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Association of apolipoprotein E phenotypes with late onset Alzheimer's disease: population based study

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Abstract

***Objective*—To determine the association between the e4 allele of apolipoprotein E and Alzheimer's disease in a randomly selected population sample.**

***Design*—Cross sectional population based study.**

***Subjects*—980 people aged 69 to 78 (349 men, 631 women).**

***Setting*—Population of Kuopio, eastern Finland.**

***Main outcome measures*—Presence of e4 allele and diagnosis of Alzheimer's disease by detailed neurological and neurophysiological evaluation.**

***Results*—46 (4·7%) subjects were classified as having probable or possible Alzheimer's disease. The frequency of the apolipoprotein E e4 allele was 0·359 in patients with Alzheimer's disease and 0·165 in subjects without dementia ($P<0·0001$). The prevalence of Alzheimer's disease was 2·9% in subjects with no e4 alleles, 7·6% in subjects with one e4 allele, and 21·4% in subjects with two e4 alleles of apolipoprotein E.**

***Conclusions*—Allele e4 of apolipoprotein is associated with Alzheimer's disease in a dose-response fashion in a randomly selected elderly population.**

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Introduction

Alzheimer's disease is a leading cause of dementia in elderly people. Genetic factors have an important role before age 60, when the disease is caused either by a mutation in the amyloid precursor protein on chromosome 21 or, more commonly, by an unidentified gene on chromosome 14.[1-2] Evidence is accumulating that apolipoprotein E is important in late onset Alzheimer's disease. Three common alleles, e2, e3, and e4 determine the six apolipoprotein E phenotypes E2/2, E2/3, E2/4, E3/3, E4/3, and E4/4. Plasma apolipoprotein E phenotypes modulate lipoprotein concentrations, particularly that of low density lipoprotein cholesterol.[3,4] Furthermore, phenotypes E4/4 and E4/3 have been associated with the risk of myocardial infarction and coronary heart disease,[5-7] particularly in young people, although there is some controversy about this.[8]

The first evidence that e4 allele of apolipoprotein E could be associated with Alzheimer's disease was published by Pericak-Vance *et al.*[9] They showed a genetic linkage to chromosome 19 in affected members of families with a history of Alzheimer's disease after the age of 60. Recently, several studies based on clinical series have shown an association between the e4 allele and Alzheimer's disease in elderly subjects.[10-14] These studies indicate that 30-40% of all Alzheimer's disease cases could be attributed to the allele. Thus, it is the most common risk factor for Alzheimer's disease known so far.

All the studies that have investigated the relation between apolipoprotein E polymorphism and Alzheimer's disease have included highly selected patients and corresponding controls. Therefore we investigated whether the association of the e4 allele with Alzheimer's disease could be found also in a randomly selected elderly population living in eastern Finland. The Finnish population is of particular interest because the frequency of the e4 allele is high in this population.[15]

Subjects and methods

We selected subjects for this study from those participating in a population based study investigating risk factors and prevalence of atherosclerotic vascular disease in elderly people. The baseline study was conducted in Kuopio, east Finland in 1986-8, and it included 1300 subjects aged 65-74 years who were randomly selected from the inhabitants of Kuopio.[16] The follow up study was performed in 1990-1, on average 3·5 years after the baseline study. Of the 1192 subjects still alive, 980 participated in the follow up examination, which also included screening for dementia. All subjects gave informed consent and the study was approved by the ethics committee of Kuopio University Hospital.

Dementia was diagnosed in three phases (box). In the first phase we used five neurophysiological tests to identify people who were potentially demented (box). These tests have been validated and a detailed descrip-

Diagnosis and classification of dementia

Phase 1: screening for dementia
Mini-mental state examination[17]
Russell's adaptation of the visual reproduction test[18]
Trail making test[19]
Verbal fluency test[20]
Buschke selective reminding test[21]

Phase 2: verification of dementia
Neuropsychological tests[22,23]
Digit span
Digit symbol subtest (Wais-R)
Digit symbol subtest (WISC-R)
Arithmetic subtest (Wais-R)
Boston naming test
Clock drawing test
Clock setting test
Copy a cube test
Block design test
Enhanced cued recall test
Fuld's adaptation of Blessed *et al*'s mental state test
Hamilton depression scale[24]
Haschinski ischaemic score[25]
Clinical and neurological examinations

Phase 3: classification of dementia
Computed tomography
Conventional and spectral power electroencephalography
Laboratory tests and cerebrospinal fluid examination

tion has been published.[26] The neuropsychological evaluation was performed by a psychologist, physician, or trained nurse during a single visit lasting about an hour and a half. Cognitive function was evaluated two to three weeks after the clinical and laboratory investigations of the follow up study.

