The role of apolipoprotein E in atherosclerosis and dementia

An epidemiological approach to genetic susceptibility

De rol van apolipoproteïne E
in atherosclerose en dementie

Een epidemiologisch onderzoek naar erfelijke aanleg

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de rector magnificus
Prof.dr P.W.C. Akkermans M.A.
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 9 september 1998 om 11:45 uur

door

Arjen Jacob Cornelis Slooter

geboren te Roermond.

Promotiecommissie

Promotor	:	Prof.dr A. Hofman
Co-promotor	:	Dr C.M. van Duijn
Overige leden	:	Prof.dr C. Van Broeckhoven
		Dr D.G. Clayton
		Prof.dr P.J. Koudstaal

Als je door niets dan de blinde drift een boek of geschrift te maken en met de inktpot te spelen begonnen bent, en de tweede stap gezet wordt uit ijdelheid of door de noodzaak eindelijk eens te worden bewonderd, volgt daaruit nog lang niet dat de laatste stap, die naar het spreken van de waarheid, ook nog zal worden genomen

Willem Frederik Hermans

Contents

Papers and manuscripts based on the studies described in this thesis

Chapter 2

Slooter AJC, Van Duijn CM. Genetic epidemiology of Alzheimer's disease. Epidemiol Rev 1997;19:107-19.

Chapter 3

Slooter AJC, Van Gool WA, Van Duijn CM. De plaats van apolipoproteïne-E-genotypering in de differentiaaldiagnostiek van dementie. Ned Tijdschr Geneeskd 1996;140:2073-6.

Slooter AJC, Breteler MMB, Ott A, Van Broeckhoven C, Van Duijn CM. APOE genotyping in differential diagnosis of Alzheimer's disease. Lancet 1996;348:334.

Chapter 4

Slooter AJC, Tang M-X, Van Duijn CM, Stern Y, Ott A, Bell K, Breteler MMB, Van Broeckhoven C, Tatemichi TK, Tycko B, Hofman A, Mayeux R. Apolipoprotein ε4 increases the risk of dementia with stroke. A population-based investigation. JAMA 1997;277:818-21.

Slooter AJC, De Knijff P, Hofman A, Cruts M, Breteler MMB, Van Broeckhoven C, Havekes LM, Van Duijn CM. Serum apolipoprotein E level is not increased in Alzheimer's disease: The Rotterdam Study. Neurosci Lett 1998;248:21-4.

Slooter AJC, Houwing-Duistermaat JJ, Van Harskamp F, Cruts M, Van Broeckhoven C, Breteler MMB, Hofman A, Stijnen Th, Van Duijn CM. Apolipoprotein E genotype and progression of Alzheimer's disease. The Rotterdam Study. Submitted.

Chapter 5

Slooter AJC, Havekes LM, Bots ML, De Knijff P, Cruts M, Grobbee DE, Hofman A, Van Broeckhoven C, Witteman JCM, Van Duijn CM. Apolipoprotein E and atherosclerosis: The Rotterdam Study. Submitted.

Chapter 6

Slooter AJC, Van Duijn CM, Bots ML, Ott A, Breteler MMB, De Voecht J, Wehnert A, De Knijff P, Havekes LM, Grobbee DE, Van Broeckhoven C, Hofman A. Apolipoprotein E genotype, atherosclerosis, and cognitive decline: The Rotterdam Study. J Neural Transm. 1998;553:17-29.

Slooter AJC, Cruts M, Ott A, Bots ML, Witteman JCM, Hofman A, Van Broeckhoven C, Breteler MMB, Van Duijn CM. Atherosclerosis is not intermediate in the association of apolipoprotein E and dementia: The Rotterdam Study. Submitted.

Ott A, Slooter AJC, Hofman A, Van Harskamp F, Witteman JCM, Van Broeckhoven C, Van Duijn CM, Breteler MMB. Smoking and the risk of dementia and Alzheimer's disease in a population-based cohort study: The Rotterdam Study. Lancet 1998;351:1840-3.

Chapter 7

Slooter AJC, Cruts M, Kalmijn S, Hofman A, Breteler MMB, Van Broeckhoven C, Van Duijn CM. Risk estimates of dementia by apolipoprotein E genotypes from a population-based incidence study: the Rotterdam Study. Arch Neurol 1998;55:964-8.

Slooter AJC, Cruts M, Breteler MMB, De Jong PTVM, Pols HAP, Witteman JCM, Hofman A, Van Broeckhoven C, Van Duijn CM. The impact of the apolipoprotein E genotype on myocardial infarction, stroke, dementia and mortality: The Rotterdam Study. To be submitted.

Chapter 8

Slooter AJC, Van Duijn CM. Apolipoprotein E and Alzheimer's disease. Findings and challenges after five years of research. To be submitted.

1

General introduction

1

General introduction

Atherosclerosis and dementia are major problems in elderly people. Atherosclerosis, the principal cause of myocardial infarction, stroke and peripheral arterial disease, is the most important cause of mortality in western countries.[1] Dementia is the major threat to the wellbeing of older people. It is an extremely devastating condition, as a state of complete dependency often follows an extended period of loss of personality and cognition.[2] Since the frequency of both atherosclerosis and dementia increases with advancing age, the two conditions are enormous problems in our ageing societies.[3,4]

High levels of total and low density lipoprotein (LDL) cholesterol and low levels of high density lipoprotein (HDL) cholesterol predispose to the development of atherosclerosis.[5] The apolipoprotein E polymorphism (APOE) is an important determinant of the level of these lipids in serum. The APOE gene has three common alleles, APOE*2, APOE*3 and APOE*4, which determine the protein isoforms apoE2, apoE3 and apoE4, respectively.[6] Compared to homozygotes for APOE*3, the most common genotype, APOE*2 is associated with lower levels of total and LDL cholesterol and with higher levels of HDL cholesterol, while APOE*4 has opposite effects.[6] However, studies on the association between APOE and atherosclerosis have been inconsistent.[7,8] Moreover, it is yet unclear to what extent APOE is related to mortality and cardiovascular diseases in the general population.

Besides its role in lipid metabolism, the APOE*4 allele is more prevalent in patients with Alzheimer's disease than in cognitively normal controls. Although this association is well established, reported risks vary tremendously across studies.[9-11] The large majority of these investigations are cross-sectional studies based on clinical patient-series. Selection bias may have played a role in these studies, as well as possible APOE related selective survival. It is yet unclear whether the APOE*2 allele may

exert a protective effect, and whether serum apoE level contributes to the aetiology of Alzheimer's disease. In addition, it remains to be determined whether the APOE genotype is also involved in other dementing diseases, and, related to the latter topic, whether APOE testing could be used in the differential diagnosis of dementia. Furthermore, the combined effects of the APOE*4 allele and atherosclerosis deserve further attention, as these are both common risk factors for dementia.[12]

The aim of this thesis was to further investigate the role of apolipoprotein E in atherosclerosis and dementia. These studies reflect an epidemiological approach to genetic susceptibility.

References

1. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993;362: 801-9.
2. Cummings JL. Dementia: the failing brain. Lancet 1995;345:1481-9.
3. Van Duijn CM. Epidemiology of the dementias: recent developments and new approaches. J Neurol Neurosurg Psychiatry 1996;60:478-488.
4. Hart- en vaatziekten in Nederland. Cijfers over ziekte en sterfte. Nederlandse Hartstichting. Den Haag, 1997.
5. Pooling Project Research Group. Relationship of blood pressure, serum cholesterol, smoking habit, relative weight and ECG abnormalities to incidence of major coronary events: final report of the pooling project. J Chron Dis 1978;31:201-306.
6. Davignon J, Gregg RE, Sing F. Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis 1988;8:1-21.
7. De Andrade M, Thandi I, Brown S, et al. Relationship of the apolipoprotein E polymorphism with carotid artery atherosclerosis. Am J Hum Genet 1995;56:1379-90.
8. Hixson JE and the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. Apolipoprotein E polymorphism affect atherosclerosis in young males. Arterioscler Thromb 1991;11:1237-44.
9. Corder EH, Saunders AM, Strittmatter WJ, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 1993;261:921-3.
10. Henderson AS, Easteal S, Jorm AF, et al. Apolipoprotein E allele ε4, dementia, and cognitive decline in a population sample. Lancet 1995;346:1387-90.
11. Myers RH, Schaefer EJ, Wilson PWF, et al. Apolipoprotein E ε4 association with dementia in a population-based study: the Framingham study. Neurology 1996;46:673-7.
12. Hofman A, Ott A, Breteler MMB, et al. Atherosclerosis, apolipoprotein E, and the prevalence of dementia and Alzheimer's disease in the Rotterdam study. Lancet 1997;349:151-4.

2

GENETIC EPIDEMIOLOGY OF ALZHEIMER'S DISEASE

2

Review on genetic epidemiology of Alzheimer's disease

Introduction

Dementia is a major health problem in the elderly. Following an extended period of loss of personality and cognition, the disease often results in a state of complete dependency. By far the most common cause of dementia is Alzheimer's disease (AD), which is clinically characterized by a gradual, progressive decline in intellectual functions.[1] Besides, psychosis, depression, agitation and anxiety are common manifestations.[2] The most frequently used diagnostic criteria for AD are described by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA).[3] The diagnosis of AD is considered to be probable when alternative causes of dementia are excluded.[3,4] According to the NINCDS-ADRDA criteria, a definite diagnosis is made when a probable diagnosis of AD is confirmed at autopsy.[3] The neuropathologic characteristics of AD are senile plaques, neurofibrillary tangles, amyloid angiopathy and neuronal loss, resulting in a decreased activity of the enzyme choline acetyltransferase.[5] Senile plaques are extracellular deposits of predominantly β-amyloid. Neurofibrillary tangles are intraneuronal inclusions, which are in part composed of abnormally phosphorylated tau proteins.

