that occurs in Alzheimer's disease.[41 53]

Also using in situ binding techniques, apoE has been found to bind to neurofibrillary tangles in dephosphorylated tissue, probably by phosphorylation dependent binding to tau.[48] However, the binding to neurofibrillary tangles does not differ between brains with different apoε alleles,[48] and the level of paired helical filament tau does not differ significantly between patients with Alzheimer's disease with and without the apoε4 allele.[27] Immunoreactivity to ApoE is found in nearly all paired helical filament positive neurons, but only in a small percentage of neurons that are immunoreactive to hyperphosphorylated tau, suggesting that apoε becomes associated with tangle bearing neurons late in the neurofibrillary tangle genesis.[54] It has been suggested that the localisation of apoE in tangle bearing neurons may be due to an increase in apoE uptake secondary to cytoskeletal abnormalities and neuronal injury.[55] The failure of some studies[36 37] to find a relation between the neurofibrillary tangle load and the E4 allele supports this suggestion.

There were no significant differences in age at onset at dementia, duration of disease, and age at death between patients with and without the apoε4 allele, within the Alzheimer's disease and vascular dementia groups. Thus these findings do not indicate a more pronounced progress of dementia in patients with one or two apoε4 alleles, once the disease has manifested itself.

It seems that the rated degree of dementia correlated with the numbers of frontal senile plaques and neurofibrillary tangles, but not with the hippocampal degenerative findings, which may indicate that frontal degenerative changes are of special importance for intellectual impairment.

In conclusion, although the apoε genotype clearly affects whether Alzheimer's disease will develop or not, the present study suggests that it has no influence on the neuropathological burden or clinical intellectual impairment, once the dementia has developed.

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