Subjects who scored 1 SD or more below the mean score in the mini-mental state examination after adjustment for education or below the cut off score (1 SD or more below the mean in normal healthy elderly subjects of similar age) in three of the other screening tests, or both, were selected for an extensive neuropsychological and neurological examination to confirm the possibility of dementia. Patients completed 12 neuropsychological tests, and the neurological examination included an evaluation of depression with the Hamilton depression scale[24] and the possible effect of cerebrovascular diseases on cognitive function with Haschinski ischaemic score.[25] The diagnosis of dementia was based on the criteria of the *Diagnostic and Statistical Manual of Mental Disorder*, third edition revised (DSM-III-R).[27]

All potentially demented subjects were admitted to the department of neurology, Kuopio University Hospital, for further studies. The final diagnosis and classification of dementia was determined by a board of two neuropsychologists and two neurologists. Dementia was classified as follows: probable or possible Alzheimer's disease; multi-infarct dementia; or secondary dementia including other causes of dementia. The diagnosis of Alzheimer's disease was based on the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA)[28] and the diagnosis of multi-infarct dementia on DSM-III-R criteria.[27] The apolipoprotein E phenotype was determined from the plasma with isoelectric focusing and immunoblotting techniques.[15,29]

TABLE I—*Numbers (percentages) of men and women with different types of dementia*

Dementia type	Men (n=349)	Women (n=631)	Total (n=980)
Alzheimer's disease	14 (4·0)	32 (5·1)	46 (4·7)
Multi-infarct dementia	6 (1·7)	3 (0·5)	9 (0·9)
Other causes	5 (1·5)	5 (0·7)	10 (1·0)
Total	25 (7·2)	40 (6·3)	65 (6·6)

TABLE II—*Frequency of alleles of apolipoprotein E in subjects without dementia and those with Alzheimer's disease (95% confidence intervals in parentheses)* *

Apolipoprotein E alleles	No dementia (n=911)	Alzheimer's disease (n=46)
e2	0·052 (0·038 to 0·066)	0·022 (–0·021 to 0·065)
e3	0·783 (0·756 to 0·810)	0·620 (0·479 to 0·761)
e4	0·165 (0·141 to 0·189)	0·359 (0·220 to 0·498)†

*Apolipoprotein phenotype was not available for four patients.
†P<0·0001.

TABLE III—*Effect of number of apolipoprotein E e4 alleles on prevalence of Alzheimer's disease*

No of alleles	No of patients	No (%) with Alzheimer's disease
0	652	19 (2·9)
1	277	21 (7·6)
2	28	6 (21·4)

STATISTICAL ANALYSIS

Statistical analyses were conducted with the SPSS/PC+ program. The results for continuous variables are given as a mean and SE. The differences between the two groups were assessed by Student's *t* test or χ^2 test as appropriate. Apolipoprotein E e4 allele frequencies were compared with *Z* statistics.[30] Confidence intervals for apolipoprotein e4 allele frequencies were calculated according to Gardner and Altman.[31] Odds ratios and their 95% confidence intervals were calculated by the Mantel-Haenszel estimate.

Results

Altogether 349 men and 631 women were included in the study. The mean (SE) age was 73·0 (0·1) years (range 69-78), and the length of education 6·6 (0·1) years. A total of 232 completed the neuropsychological tests to verify dementia, and 66 were admitted for classification of dementia.

Table I shows the prevalence of different types of dementias by sex. Altogether 6·6% (65) of subjects (7·2% of men, 6·3% of women) had dementia. Alzheimer's disease was diagnosed in 4·7% (46) of subjects (4·0% of men, 5·1% of women). In 38 of these 46 patients the diagnosis of Alzheimer's disease was new. Alzheimer's disease was familial in eight patients and sporadic in 38 patients. Multi-infarct dementia was substantially less common, occurring only in 0·9% (9) of the subjects (1·7% of men, 0·5% of women).