The prevalence of AD increases with advancing age. It affects less than 1 percent of 60-64 year olds, and up to 40 percent of those over age 85.[6] Also the incidence increases with aging, and is estimated to be 1 per 1000 person years in 60-64 year olds, and 25 per 1000 person years in individuals older than 85 years.[7] In AD research, patients with early onset are often distinguished from those with late onset. There is however no uniform definition, and frequently used cut-off points are the age of 60, 65 and 70 years. Besides age, previous head injury or depression as well as

low educational level, atherosclerosis and exposure to aluminium were found to be risk factors for AD.[7-9] The use of anti-inflammatory drugs or estrogens seems to decrease the risk of AD.[7,10] Smokers were also found to have a decreased risk in cross-sectional studies,[11] but at an increased risk in a follow-up study.[12]

Besides, genetic factors play a role in the etiology of AD. Familial clustering has long been recognized,[13] and a positive family history of dementia is one of the most consistent risk factors.[14] A distinction is often made between patients with familial and sporadic AD. Again, there is no uniform definition of these forms of disease. The frequently used criterion of a positive family history of dementia, however, does not necessarily indicate genetic susceptibility. Many people, and not just those individuals who are genetically predisposed, develop AD in old age. On the other hand, there is significant likelihood that genetically susceptible relatives decease before the onset of AD. Given these limitations, it may not be surprising that clinical manifestations of the two AD types appeared to be similar.[15,16] However, patients with familial AD may have a more rapid disease progression,[17] and an earlier onset than cases with sporadic AD.[16]

The first step towards the localization of the genes implicated in AD (or their alternative forms, alleles) was achieved through linkage analysis, which requires no a priori knowledge of the pathogenesis. With this technique, one can study in large families the extend to which a localized marker allele is inherited together with a putative disease allele. Several mutations have now been identified that are by themselves sufficient to cause AD (i.e. autosomal dominant mutations). In addition, a number of genes have been studied because they code for proteins which are part of the AD pathology. Through this candidate gene approach, common variations in genes (i.e. polymorphisms) were identified which increase the risk of AD, but are by themselves not sufficient to cause the disease. These genes are referred to as susceptibility genes.

In this review, the genes involved in AD will be discussed in light of their relative contribution to the development of disease and their putative role in the pathogenesis. First, we will consider the autosomal dominant mutations and the susceptibility genes. Next we will review the interaction between different genes and environmental risk factors for AD. Finally, we will discuss the clinical and public health implications.

Autosomal dominant mutations

Amyloid precursor protein

The localization of the first gene involved in AD was hinted to by the observation that AD is very common in middle-aged patients with trisomy 21 (Down's

syndrome).[18,19] As most Down's syndrome patients carry three copies of chromosome 21 instead of two, this finding indicated that overexpression of one or more genes on chromosome 21 may lead to the development of AD. In 1987, the gene encoding for the amyloid precursor protein (APP) was isolated and localized on chromosome 21 (region 21q11.2-21q21.2).[20] APP is the precursor for β-amyloid, a protein that accumulates in senile plaques and cerebral blood vessels in AD brains. Therefore, the APP gene was considered to be a candidate for AD despite the fact that the causal relation between senile plaque formation and the development of AD was an issue of debate. Arguing strongly against a causal relation is the absence of a relation between severity of dementia in AD patients and β-amyloid burden.[21] Moreover, senile plaques can also be observed in brains from nondemented elderly.[22]

In 1991, a mutation in the APP gene was identified which leads to AD.[23] Since then, several other APP mutations have been found that cause autosomal dominant forms of AD with an early onset.[24] Each of these variations change the coding for one amino acid. At position 717, three different substitutions of valine have been found: to isoleucine (Val717→Ile),[23,25-29] to phenylalanine (Val717→Phe),[30] and to glycine (Val717→Gly).[31] Other mutations include a substitution of asparagine instead of lysine at position 670 (Lys670→Asn),[32] methionine for leucine at position 671 (Met671→Leu), and alanine instead of glycine at 692 (Ala692→Gly).[33]

It has become clear that mutations in the APP gene may explain only a limited number of patients with AD.[34-39] The Val717→Ile mutation was found in only nine families[23,25-29]; these include families from European as well as Japanese origin, which suggests that several independent mutations may have occurred. The other APP gene mutations have been observed in one family each. Although the clinical and public health implications of these rare mutations are thus limited, the finding that APP mutations lead to AD implies that ß-amyloid is most likely involved in the disease etiology. Several studies have examined the consequences of the APP mutations to the ß-amyloid protein (i.e., the product of the APP gene). The consequence of the Val717→Ile mutation may be that longer ß-amyloid fragments are generated, which aggregate more rapidly.[40] For the Lys670→Asn and the Ala692→Gly mutations, the secretion of ß-amyloid appears to be increased.[41-43] Although several functions have been proposed for ß-amyloid and APP, their function is not yet known.[44] APP resembles, on the basis of its amino acids sequence a cell-surface receptor.[45]

APP mutations are not solely related to AD but may also lead to other disorders. A mutation in codon 693 of the APP gene, replacing glutamic acid by glutamine (Glu693→Gln), causes Hereditary Cerebral Hemorrhage with Amyloidosis of the Dutch type, an autosomal dominant form of hemorrhage due to amyloidosis.[46,47] The Ala692→Gly mutation results in AD and in cerebral hemorrhages due to congophylic amyloid angiopathy.[33]

Presenilins

Since APP could only explain the development of AD in a small number of families, several groups have searched for other implicating genes. In 1992, the second AD gene was localized on chromosome 14.[48] However, this gene could not be isolated until 1995.[49] It was designated presenilin 1 (PS-1) because of the presenile onset of disease in the families studied. It has been suggested that mutations in this PS-1 gene may cause up to 70 percent of all autosomal dominant AD with onset before 55 years.[49-51] However, screening of a population-based sample of early onset AD patients, revealed PS-1 mutations in not more than 7 percent.[52] At present, over 40 different mutations in PS-1 have been described.[49-60] The diverse ethnic origin of patients with the same PS-1 variation suggests a high frequency of new mutations. Therefore, it cannot be excluded that mutations in the PS-1 gene can also be found in AD patients with the sporadic form (i.e., cases with no family history of dementia). Mutations in the PS-1 gene may also lead to AD with a late onset. Carriers of two type 1 alleles at intron 8, a polymorphism that is not expressed, were found to have an increased risk for the late onset form of AD.[61] However, this finding could not be confirmed by others.[62] The exact proportion of AD patients that can be ascribed to PS-1 mutations has yet to be determined.

Within months after the identification of PS-1, a similar gene on chromosome 1 was localized, which was termed presenilin 2 (PS-2).[58] At present, two PS-2 mutations that cause AD have been found. But, PS-2 mutations appear to be a rare cause of early onset AD. One of the PS-2 mutations was identified in seven families of Volga-German origin.[63] The second PS-2 mutation was found in an Italian AD family.[58]

The role of the presenilins in the pathogenesis of AD is unknown. The gene product of PS-1 is a membrane protein.[51] PS-1 has been found in senile plaques from AD patients, and not only in those with a mutation in the PS-1 gene.[64] It has been suggested that a mutation in PS-1 leads to greater secretion of long ß-amyloid which aggregates more rapidly.[60,65]

Susceptibility genes

Apolipoprotein E

Before the discovery that apolipoprotein E (apoE=protein, APOE=gene) is involved in the etiology of AD, apoE had been studied because of its pivotal role in lipid metabolism.[66] The APOE gene is localized on chromosome 19 (region 19q13.2) and has three common alleles (APOE*2, APOE*3 and APOE*4) that code for three different isoforms (apoE2, apoE3 and apoE4, respectively).[66] The apoE isoforms are

structurally very similar, but differ in one or two amino acids: apoE3 has cysteine on position 112 and arginine on position 158, apoE4 has arginine on these positions, and apoE2 cysteine.[67] In populations of European ancestry, the APOE*3 allele has a frequency of 0.77. APOE*2 and APOE*4 are less common (allele frequencies respectively 0.08 and 0.15 in populations of European origin).[68] APOE*4 is more rare in Chinese and Japanese populations (0.06-0.12),[68] and more frequent in Africans (0.21) and in Finns (0.23).[68,69]

Strength of association In 1991, linkage to chromosome 19 was reported in families with late onset AD.[70] One of the genes located in this region is APOE. As the apoE protein was also found in senile plaques, APOE was tested as a candidate gene for AD.[71] In 1993, Strittmatter and colleagues reported that the APOE*4 allele frequency was significantly increased in familial AD patients derived from a brain bank.[71] Since then, numerous research groups confirmed the increased frequency of the APOE*4 allele among AD patients.[72-97] In a meta-analysis of the findings up to early 1995,[98] both early and late onset AD as well as sporadic and familial types, were associated with APOE*4 (*table*). However, an increased APOE*4 frequency in familial early onset AD has not been reported consistently.[93,99-101] Part of the discrepancy between studies on familial early onset AD can be explained by the lack of uniform criteria for familial disease.

Several studies showed that subjects with two copies of the APOE*4 allele (homozygotes) have a higher risk compared with those with one copy (heterozygotes) (odds ratios vary from 4.9-34.3 and 1.6-5.1 respectively).[72-75,80-82,86,87,91,97]

Table – APOE*4 allele frequencies in various AD types*

Type AD	Number of studies	Number of patients	APOE*4 frequency	95% confidence interval
Early onset familial AD†	2	143	0.42	0.36 to 0.48
Early onset sporadic AD	4	168	0.28	0.23 to 0.33
Late onset familial AD	8	601	0.48	0.45 to 0.51
Late onset sporadic AD	24	1562	0.37	0.35 to 0.39
Nondemented controls	1	2000	0.14	0.12 to 0.16

* Based on a meta-analysis.[98]

† Cases were not examined for all currently known autosomal dominant mutations, later studies did not find an association.[93,99-101]

There is some evidence that the APOE genotype effects the age at AD onset. APOE*4 homozygotes were found to have a 3-16 years earlier onset as compared with non-APOE*4 carriers.[73,81,87,102-106] The age of onset effect was however not observed consistently.[82,96,97,107-109] Also the role of the APOE*2 allele in AD is a matter of controversy. Several studies reported a decreased APOE*2 frequency in AD patients (0.01-0.05), independent of the effect of APOE*4, which suggests a protective effect.[75,92,110-113] In contrast, others found an increased frequency of APOE*2 (0.13-0.14).[114,115]

Heterogeneity of studies Despite the large number of investigations compatible with an increased risk of AD for carriers of APOE*4, the strength of this association varies considerably. The differences across studies are probably the result of the differences in ascertainment of cases (clinic- versus population-based, prevalent versus incident), the heterogeneity in case-series studied (familial or sporadic, late onset versus early onset) and the diagnostic criteria used (possible, probable or definite AD). The large majority of studies used clinic-based patient-series, and all studies except two[116,117] are based on prevalent cases. Thus, selection may also have occurred because of referral bias and survival bias associated with the APOE genotype.

Possible bias An important aspect regarding referral bias is that APOE may, in part, determine the symptoms of AD,[118-120] which may effect clinical referral. Also, the fact that APOE*4 carriers more often have a positive family history of dementia,[80,91] could lead to referral bias.[121] Compared with AD cases from a community-based study, patients from a memory clinic indeed had a higher APOE*4 allele frequency, an earlier disease onset, and a more advanced state at assessment.[122]

With regard to putative prevalence-incidence bias, investigations of the role of APOE in progression and survival of AD are relevant. However, studies on APOE and the rate of progression of AD have yielded inconclusive results.[102,103,107,123-127] No relation between APOE and mortality has been found by some groups,[109,128] while others described an increased survival in AD patients with APOE*4,[102,103,114,123] and a decreased survival for early onset AD patients with APOE*2.[114] A problem in the interpretation of these studies is that each of these investigations is based on prevalent patients who may have entered the study at different stages of disease. Also the characteristics of the AD patients and the definition of disease duration varied considerably. However, if the finding that APOE*4 carriers survive longer is correct, the risk of AD associated with APOE*4 may be inflated in studies of prevalent patients conducted to date.[129]

Incidence studies Indeed, in two population-based studies on incident patients, in which participants were included before onset of dementia, a relatively low APOE*4 frequency had been observed.[116,117] However, one of these studies focussed on dementia and not AD specifically,[116] which may explain in part the low APOE*4 frequencies observed (0.17 in patients using DSM-III-R criteria,[130] and 0.26 in cases diagnosed with ICD-10 criteria).[131] However, also the other study on incident AD patients reported a low APOE*4 frequency (0.13),[117] and used the NINCDS-ADRDA criteria.[3] As this latter study was performed among Italian-Americans in whom APOE*4 is relatively rare, the frequency of this allele in AD cases was still significantly increased compared with the unaffected controls (0.08).[117] Age- and sex-specific risk estimates associated with the various APOE genotypes await large scale incidence studies.

Causal inference: studies of different populations An important issue to resolve is whether APOE is causally related to AD or whether another, neighboring gene exerts the pathogenic effect. The latter situation is known as linkage disequilibrium. Indeed, polymorphisms flanking the APOE gene have been found to be associated with AD in some studies.[92,93,132] Investigations of different populations may shed light on the possibility of linkage disequilibrium. If APOE*4 is causally related to AD, one expects to find an association in each population studied, assuming that there are no strong effect modifiers which differ across populations. The association between APOE*4 and AD has been confirmed in various populations, including those of African, Chinese and Japanese origin.[133-137] However, findings in persons of African origin have been inconsistent. In a small study on Nigerians, no association between APOE*4 and AD was observed.[69] Whereas one study in African-Americans reported that APOE*4 was a strong risk factor for AD,[133] others found a lower APOE*4 associated risk of AD for African-Americans compared with Caucasians.[134]

Causal inference: experimental studies In spite of the weak associations in the studies on incident cases,[116,117] and on patients of African descent,[69,133,134] there is support from experimental studies that APOE is causally related to AD. It has been shown that the ß-amyloid burden is related to the APOE genotype.[84,85,96,103,138,139] The apoE isoforms may have a specific effect on the formation, aggregability,[140-143] or clearance,[84,144] of ß-amyloid. Also isoforms specific differences in the binding to ß-amyloid have been reported, though not consistently.[145-147] The apoE4 isoform may bind less strongly than apoE3 to tau and MAP2c proteins, which may result in the development of neurofibrillary tangles,[148,149] another neuropathologic feature of AD. Alternatively, the transport of lipids could be compromised in APOE*4 carriers, leading to a poor reinnervation after neuronal cell loss.[150] The cholinergic neuro-

transmitter system relies heavily on lipid homeostasis,[150] and the choline acetyl-transferase activity in the AD brain may be inversely related to the number of APOE*4 alleles.[151,152] In vitro studies suggest that apoE3 facilitates and apoE4 inhibits neurite outgrowth.[153,154] Recently, apoE4 has been shown to have decreased antioxidant activity, and apoE2 increased activity, as compared with apoE3.[155] Apart from structural differences between the apoE isoforms, it was found that the apoE content decreases with increasing APOE*4 allele copies in the AD brain.[156]

Other susceptibility genes

A number of genes have been studied because of the possible role of the gene product in the AD pathology. Like apoE, α_1-antichymotrypsin (ACT) binds to ß-amyloid, and serves as a stimulatory factor in the polymerization of ß-amyloid.[142] In the AD brain, ACT is expressed particularly in areas that develop ß-amyloid deposits.[157] Therefore, the ACT gene was considered to be a candidate gene involved for AD. Although in one study, homozygotes for the ACT*A allele were found to have an 1.5-fold increased risk of AD as compared with other subjects,[158] this could not be confirmed elsewhere.[159]

The association between APOE*4 and AD led to studies of genes whose products bind to apoE. The Very Low Density Lipoprotein receptor (VLDL-r) is one of the receptors for lipoproteins containing apoE.[160] Subjects homozygous for the A2 allele of the VLDL-r gene had a two- to threefold increased risk of AD in a Japanese study.[161] However, these findings could not be verified in Caucasians.[162,163]

There is some evidence that the gene encoding the precursor protein of non-amyloid-ß component (NACP) may be involved in the pathogenesis of AD. Among carriers of APOE*4, the NACP allele 2 was found to be more frequent in non-demented elderly than in AD patients.[164] The observation that the NACP allele 2 may exert a protective effect remains to be confirmed.

The CYP2D6B allele may increase the risk of Parkinson's disease and the Lewy Body variant of AD,[165,166] although not consistently.[167] The protein product of CYP2D6 is involved in detoxifying environmental toxins.[168] As there are similarities between AD and Parkinson's disease and familial aggregation of these disorders has been observed,[7] CYP2D6B was studied in AD. The CYP2D6B allele was found to be associated with milder synaptic pathology in AD brains.[169] A recent study suggests that the frequency of this allele was comparable in AD patients and in controls. Therefore, the role of the CYP2D6B allele in the development of AD may also be limited.

Interaction of genes and environmental factors

As large numbers of patients and controls are needed to study rare gene-gene and gene-environment interactions, studies up till now focused on the most frequent genetic risk factor for AD, the APOE*4 allele. Several studies found the strongest effects of APOE*4 on AD to occur in those with a positive family history of dementia.[80,91,96,98,170] However, for most dominant mutations, no interaction with APOE*4 was observed.[61,93,99-101] The exception may be the Val717→Ile APP mutation, for which APOE appears to effect the age of AD onset.[171,172] Patients with APOE*2 had a later onset age,[171,172] while disease onset was earlier in cases with APOE*4.[172] It is controversial whether there is interaction between APOE and the putative susceptibility genes ACT, VLDL-r and NACP.[158,159,161-164,173]

There is some evidence that the association between APOE*4 and AD may be modified by gender.[73,174-176] In a study of familial AD, women who carry one APOE*4 allele had a similar risk of AD as women carrying two APOE*4 alleles.[177] In men, to the contrary, no significant differences were observed between APOE*4 heterozygotes and non-APOE*4 carriers.[103,177] However, a gender specific APOE*4 associated risk of AD was not found by others.[178] The interpretation of these findings is hampered by the fact that these are based on prevalent patients, and may be the result of gender specific differences in cardiovascular mortality related to APOE*4.[179]

The odds ratio for AD associated with APOE*4 may decrease with age.[75,113,180] In very elderly subjects the APOE*4 frequency was similar in AD patients and cognitively normal individuals.[181] There are several explanations possible including age-dependent expression of the APOE gene and interaction of APOE*4 with an age dependent factor. However, in one of the population-based studies of incident AD patients, the APOE*4 related risk of AD did not vary with age.[117] Further studies of age-specific risk are clearly needed.

Serum cholesterol levels are partly determined by genetic factors, including the APOE gene.[68] It has been suggested that total cholesterol level may modulate the APOE*4 associated risk of AD.[175] Also the presence of generalized atherosclerosis seems to potentiate the effects of APOE*4 on the risk of AD.[8]

A previous head injury may be another environmental factor involved in the etiology of AD.[9] A synergistic interaction was observed between the effects of traumatic head injury and APOE*4.[182] APOE*4 carriers without a previous head injury had a twofold increased risk of AD, while APOE*4 carriers with a head injury were on a tenfold increased risk.[182] This observation was supported by a postmortem study which suggested that deposition of ß-amyloid after head injury may depend on the number of APOE*4 alleles.[183]

A recent study suggests that reactivation of herpes simplex virus type 1 in the brain, is only associated with the development of AD when the APOE*4 allele is present.[184] If confirmed, this finding indicates another gene-environment interaction.

In several case-control studies, an inverse relation between smoking and AD has been reported.[11] The putative protective effect of smoking may be limited to APOE*4 carriers with a family history of dementia.[185] This relation should be further investigated, since previous studies have been subject to selection bias, survival bias and recall bias. Indeed, in a recent follow-up study, smokers were found to have an increased risk of dementia, especially in the absence of APOE*4.[12]

Estrogen use during menopause may lower the risk of AD.[10] Among women with APOE*4, the protective effect of estrogen was stronger compared with women without APOE*4, although these differences were not statistically significant.[10] In a study of AD patients with early onset, the protective effect of estrogen use was found to be limited to women who carried APOE*4.[186]

Limited information is available on the interaction of APOE with the use of nonsteroid anti-inflammatory drugs. Although the strongest evidence for an association was found in persons without APOE*4, there is no significant evidence for effect modification.[187]

Clinical and public health implications

There are three potential applications of testing for the presence of AD mutations: 1. for predictive testing in unaffected individuals in order to identify persons at high risk of AD, 2. as a diagnostic test, and 3. to select demented patients for therapy. Despite the high risk of AD associated with the known dominant mutations in the APP, PS-1, and PS-2 genes, testing will be not be valuable because these mutations are very rare. Only in patients from families with an autosomal dominant form of AD, screening for these mutations may be useful. This section will therefore focus on APOE*4, by far the most frequent genetic risk factor for AD.

Predictive testing Many authors have argued against the use of APOE genotyping in the prediction of whether or not someone will develop AD.[188-193] An important argument is that reliable APOE specific estimates of the lifetime risk of AD are lacking. Estimates, based on data from various case-series, indicate that APOE*4 carriers have a substantial chance of *not* developing AD during a lifetime.[192] Although there is some evidence that APOE typing can identify asymptomatic people at high risk of AD,[194,195] in the absence of preventive strategies, presymptomatic testing seems to be of little use and to be unethical.