Table II shows the frequency of apolipoprotein E alleles in patients with Alzheimer's disease and in subjects without dementia. The e4 allele was twice as common in patients with Alzheimer's disease as in subjects without dementia (0·359 *v* 0·165, P<0·0001). Multi-infarct dementia or other causes of dementia were not associated with a high frequency of e4 allele (data not shown). To investigate the gene dose effect of the e4 allele on the prevalence of Alzheimer's disease we determined the prevalence of Alzheimer's disease by the number of alleles (none=652, one=277, two=28). Table III shows there was a large increase in the proportion of patients with Alzheimer's disease with an increasing apolipoprotein E e4 gene dose. Odds ratios for Alzheimer's disease were 2·7 (95% confidence interval 1·4 to 5·2) and 9·1 (3·5 to 23·4) with the presence of one and two e4 alleles, respectively, compared with no e4 alleles.

Discussion

Recent reports have shown that patients with Alzheimer's disease have significantly higher frequency of the e4 allele of apolipoprotein E than corresponding controls.[10-13] These studies, however, can be criticised because they have been based on clinical series of patients with Alzheimer's disease rather than on population based cohorts. Our study of

a random sample of 980 elderly Finnish subjects showed that the presence of the e4 allele is associated strongly with Alzheimer's disease. Furthermore, there was a clear gene dose effect of the e4 allele. Our results therefore confirm those in selected patients and extend the findings to randomly selected populations.

We found that the e4 allele frequency was twice as high in patients with Alzheimer's disease as in subjects without dementia (0·359 *v* 0·165). Other studies have reported a higher allele frequency ranging from 0·36 to 0·50.

The prevalence of Alzheimer's disease in this study was 4·7%, and it was the commonest type of dementia. Multi-infarct dementia was uncommon (1·7% in men, 0·5% in women), and we may have underestimated the true frequency of multi-infarct dementia because only subjects capable of visiting outpatient clinics were included. The frequency of Alzheimer's disease agrees with a previous study from Finland that found a prevalence of Alzheimer's disease of 2·7% in people aged 70-74 and 5·1% in those aged 75-79.[32]

The prevalence of Alzheimer's disease in the Finnish population is similar to that in other populations.[33] Therefore, although the prevalence of the e4 allele is high in the Finnish population,[15 34] it does not seem to cause an excess of Alzheimer's disease. How can we explain this finding? The e4 allele is also associated with high total and low density lipoprotein cholesterol concentrations[3 4] and coronary heart disease.[5-7] Hypercholesterolaemia and coronary heart disease are common in the Finnish population and the high frequency of the e4 allele may contribute to the excess risk of coronary heart disease, particularly in middle age. This theory is supported by the observation that the frequency of e4 allele in our study in subjects without dementia was 0·165 compared with 0·244 previously reported in middle aged Finnish subjects.[15] Furthermore, the e4 allele frequency among the Finnish nonagenarians was only half that in middle aged Finnish subjects.[35] Thus, the e4 allele seems to have different effects on morbidity and mortality depending on age. In middle aged people the e4 allele is likely to cause an excess of coronary heart disease and in elderly people it is associated with Alzheimer's disease in subjects who have survived coronary heart disease.

Finally, we showed a clear dose-response relation between the number of e4 alleles and the prevalence of Alzheimer's disease. The presence of one allele increased the risk 2·7-fold and the presence of two alleles 9·3-fold. The dose-response relation between the e4 allele and Alzheimer's disease further supports the essential role of the allele in the pathogensis of Alzheimer's disease. Further studies are needed to determine whether the association is direct or indirect.

Clinical implications

- Alzheimer's disease has been associated with the e4 allele of apolipoprotein E in clinical series
- In our population based study the frequency of the e4 allele was twice as high in patients with Alzheimer's disease as in patients without dementia
- There was a clear dose-response relation between the number of e4 alleles and the prevalence of Alzheimer's disease
- The e4 allele seems to be a risk factor for Alzheimer's disease. Studies are needed to determine the reason for the increased risk

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