Diagnosis It has been suggested that APOE testing be used in the diagnosis of AD.[189] Although APOE genotyping may further increase diagnostic certainty in a population of *probable AD* patients,[196] its value in the differential diagnosis of *dementia* is limited as APOE*4 may also be associated with other dementing illnesses than AD.[197] Increased APOE*4 frequencies have been reported in patients with vascular dementia,[94,198,199] Lewy Body disease,[200-203] Parkinson's disease dementia,[204] frontal lobe dementia,[205,206] and Creutzfeldt-Jakob disease.[207] Findings on APOE and the non-AD dementias are somewhat controversial however,[208-214] with the exception of Lewy Body disease. The probable association between APOE*4 and the most important alternative diagnosis for AD, vascular dementia,[94,198,199] may limit the utility of APOE testing in the differential diagnosis of dementia. There is an ongoing debate on this issue. The American College of Medical Genetics/American Society of Human Genetics and others did not recommend APOE genotyping in the diagnosis of AD.[190] The National Institute on Aging/Alzheimer's Association Working Group concluded that physicians may choose to use APOE genotyping as an adjunct to other tests currently employed for AD diagnosis.[191]

Therapy It has further been suggested that APOE genotyping may be valuable in the evaluation of therapy. Tacrine, an acetylcholinesterase inhibitor,[215] was found to be less effective in demented APOE*4 carriers, as compared with patients who do not carry an APOE*4 allele.[151] However, in this small study, patients were selected from a group who completed the trial, and who showed maximal change.[151] Diagnostic criteria were not described, and it is not clear whether there were baseline differences in the stage of disease between patients with and without APOE*4.[151] Besides, several outcomes were used with no adjustment for multiple testing.[151] This finding is therefore preliminary and needs to be confirmed by others.

Discussion

In recent years, remarkable progress has been made in the unraveling of the genetic basis of AD. The possible interaction of various AD genes and environmental factors is schematically represented in the *figure*. Yet, research on the genetics of AD is far from completed. Various mutations in three genes (APP, PS-1 and PS-2) have been identified which can lead to AD, but these are all extremely rare. The APOE*4 allele is a common risk factor, but despite the overwhelming evidence for an increased frequency of this allele in AD patients, there are currently no reliable age and gender specific risk estimates available. The risk of AD associated with the APOE*2 allele also remains to be resolved. Up till now, findings on other susceptibility genes have been difficult to reproduce.

It is to be expected that other, yet unknown genes are involved in the etiology of AD. Familial aggregation in the general population or in genetically isolated populations could not be explained by already known AD genes only.[52,80,91,216,217] The yet unknown AD genes are most likely implicated in the etiology through more complex mechanisms than the ones identified to date.

Classical linkage analysis does not seem to be a promising technique to detect these new genes, as families with AD patients in multiple generations are rare, and linkage analyses did not yield clues.[48] An alternative approach is to examine affected sib-pairs, but this requires a large number of siblings.[218] In recent years, much attention has been paid to the possibility of localizing disease genes using case-series instead of families.[218] The statistical power of these studies in the general population is limited, but the situation is more favorable in isolated populations were there is usually less genetic variability.[219] However, extrapolation of these findings to the general population may be limited. As experimental research will identify more and more details of the protein chemistry of AD, and the Human Genome Project advances further in identifying genes, the opportunities for candidate-gene studies will increase. However, all of the above techniques for the identification of new genes are subject to false positive findings. As there is usually no a priori hypothesis as to which allele is associated with the disease, and a large number of alleles can be tested, problems related to multiple testing are encountered.

An important aim in genetic-epidemiologic research will be to determine the contribution of newly identified genes to the occurrence of disease. For this purpose,

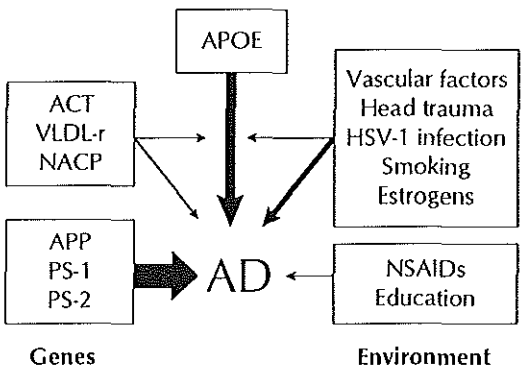

Figure – Schematic representation of various genetic and environmental factors involved in the etiology of Alzheimer's disease (AD).
The thickness of the arrows indicates the evidence of a causal association. APP indicates the amyloid precursor protein gene, PS-1 the presenilin 1 gene, PS-2 the gene for presenilin 2, ACT denotes the α_1-antichymotrypsin gene, VLDL-r the gene for the very low density lipoprotein receptor, NACP the gene for non-amyloid-ß component of amyloid precursor protein, and APOE denotes the apolipoprotein E gene. HSV-1 indicates herpes simplex virus type 1, and NSAIDs denotes non-steroidal anti-inflammatory drugs.

population-based studies are needed in order to overcome referral bias. The problem of survival bias can only be overcome in a follow-up setting. However, in these surveys it has been difficult to implement neuropathologic confirmation of the diagnosis. An estimated 10 percent of clinically diagnosed AD patients appear to have another dementing illness at autopsy.[220] This may result in diagnostic misclassification which reduces statistical power. Nevertheless the problem of misclassification in population-based studies is outweighed by the opportunity to study gene-environment interactions, using prospectively collected data on exposure.

Further research will thus be important to unravel the pathogenesis of AD, and eventually to develop effective therapy. Transgenic animals carrying AD mutations can be useful for this purpose. Given the developments in pharmacology and genetics, it is likely that genetic testing will be used to identify subgroups that will benefit from new therapeutic interventions. For susceptibility genes, unraveling interactions with preventable risk factors may ultimately lead to a new area in genetic-epidemiology, prevention of disease in genetically susceptible groups.

References

1. Alzheimer A. Über eine eigenartige Erkrankung der Hirnride. (German). Allg Z Psychiatr 1907;64:146-8. [English translation: Arch Neurol 1969;21:109-10.]
2. Cummings JL. Dementia: the failing brain. Lancet 1995;345:1481-9.
3. McKhann G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's disease. Neurology 1984;34:39-44.
4. Corey-Bloom J, Thal LJ, Galasko D, et al. Diagnosis and evaluation of dementia. Neurology 1995;45:211-8.
5. Mirra SS, Heyman A, McKeel D, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology 1991;41:479-86.
6. Breteler MMB, Claus JJ, Van Duijn CM, et al. Epidemiology of Alzheimer's disease. Epidemiol Rev 1992;14:59-82.
7. Van Duijn CM. Epidemiology of the dementias: recent developments and new approaches. J Neurol Neurosurg Psychiatry 1996;60:478-488.
8. Hofman A, Ott A, Breteler MMB, et al. Atherosclerosis, apolipoprotein E and the prevalence of dementia and Alzheimer's disease in a population-based study: the Rotterdam study. Lancet 1997;349:151-4.
9. Mortimer JA, Van Duijn CM, Chandra V, et al. Head trauma as a risk factor for Alzheimer's disease: a collaborative re-analysis of case-control studies. Int J Epidemiol 1991;20:S28-S35.
10. Tang M-X, Jacobs D, Stern Y, et al. Effect of oestrogen during menopause on risk and age at onset of Alzheimer's disease. Lancet 1996;348:429-32.

11. Lee PN. Smoking and Alzheimer's disease: a review of the epidemiological evidence. Neuroepidemiology 1994;13:131-44.
12. Ott A, Slooter AJC, Hofman A, et al. Smoking and the risk of dementia and Alzheimer's disease in a population-baseed cohort study. The Rotterdam study. Lancet 1998;351:1840-3.
13. Åkeson HO. A population study of senile and arteriosclerotic psychosis. Hum Hered 1969;19:546-66.
14. Van Duijn CM, Clayton D, Chandra V, et al. Familial aggregation of Alzheimer's disease and related disorders: a collaborative re-analysis of case-control studies. Int J Epidemiol 1991;20 (suppl 2):S13-S20.
15. Lippa CF, Saunders AM, Smith TW, et al. Familial and sporadic Alzheimer's disease: neuropathology cannot exclude a final common pathway. Neurology 1996;46:406-12.
16. Duara R, Lopez-Alberola RF, Barker WW, et al. A comparison of familial and sporadic Alzheimer's disease. Neurology 1993;43:1377-84.
17. Farrer LA, Cupples LA, Van Duijn CM, et al. Rate of progression of Alzheimer's disease is associated with genetic risk. Arch Neurol 1995;52:918-23.
18. Oliver C, Holland AJ. Down's syndrome and Alzheimer's disease: a review. Psychol Med 1986;16:307-22.
19. Wisniewski KE, Wisniewski HM, Wen GY. Occurrence of neuropathological changes and dementia of the Alzheimer type in Down's syndrome. Ann Neurol 1985;17:278-82.
20. Tanzi RE, Gusella JF, Watkins PC, et al. Amyloid ß protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer locus. Science 1987;235:880-4.
21. Gómez-Isla T, Hollister R, West H, et al. Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. Ann Neurol 1997;41:17-24.
22. Crystal H, Dickson D, Fuld P, et al. Clinico-pathologic studies in dementia: nondemented subjects with pathologically confirmed Alzheimer's disease. Neurology 1988;38:1682-7.
23. Goate A, Chartier-Harlin M-C, Mullan M, et al. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature 1991;349:704-6.
24. Van Broeckhoven C. Molecular genetics of Alzheimer disease: identification of genes and gene mutations. Eur Neurol 1995;35:8-19.
25. Naruse S, Igarashi S, Aoki K, et al. Mis-sense mutation Val→Ile in exon 17 of amyloid precursor protein gene in Japanese familial Alzheimer's disease. Lancet 1991;337:978-9.
26. Yoshioka Y, Miki T, Katsuya T, et al. The ^{717}Val→Ile substitution in amyloid precursor protein is associated with familial Alzheimer's disease regardless of ethnic groups. Biochem Biophys Res Commun 1991;178:1141-6.
27. Fidani L, Rooke K, Chartier-Harlin M, et al. Screening for mutations in the open reading frame and promotor of the ß-amyloid precursor protein gene in familial Alzheimer's disease: identification of a further family with APP717 Val→Ile. Hum Molec Genet 1992;1: 165-8.
28. Karlinsky H, Vaula G, Haines JL, et al. Molecular and prospective phenotypic characterization of a pedigree with familial Alzheimer's disease and a missense mutation in codon 717 of the ß-amyloid precursor protein gene. Neurology 1992;42:1445-53.
29. Sorbi S, Nacmias B, Forleo P, et al. APP717 and Alzheimer's disease in Italy. Nat Genet (Letter). 1993;4:10.
30. Murrell J, Farlow M, Ghetti B, et al. A mutation in the amyloid precursor protein associated with hereditary Alzheimer's disease. Science 1991;254:97-9.

31. Chartier-Harlin M-C, Crawford F, Houlden H, et al. Early-onset Alzheimer's disease caused by mutations at codon 717 of the ß-amyloid precursor protein gene. Nature 1991;353:844-6.
32. Mullan M, Crawford F, Axelman K, et al. A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of ß-amyloid. Nat Genet 1992;1:345-7.
33. Hendriks L, Van Duijn CM, Cras P, et al. Presenile dementia and cerebral haemorrhage linked to a mutation at codon 692 of the ß-amyloid precursor protein gene. Nat Genet 1992;1:218-221.
34. Van Broeckhoven C, Genthe AM, Vandenberghe A, et al. Failure of familial Alzheimer's disease to segregate with the A4 amyloid gene in several European families. Nature 1987; 329:153-5.
35. Tanzi RE, St George-Hyslop PH, Haines JL, et al. The genetic defect in familial Alzheimer's disease is not tightly linked to the amyloid ß protein gene. Nature 1987;329: 156-7.
36. Schellenberg GD, Bird TD, Wijsman EM, et al. Absence of linkage of chromosome 21q21 markers to familial Alzheimer's disease. Science 1988;241:1507-10.
37. Pericak-Vance MA, Yamaoka LH, Haynes CS, et al. Genetic linkage studies in Alzheimer's disease families. Exp Neurol 1988;102:271-9.
38. St George-Hyslop PH, Haines JL, Farrer LA, et al. Genetic linkage studies suggest that Alzheimer's disease is not a single homogeneous disorder. Nature 1990;347:194-7.
39. Schellenberg GD, Pericak-Vance MA, Wijsman EM, et al. Linkage analysis of familial Alzheimer's disease using chromosome 21 markers. Am J Hum Genet 1991;48:563-83.
40. Jarret JT, Lansbury PT. Seeding 'one-dimensional crystallization' of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie? Cell 1993;73:1055-8.
41. Cai X, Golde TE, Younkin SG. Release of excess amyloid-β protein from a mutant amyloid-ß protein precursor. Science 1993;259:514-6.
42. Citron M, Oltersdorf T, Haass C, et al. Mutation of the β-amyloid precursor protein in familial Alzheimer's disease increases β-protein production. Nature 1992;360:672-4.
43. Haass C, Hung AY, Selkoe DJ, et al. Mutations associated with a locus for familial Alzheimer's disease result in alternative processing of amyloid beta-protein precursor. J Biol Chem 1994;269:17741-8.
44. Kosik KS. Alzheimer's disease: a cell biological perspective. Science 1992;256:780-3.
45. Kang J, Lemaire H, Unterbeck A, et al. The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. Nature 1987;325:733-6.
46. Levy E, Carman MD, Fernandez-Madrid IJ, et al. Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch type. Science 1990;248:1124-6.
47. Van Broeckhoven C, Haan J, Bakker E, et al. Amyloid-ß protein precursor gene and hereditary cerebral hemorrhage with amyloidosis (Dutch). Science 1990;248:1120-2.
48. Schellenberg GD, Bird TD, Wijsman EM, et al. Genetic linkage evidence for a familial Alzheimer's disease locus on chromosome 14. Science 1992;258:668-71.
49. Sherrington R, Rogaeva EI, Liang Y, et al. Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. Nature 1995;375:754-60.
50. Alzheimer's Disease Collaborative Group. The structure of the presenilin 1 (S182) gene and identification of six novel mutations in early onset AD families. Nat Genet 1995;11:219-22.

51. Van Broeckhoven C. Presenilins and Alzheimer disease. Nat Genet 1995;11:230-2.
52. Van Broeckhoven C, Backhovens H, Wehnert A, et al. Estimation of the mutation frequency of presenilin-1 in a population-based sample of early-onset Alzheimer's disease. Neurobiol Ageing (Abstract). 1996;17:S194.
53. Cruts M, Backhovens H, Wang S-Y, et al. Molecular genetic analysis of familial early-onset Alzheimer's disease linked to chromosome 14q24.3. Hum Molec Genet 1995;4:2463-71.
54. Campion D, Flaman J-M, Brice A, et al. Mutations of the presenilin I gene in families with early-onset Alzheimer's disease. Hum Molec Genet 1995;4:2373-7.
55. Perez-Tur J, Froelich S, Prihar G, et al. A mutation in Alzheimer's disease destroying a splice acceptor site in the presenilin-1 gene. NeuroReport 1995;7:297-301.
56. Pérez-Tur J, Wavrant-De Vrieze F, Lambert JC, et al. Presenilin-1 polymorphism and Alzheimer's disease. Lancet (Letter). 1996;347:1560-1.
57. Wasco W, Pettingell WP, Jondro PD, et al. Familial Alzheimer's disease chromosome 14 mutations. Nat Med (Letter). 1995;11:848.
58. Rogaev EI, Sherrington R, Rogaeva EA, et al. Familial Alzheimer's disease in kindreds with a missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. Nature 1995;376:775-8.
59. Boteva K, Vitek M, Mitsuda H, et al. Mutation analysis of presenilin 1 gene in Alzheimer's disease. Lancet (Letter). 1996;347:130-1.
60. Hardy J. Amyloid, the presenilins and Alzheimer's disease. Trends Neurosci 1997;20:154-9.
61. Wragg M, Hutton M, Talbot C, et al. Genetic association between intronic polymorphism in presenilin-1 gene and late-onset Alzheimer's disease. Lancet 1996;347:509-12.
62. Scott WK, Growdon JH, Roses AD, et al. Presenilin-1 polymorphism and Alzheimer's disease. Lancet (Letter). 1996;347:1186-7.
63. Levy-Lahad E, Wasco W, Poorkaj P, et al. Candidate gene for the chromosome 1 familial Alzheimer's disease locus. Science 1995;269:973-7.
64. Wisniewski T, Palha JA, Ghiso J, et al. S182 protein in Alzheimer's disease neuritic plaques. Lancet (Letter). 1995;346:1366.
65. Mann DMA, Iwatsubo T, Cairns NJ, et al. Amyloid ß protein (Aß) deposition in chromosome 14-linked Alzheimer's disease: predominance of $Aß_{42(43)}$. Ann Neurol 1996;40:149-56.
66. Mahley RW. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science 1988;240:622-30.
67. Rall SC, Weisgraber KH, Mahley RW. Human apolipoprotein E. The complete amino acid sequence. J Biol Chem 1982;257:4171-8.
68. Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis 1988;8:1-21.
69. Osuntokun BO, Sahota A, Ogunniyi AO, et al. Lack of an association between apolipoprotein E ε4 and Alzheimer's disease in elderly Nigerians. Ann Neurol 1995;38:463-5.
70. Pericak-Vance MA, Bebout JL, Gaskell PC, et al. Linkage studies in familial Alzheimer's disease: evidence for chromosome 19 linkage. Am J Hum Genet 1991;48:1034-54.
71. Strittmatter WJ, Saunders AM, Schmechel D, et al. Apolipoprotein E: high-avidity binding to ß-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci USA 1993;90:1977-81.
72. Mayeux R, Stern Y, Ottman R, et al. The apolipoprotein ε4 allele in patients with Alzheimer's disease. Ann Neurol 1993;34:752-4.

73. Poirier J, Davignon J, Bouthillier D, et al. Apolipoprotein E polymorphism and Alzheimer's disease. Lancet 1993;342:697-9.
74. Kuusisto J, Koivisto K, Kervinen K, et al. Association of apolipoprotein E phenotypes with late onset Alzheimer's disease: population based study. BMJ 1994;309:636-8.
75. Corder EH, Saunders AM, Risch NJ, et al. Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. Nat Genet 1994;7:180-4.
76. Saunders AM, Strittmatter WJ, Schmechel D, et al. Association of apolipoprotein E allele ε4 with late-onset familial and sporadic Alzheimer's disease. Neurology 1993;43:1467-72.
77. Houlden H, Crook R, Duff K, et al. Confirmation that the apolipoprotein E4 allele is associated with late onset, familial Alzheimer's disease. Neurodegeneration 1993;2:283-6.
78. Czech C, Mönning U, Tienari PJ, et al. Apolipoprotein E-ε4 allele and Alzheimer's disease. Lancet (Letter). 1993;342:1309-10.
79. Payami H, Kaye J, Heston LL, et al. Apolipoprotein E genotype and Alzheimer's disease. Lancet (Letter). 1993;342:738.
80. Jarvik GP, Larson EB, Goddard K, et al. Influence of apolipoprotein E genotype on the transmission of Alzheimer disease in a community-based sample. Am J Hum Genet 1996;58:191-200.
81. Lehtovirta M, Helisalmi S, Mannermaa A, et al. Apolipoprotein E polymorphism and Alzheimer's disease in Eastern Finland. Neurosci Lett 1995;185:13-5.
82. Liddell M, Williams J, Bayer A, et al. Confirmation of association between the e4 allele of apolipoprotein E and Alzheimer's disease. J Med Genet 1994;31:197-200.
83. Dai XY, Nanko S, Hattori M, et al. Association of apolipoprotein E4 with sporadic Alzheimer's disease is more pronounced in early onset type. Neurosci Lett 1994;175:74-6.
84. Rebeck GW, Reiter JS, Strickland DK, et al. Apolipoprotein E in sporadic Alzheimer's disease: allelic variation and receptor interactions. Neuron 1993;11:575-80.
85. Polvikoski T, Sulkava R, Haltia M, et al. Apolipoprotein E, dementia, and cortical deposition of ß-amyloid protein. N Engl J Med 1995;333:1242-7.
86. Myers RH, Schaefer EJ, Wilson PWF, et al. Apolipoprotein E ε4 association with dementia in a population-based study: the Framingham study. Neurology 1996;46:673-7.
87. Tsai M-S, Tangalos EG, Petersen RC, et al. Apolipoprotein E: risk factor for Alzheimer disease. Am J Hum Genet 1994;54:643-9.
88. Adroer R, Santacruz P, Blesa R, et al. Apolipoprotein E4 allele frequency in Spanish Alzheimer and control cases. Neurosci Lett 1995;189:182-6.
89. Lucotte G, Visvikis S, Leininger-Müler B, et al. Association of apolipoprotein E allele ε4 with late-onset sporadic Alzheimer's disease. Am J Med Genet 1994;54:286-8.
90. Okuizumi K, Onodera O, Tanaka H, et al. ApoE-ε4 and early-onset Alzheimer's. Nat Genet 1994;7:101.
91. Van Duijn CM, De Knijff P, Cruts M, et al. Apolipoprotein E4 allele in a population-based study of early-onset Alzheimer's disease. Nat Genet 1994;7:74-8.
92. Chartier-Harlin M-C, Parfitt M, Legrain S, et al. Apolipoprotein E, ε4 allele as a major risk factor for sporadic early and late-onset forms of Alzheimer's disease: analysis of the 19q13.2 chromosomal region. Hum Mol Genet 1994;3:569-74.
93. Yu C-E, Payami H, Olson JM, et al. The apolipoprotein E/CI/CII gene cluster and late-onset Alzheimer disease. Am J Hum Genet 1994;54:631-42.

94. Noguchi S, Murakami K, Yamada N. Apolipoprotein E genotype and Alzheimer's disease. (Letter). Lancet 1993;342:737.
95. Brousseau T, Legrain S, Berr C, et al. Confirmation of the ε4 allele of the apolipoprotein E gene as a risk factor for late-onset Alzheimer's disease. Neurology 1994;44:342-4.
96. Zubenko GS, Stiffler S, Stabler S, et al. Association of the apolipoprotein E ε4 allele with clinical subtypes of autopsy-confirmed Alzheimer's disease. Am J Med Genet 1994;54:199-205.
97. St Clair D, Rennie M, Slorach E, et al. Apolipoprotein E ε4 allele is a risk factor for familial and sporadic presenile Alzheimer's disease in both homozygote and heterozygote carriers. J Med Genet 1995;32:642-4.
98. Van Gool WA, Evenhuis HM, Van Duijn CM, et al. A case-control study of apolipoprotein E genotypes in Alzheimer's disease associated with Down's syndrome. Ann Neurol 1995;38:225-30.
99. Haan J, Van Broeckhoven C, Van Duijn CM, et al. The apolipoprotein E ε4 allele does not influence the clinical expression of the amyloid precursor protein gene codon 693 or 692 mutations. Ann Neurol 1994;36:434-7.
100. Van Broeckhoven C, Backhovens H, Cruts M, et al. APOE genotype does not modulate age of onset in families with chromosome 14 encoded Alzheimer's disease. Neurosci Lett 1994;169:179-80.
101. Saunders AM, Schmader K, Breitner JCS, et al. Apolipoprotein E ε4 allele distributions in late-onset Alzheimer's disease and in other amyloid-forming diseases. Lancet 1993;342:710-1.
102. Frisoni GB, Govoni S, Geroldi C, et al. Gene dose of the ε4 allele of apolipoprotein E and disease progression in sporadic late-onset Alzheimer's disease. Ann Neurol 1995;37:596-604.
103. Gomez-Isla T, West HL, Rebeck GW, et al. Clinical and pathological correlates of apolipoprotein E ε4 in Alzheimer's disease. Ann Neurol 1996;39:62-70.
104. Corder EH, Saunders AM, Strittmatter WJ, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 1993;261:921-3.
105. Houlden H, Crook R, Hardy J, et al. Confirmation that familial clustering and age of onset in late onset Alzheimer's disease are determined at the apolipoprotein E locus. Neurosci Lett 1994;174:222-4.
106. Lucotte G, Turpin J-C, Landais P. Apolipoprotein E-ε4 allele doses in late-onset Alzheimer's disease. Ann Neurol 1994;36:681-2.
107. Dal Forno G, Rasmusson X, Brandt J, et al. Apolipoprotein E genotype and rate of decline in probable Alzheimer's disease. Arch Neurol 1996;53:345-50.
108. Levy-Lahad E, Lahad A, Wijsman EM, et al. Apolipoprotein E genotypes and age of onset in early-onset familial Alzheimer's disease. Ann Neurol 1995;38:678-80.
109. Norrman J, Brookes AJ, Yates C, et al. Apolipoprotein E genotype and its effect on duration and severity of early and late onset Alzheimer's disease. Br J Psychiatry 1995;167:533-6.
110. Talbot C, Lendon C, Craddock N, et al. Protection against Alzheimer's disease with apoE ε2. Lancet (Letter). 1994;343:1432-3.
111. West HL, Rebeck GW, Hyman BT. Frequency of the apolipoprotein E ε2 allele is diminished in sporadic Alzheimer disease. Neurosci Lett 1994;175:46-8.

112. Smith AD, Johnston C, Sim E, et al. Protective effect of apo E ε2 in Alzheimer's disease. Lancet (Letter). 1994;344:473-4.
113. Duara R, Barker WW, Lopez-Alberola R, et al. Alzheimer's disease: interaction of apolipoprotein E genotype, family history of dementia, gender, education, ethnicity, and age of onset. Neurology 1996;46:1575-9.
114. Van Duijn CM, De Knijff P, Wehnert A, et al. The apolipoprotein E ε2 allele is associated with an increased risk of early-onset Alzheimer's disease and a reduced survival. Ann Neurol 1995; 37:605-10.
115. Sorbi S, Nacmias B, Forleo P, et al. ApoE allele frequencies in Italian sporadic and familial Alzheimer's disease. Neurosci Lett 1994;177:100-2.
116. Henderson AS, Easteal S, Jorm AF, et al. Apolipoprotein E allele ε4, dementia, and cognitive decline in a population sample. Lancet 1995;346:1387-90.
117. Evans DA, Beckett LA, Field TS, et al. Apolipoprotein E ε4 and incidence of Alzheimer disease in a community population of older persons. JAMA 1997;277:822-4.
118. Ramachandran G, Marder K, Tang M, et al. A preliminary study of apolipoprotein E genotype and psychiatric manifestations of Alzheimer's disease. Neurology 1996;47:256-9.
119. Lehtovirta M, Soininen H, Helisalmi S, et al. Clinical and neuropsychological characteristics in familial and sporadic Alzheimer's disease. Relation to apolipoprotein E polymorphism. Neurology 1996;46:413-9.
120. Bronzova J, Van Duijn CM, Havekes LM, et al. Apolipoprotein E genotype and concomitant clinical features in early-onset Alzheimer's disease. J Neurol 1996;243:465-8.
121. Mullan M, Scibelli P, Duara R, et al. Familial and population-based studies of apolipoprotein E and Alzheimer's disease. Ann N Y Acad Sci 1996;802:16-26.
122. Tsuang D, Kukull W, Sheppard L, et al. Impact of sample selection on APOE ε4 allele frequency: a comparison of two Alzheimer's disease samples. J Am Geriatr Soc 1996;44: 704-7.
123. Basun H, Grut M, Winblad B, et al. Apolipoprotein ε4 allele and disease progression in patients with late-onset Alzheimer's disease. Neurosci Lett 1995;183:32-4.
124. Kurz A, Egensperger R, Haupt M, et al. Apolipoprotein E ε4 allele, cognitive decline, and deterioration of everyday performance in Alzheimer's disease. Neurology 1996;47:440-3.
125. Growdon JH, Locascio JJ, Corkin S, et al. Apolipoprotein E genotype does not influence rates of cognitive decline in Alzheimer's disease. Neurology 1996;47:444-8.
126. Asada T, Kariya T, Yamagata Z, et al. ApoE ε4 allele and cognitive decline in patients with Alzheimer's disease. Neurology 1996;47:603.
127. Plassman BL, Breitner JCS. Apolipoprotein E and cognitive decline in Alzheimer's disease. Neurology 1996;47:317-20.
128. Corder EH, Saunders AM, Strittmatter WJ, et al. Apolipoprotein E, survival in Alzheimer's disease patients, and the competing risks of death and Alzheimer's disease. Neurology 1995;45:1323-8.
129. Riggs JE, Keefover RW. The association between apolipoprotein E allele ε4 and late-onset Alzheimer's disease: pathogenic relationship or differential survival bias. Arch Neurol (Letter). 1994;51:750.
130. American Psychiatric Association. Diagnostic and statistical manual of mental disorders 3rd ed, revised. DSM-III-R. Washington DC: APA, 1987.

131. World Health Organization. The ICD-10 classification of mental and behavioral disorders. Diagnostic criteria for research. Geneva: WHO, 1993.
132. Tsuda T, Lopez R, Rogaev EA, et al. Are the associations between Alzheimer's disease and polymorphisms in the apolipoprotein E and the apolipoprotein CII genes due to linkage disequilibrium? Ann Neurol 1994;36:97-100.
133. Hendrie HC, Hall KS, Hui S, et al. Apolipoprotein E genotypes and Alzheimer's disease in a community study of elderly African Americans. Ann Neurol 1995;37:118-20.
134. Maestre G, Ottman R, Stern Y, et al. Apolipoprotein E and Alzheimer's disease: ethnic variation in genotypic risks. Ann Neurol 1995;37:254-9.
135. Mak YT, Chiu H, Woo J, et al. Apolipoprotein E genotype and Alzheimer's disease in Hong Kong elderly Chinese. Neurology 1996;46:146-9.
136. Hong CJ, Liu TY, Liu H-C, et al. ε4 allele of apolipoprotein E increases risk of Alzheimer's disease in a Chinese population. Neurology 1996;46:1749-51.
137. Yoshizawa T, Yamakawa-Kobayashi K, Komatsuzaki Y, et al. Dose-dependent association of apolipoprotein E allele ε4 with late-onset, sporadic Alzheimer's disease. Ann Neurol 1994;36:656-9.
138. Nagy Z, Esiri MM, Jobst KA, et al. Influence of the apolipoprotein E genotype on amyloid deposition and neurofibrillary tangle formation in Alzheimer's disease. Neuroscience 1995;69:757-61.
139. Schmechel DE, Saunders AM, Strittmatter WJ, et al. Increased amyloid ß-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. Proc Natl Acad Sci USA 1993;90:9649-53.
140. Sanan DA, Weisgraber KH, Russell SJ, et al. Apolipoprotein E associates with ß amyloid peptide of Alzheimer's disease to form novel monofibrils. Isoform apoE4 associates more efficiently than apoE3. J Clin Ivest 1994;94:860-9.
141. Evans KC, Berger EP, Cho C-G, et al. Apolipoprotein E is a kinetic but not a thermodynamic inhibitor of amyloid formation: implications for the pathogenesis and treatment of Alzheimer disease. Proc Natl Acad Sci USA 1995;92:763-7.
142. Ma J, Yee A, Brewer HB Jr, et al. Amyloid-associated proteins α_1-antichymotrypsin and apolipoprotein E promote assembly of Alzheimer ß-protein into filaments. Nature 1994;372:92-4.
143. Castaño EM, Prelli F, Wisniewski T, et al. Fibrillogenesis in Alzheimer's disease of amyloid ß peptides and apolipoprotein E. Biochem J 1995;306:599-604.
144. Rebeck GW, Harr SD, Strickland DK, et al. Multiple, diverse senile plaque-associated proteins are ligands of an apolipoprotein E receptor, the α_2-macroglobulin receptor/low-density-lipoprotein receptor-related protein. Ann Neurol 1995;37:211-7.
145. Strittmatter WJ, Weisgraber KH, Huang DY, et al. Binding of human apolipoprotein E to synthetic amyloid ß peptide: isoform-specific effects and implications for late-onset Alzheimer disease. Proc Natl Acad Sci USA 1993;90:8098-8102.
146. LaDu MJ, Falduto MT, Manelli AM, et al. Isoform-specific binding of apolipoprotein E to ß-amyloid. J Biol Chem 1994;269:23403-6.
147. Zhou Z, Smith JD, Greengard P, et al. Alzheimer amyloid-ß peptide forms denaturant-resistent complex with type ε3 but not type ε4 isoform of native apolipoprotein E. Mol Med 1996;2:175-80.

148. Strittmatter WJ, Saunders AM, Goedert M, et al. Isoform-specific interactions of apolipoprotein E with microtuble-associated protein tau: implications for Alzheimer's disease. Proc Natl Acad Sci USA 1994;91:11183-6.
149. Huang DY, Goedert M, Jakes R, et al. Isoform-specific interactions of apolipoprotein E with the microtubule-associated protein MAP2c: implications for Alzheimer's disease. Neurosci Lett 1994;182:55-8.
150. Poirier J. Apolipoprotein E in animal models of CNS injury and in Alzheimer's disease. Trends Neurosci 1994;17:525-30.
151. Poirier J, Delisle M-C, Quirion R, et al. Apolipoprotein E4 allele as a predictor of cholinergic deficits and treatment outcome in Alzheimer disease. Proc Natl Acad Sci USA 1995;92: 12260-4.
152. Soininen H, Kosunen O, Helisalmi S, et al. A severe loss of choline acetyltransferase in the frontal cortex of Alzheimer patients carrying apolipoprotein ε4 allele. Neurosci Lett 1995; 187:79-82.
153. Nathan BP, Chang K-C, Bellosta S, et al. The inhibitory effect of apolipoprotein E4 on neurite outgrowth is associated with microtubule depolymerization. J Biol Chem 1995;270: 19791-9.
154. Mahley RW, Nathan BP, Bellosta S, et al. Apolipoprotein E: impact of cytoskeletal stability in neurons and the relationship to Alzheimer's disease. Curr Opin Lipidol 1995;6:86-91.
155. Miyata M, Smith J. Apolipoprotein E allele-specific antioxidant activity and effects on cytotoxicity by oxidative insults and ß-amyloid peptides. Nat Genet 1996;14:55-61.
156. Bertrand P, Poirier J, Oda T, et al. Association of apolipoprotein E genotype with brain levels of apolipoprotein E and apolipoprotein J (clusterin) in Alzheimer disease. Mol Brain Res 1995;33:174-8.
157. Abraham CR, Selkoe DJ, Potter H. Immunochemical identification of the serine protease inhibitor α1-antichymotrypsin in the brain amyloid deposits of Alzheimer's disease. Cell 1988;52:487-501.
158. Kamboh MI, Sanghera DK, Ferrell RE, et al. APOE*4-associated Alzheimer's disease risk is modified by α1-antichymotrypsin polymorphism. Nat Genet 1995;10:486-8.
159. Haines JL, Pritchard ML, Saunders AM, et al. No genetic effect of α1-antichymotrypsin in Alzheimer disease. Genomics 1996;33:53-6.
160. Takahashi S, Kawarabayasi Y, Nakai T, et al. Rabbit very low density lipoprotein receptor: a low density lipoprotein receptor-like protein with distinct ligand specificity. Proc Natl Acad Sci USA 1992;89:9252-6.
161. Okuizumi K, Onodera O, Namba Y, et al. Genetic association of the very low density lipoprotein (VLDL) receptor gene with sporadic Alzheimer's disease. Nat Genet 1995;11:207-9.
162. Okuizumi K, Onodera O, Seki K, et al. Lack of association of very low density lipoprotein receptor gene polymorphism with caucasian Alzheimer's disease. Ann Neurol 1996;40: 251-4.
163. Chung H, Roberts CT, Greenberg S, et al. Lack of association of trinucleotide repeat polymorphisms in the very-low-density lipoprotein receptor gene with Alzheimer's disease. Ann Neurol 1996;39:800-3.
164. Xia Y, De Silva HAR, Rosi BL, et al. Genetic studies in Alzheimer's disease with an NACP/α-Synuclein polymorphism. Ann Neurol 1996;40:207-15.

165. Armstrong M, Daly AK, Cholerton S, et al. Mutant debrisoquine hydroxylation genes in Parkinson's disease. Lancet 1992;339:1017-8.
166. Saitoh T, Xia Y, Chen X, et al. The CYP2D6B mutant allele is overrepresented in the lewy body variant of Alzheimer's disease. Ann Neurol 1995;37:110-2.
167. Gasser T, Müller-Myhsok B, Supala A, et al. The CYP2D6B allele is not overrepresented in a population of German patients with idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry 1996;61:518-20.
168. Evans DAP, Mahgoub A, Sloan TP, et al. A family and population study of the genetic polymorphism of debrisoquine oxidation in a white British population. J Med Genet 1980; 17:102-5.
169. Chen X, Xia Y, Alford M, et al. The CYP2D6B allele is associated with a milder synaptic pathology in Alzheimer's disease. Ann Neurol 1995;38:653-8.
170. Lannfelt L, Lilius L, Nastase M, et al. Lack of association between apolipoprotein E allele ε4 and sporadic Alzheimer's disease. Neurosci Lett 1994;169:175-8.
171. Nacmias B, Latorraca S, Piersanti P, et al. ApoE genotype and familial Alzheimer's disease: a possible influence on age of onset in APP717→Ile mutated families. Neurosci Lett 1995;183:1-3.
172. Sorbi S, Nacmias B, Forleo P, et al. Epistatic effect of APP717 mutation and apolipoprotein E genotype in familial Alzheimer's disease. Ann Neurol 1995;38:124-7.
173. Talbot C, Houlden H, Craddock N, et al. Polymorphism in AACT gene may lower age of onset of Alzheimer's disease. NeuroReport 1996;7:534-6.
174. Payami H, Montee K, Kaye J, et al. Alzheimer's disease, apolipoprotein E and gender. JAMA (Letter). 1994;271:1316-7.
175. Jarvik GP, Wijsman EM, Kukull WA, et al. Interactions of apolipoprotein E genotype, total cholesterol level, age, and sex in prediction of Alzheimer's disease: a case-control study. Neurology 1995;45:1092-6.
176. Farrer LA, Cupples LA, Van Duijn CM, et al. Apolipoprotein E genotype in patients with Alzheimer's disease: implications for the risk of dementia among relatives. Ann Neurol 1995;38:797-808.
177. Payami H, Zareparsi S, Montee KR, et al. Gender difference in apolipoprotein E - associated risk for familial Alzheimer disease: a possible clue to the higher incidence of Alzheimer disease in women. Am J Hum Genet 1996;58:803-11.
178. Corder EH, Saunders AM, Strittmatter WJ, et al. The apolipoprotein E E4 allele and sex-specific risk of Alzheimer's disease. JAMA (Letter). 1995;273:373-4.
179. Reilly SL, Ferrell RE, Kottke BA, et al. The gender-specific apolipoprotein E genotype influence on the distribution of lipids and apolipoproteins in the population of Rochester, MN. I. Pleiotropic effects on means and variances. Am J Hum Genet 1991;49:1155-66.
180. Rebeck GW, Perls TT, West HL, et al. Reduced apolipoprotein ε4 allele frequency in the oldest old Alzheimer's patients and cognitively normal individuals. Neurology 1994; 44:1513-6.
181. Sobel E, Louhija J, Sulkava R, et al. Lack of association of apolipoprotein E allele ε4 with late-onset Alzheimer's disease among Finnish centenarians. Neurology 1995;45:903-7.
182. Mayeux R, Ottman R, Maestre G, et al. Synergistic effects of traumatic head injury and apolipoprotein-ε4 in patients with Alzheimer's disease. Neurology 1995;45:555-7.
183. Nicoll JAR, Roberts GW, Graham DI. Apolipoprotein E ε4 allele is associated with depo-

sition of amyloid ß-protein following head injury. Nat Med 1995;1:135-7.
184. Itzhaki RF, Lin W-R, Shang D, et al. Herpes simplex virus type 1 in brain and risk of Alzheimer's disease. Lancet 1997;349:241-4.
185. Van Duijn CM, Havekes LM, Van Broeckhoven C, et al. Apolipoprotein E genotype and association between smoking and early onset Alzheimer's disease. BMJ 1995;310:627-31.
186. Van Duijn CM, Meijer H, Witteman JCM, et al. Estrogen, apolipoprotein E and the risk of Alzheimer's disease. Neurobiol Aging (Abstract). 1996;17:S79.
187. Breitner JC, Welsh KA, Helms MJ, et al. Delayed onset of Alzheimer's disease with nonsteroidal anti-inflammatory and histamine H2 blocking drugs. Neurobiol Aging 1995;16:523-530.
188. Bird TD. Apolipoprotein E genotyping in the diagnosis of Alzheimer's disease: a cautionary view. Ann Neurol 1995;38:2-4.
189. Roses AD. Apolipoprotein E genotyping in the differential diagnosis, not prediction, of Alzheimer's disease. Ann Neurol 1995;38:6-14.
190. American College of Medical Genetics/American Society of Human Genetics Working Group on ApoE and Alzheimer Disease. Statement on use of apolipoprotein E testing for Alzheimer disease. JAMA 1995;274:1627-9
191. National institute on aging/Alzheimer's association working group. Apolipoprotein E genotyping in Alzheimer's disease. Lancet 1996;347:1091-5.
192. Seshadri S, Drachman DA, Lippa CF. Apolipoprotein E ε4 allele and the lifetime risk of Alzheimer's disease: what physicians know, and what they should know. Arch Neurol 1995; 52:1074-9.
193. Hyman BT, Gomez-Isla T, Briggs M, et al. Apolipoprotein E and cognitive change in an elderly population. Ann Neurol 1996;40:55-66.
194. Small GW, Mazziotta JC, Collins MT, et al. Apolipoprotein E type 4 allele and cerebral glucose metabolism in relatives at risk for familial Alzheimer disease. JAMA 1995;273:942-7.
195. Reiman EM, Caselli RJ, Yun LS, et al. Preclinical evidence of Alzheimer's disease in persons homozygous for the ε4 allele for apolipoprotein E. N Engl J Med 1996;334:752-8.
196. Saunders AM, Hulette C, Welsh-Bohmer KA, et al. Specificity, sensitivity, and predictive value of apolipoprotein-E genotyping for Alzheimer's disease. Lancet 1996;348:90-3.
197. Slooter AJC, Breteler MMB, Ott A, et al. APOE genotyping in differential diagnosis of Alzheimer's disease. Lancet (Letter). 1996;348:334.
198. Slooter AJC, Tang M-X, Van Duijn CM, et al. Apolipoprotein E ε4 and the risk of dementia with stroke. A population-based investigation. JAMA 1997;277:818-21.
199. Frisoni GB, Calabresi L, Geroldi C, et al. Apolipoprotein E ε4 allele in Alzheimer's disease and vascular dementia. Dementia 1994;5:240-2.
200. Lippa CF, Smith TW, Saunders AM, et al. Apolipoprotein E genotype and lewy body disease. Neurology 1995;45:97-103.
201. Benjamin R, Leake A, Edwardson JA, et al. Apolipoprotein E genes in lewy body and Parkinson's disease. Lancet (Letter). 1994;343:1565.
202. Pickering-Brown SM, Mann DMA, Bourke JP, et al. Apolipoprotein E4 and Alzheimer's disease pathology in lewy body disease and in other ß-amyloid-forming diseases. Lancet (Letter). 1994;343:1155.

203. McKeith IG, Morris CM. Apolipoprotein E genotyping in Alzheimer's disease. Lancet (Letter). 1996;347:1775.
204. Arai H, Muramatsu T, Higuchi S, et al. Apolipoprotein E gene in Parkinson's disease with or without dementia. (Letter). Lancet 1994;344:889.
205. Stevens M, Van Duijn CM, De Knijff P, et al. Apolipoprotein E gene and sporadic frontal lobe dementia. Neurology 1997;48:1526-9.
206. Schneider JA, Gearing M, Robbins RS, et al. Apolipoprotein E genotype in diverse neurodegenerative disorders. Ann Neurol 1995;38:131-5.
207. Amouyel P, Vidal O, Launay JM. The apolipoprotein E alleles as major susceptibility factors for Creutzfeldt-Jakob disease. Lancet 1994;344:1315-8.
208. Sakoda S, Kuriyama M, Osame M, et al. Apolipoprotein E ε4. Neurology 1994;44:2420.
209. Stengård JH, Pekkanen J, Sulkava R, et al. Apolipoprotein E polymorphism, Alzheimer's disease and vascular dementia among elderly Finnish men. Acta Neurol Scand 1995;92:297-8.
210. Koller WC, Glatt SL, Hubble JP, et al. Apolipoprotein E genotypes in Parkinson's disease with and without dementia. Ann Neurol 1995;37:242-5.
211. Marder K, Maestre G, Cote L, et al. The apolipoprotein ε4 allele in Parkinson's disease with and without dementia. Neurology 1994;44:1330-1.
212. Nakagawa Y, Kitamoto T, Furukawa H, et al. Apolipoprotein E in Creutzfeldt-Jakob disease. Lancet 1995;345:68.
213. Pickering-Brown SM, Siddons M, Mann DMA, et al. Apolipoprotein E allelic frequencies in patients with lobar atrophy. Neurosci Lett 1995;188:205-7.
214. Zerr I, Helmhold M, Weber T. Apolipoprotein E in Creutzfeldt-Jakob disease. Lancet 1995;345:68-9.
215. Davis KL, Powchik P. Tacrine. Lancet 1995;345:625-30.
216. Pericak-Vance MA, Johnson CC, Rimmler JB, et al. Alzheimer's disease and apolipoprotein E-4 allele in an Amish population. Ann Neurol 1996;39:700-4.
217. Rosenberg RN, Richter RW, Risser RC, et al. Genetic factors for the development of Alzheimer disease in the Cherokee indian. Arch Neurol 1996;53:997-1000.
218. Lander ES, Schork NJ. Genetic dissection of complex traits. Science 1994;265:2037-48.
219. Houwen RHJ, Baharloo S, Blankenship K, et al. Genome screening by searching for shared segments: mapping a gene for benign recurrent intrahepatic cholestasis. Nat Genet 1994;8:380-6.
220. Galasko D, Hansen LA, Katzman R, et al. Clinical-neuropathological correlations in Alzheimer's disease and related dementias. Arch Neurol 1994;51:888-95.

3

APOLIPOPROTEIN E GENOTYPING AND DIFFERENTIAL DIAGNOSIS OF DEMENTIA

3.1

APOE testing in the differential diagnosis of dementia

Considerations based on existing literature

Introduction

In Alzheimer's disease, there is a gradual decline in cognitive functions, often accompanied by alterations in personality and behaviour.[1,2] Despite these characteristics it can be difficult to differentiate Alzheimer's disease form other types of dementia, especially in its early stages. Although there is not yet effective treatment available, it is important to make the right diagnosis. Firstly, reversible dementing illnesses should be diagnosed and treated. Secondly, given the devastating course, it is crucial to correctly inform the demented patient and his family which future lies ahead.

A diagnosis of Alzheimer's disease is usually made in two steps. Firstly, it is investigated whether a dementia syndrome is present, usually using the DSM-III-R criteria.[3] This is a clinical diagnosis, in which neuropsychological testing can play an important role. Secondly, it is investigated whether the clinical picture fits that of Alzheimer's disease, while other causes of dementia are excluded. For the latter purpose, various diagnostic tools can be used.[4] The association between the APOE*4 allele of the apolipoprotein E gene (APOE),[5,6] brought some investigators to recommended APOE testing as a differential diagnostic tool.[7]

If APOE testing can truly distinguish Alzheimer patients from other demented individuals, it implies that APOE*4 is differentially associated with Alzheimer's disease than with other dementias. As the value of APOE typing in the differential diagnosis of dementia has not yet been investigated in patients who are referred

because of a possible dementia syndrome, this article aims to explore this issue on the basis of the existing literature. Firstly, the association between the APOE*4 allele and the non-Alzheimer's disease dementias will be reviewed. Secondly, some methodological issues will be discussed.

Apolipoprotein E and the non-Alzheimer's disease dementias

Vascular dementia, dementia due to cerebrovascular disorders, is after Alzheimer's disease the most common cause of dementia.[8] Classically, it is characterised by a sudden onset and focal neurological deficits. Different diagnostic criteria can be used, but the overlap is poor.[9] As atherosclerosis seems also to be related to Alzheimer's disease,[10] the distinction from vascular dementia is often difficult, and may be artificial. Among patients with vascular dementia, an increased frequency of the APOE*4 allele has been observed: 0.21-0.46 versus 0.15 in the general population.[11-13] However, others did not find an association between APOE*4 and vascular dementia (allele frequency 0.06-0.14).[14-16] It should be noted however, that studies supporting the association between APOE*4 and vascular dementia are methodologically superior. Therefore it seems to be likely that APOE*4 is related to vascular dementia.

Diffuse Lewy body disease was found to be associated in neuropathological studies with an increased APOE*4 allele frequency (0.22-0.42).[17-19] Diffuse Lewy body disease is a type of dementia characterised by confusion, anxiety, hallucinations and parkinsonism. Lewy body disease is however a controversial entity, and may be a variant of Alzheimer's disease.

Dementia after the onset of Parkinson's disease is often characterised by mental slowing, decreased flexibility, and a deficit in recalling, while recognition may be unaltered. Still, the distinction with Alzheimer's disease may be difficult, as also Alzheimer patients may develop parkinsonism.[20] It is still unclear whether Parkinson's disease dementia is related to APOE*4: an increased allele frequency has been described (0.32),[21,22] but this was not confirmed by others.[23,24] Of these studies, two were population-based,[22,24] and contradicting.

It remains to be clarified whether frontal lobe dementia is association with APOE*4. Two publications report an increased APOE*4 frequency (0.25-0.38),[25,26] a third one does not (0.09).[27] The clinical picture of frontal lobe dementia is, in its early stages, dominated by alterations in personality and behaviour; memory disorders usually occur later on.

Cognitive impairment is frequently found in depressive patients. The loss of motivation and attention, may result in a decreased performance with cognitive testing. Disorders of mood are also frequent in the early stages of dementia. Therefore, the distinction between early dementia and depression in the elderly can be difficult. The APOE*4 allele frequency was found to be increased in a small study among depressive elderly people.[28] This finding remains to be confirmed by others.

Discussion

In order to be useful in diagnosis, it is essential that a test result changes diagnostic certainty. Therefore, we can compare the pre-test probability (or *a priori* probability) with the post-test probability (or *a posteriori* probability). The *a priori* probability of Alzheimer's disease, P(AD), in a demented patient is 50-70%, the proportion Alzheimer patients among all demented subjects.[1,8] Using Bayes' paradigm the *a posteriori* probability P(AD|APOE*4) can be calculated, which is the probability of Alzheimer's disease given carriership of APOE*4:

$$P(AD|APOE*4) = \frac{P(AD)\cdot P(APOE*4|AD)}{P(AD)\cdot P(APOE*4|AD) + P(\text{no AD})\cdot P(APOE*4|\text{no AD})}$$

The gain (the difference between the a priori and the a posteriori probability) increases with a decreasing denominator of the above ratio. This denominator decreases when P(no APOE*4|no AD) increases, because P(APOE*4|no AD) + P(no APOE*4|no AD) = 1. P(no APOE*4|no AD) is known as the specificity. Therefore, the larger the specificity the larger the diagnostic gain.

As outlined above, APOE*4 seems to be associated with the most important alternative diagnosis for Alzheimer's disease, vascular dementia, and possibly also with other dementing diseases. It is therefore not to be expected that the specificity of APOE testing among dementia patients will be high. The gain of diagnostic certainty will thus not be substantial.

Theoretically, it is not be expected that the presence or absence of a risk factor can easily be used for diagnostic purposes.[29] A test should be positive in the diseased (i.e. high sensitivity), and negative in those without the disease (high specificity). The sensitivity can be written as

$$P(APOE*4|AD) = \frac{P(APOE*4)\cdot P(AD|APOE*4)}{P(APOE*4)\cdot P(AD|APOE*4) + P(\text{no}APOE*4)\cdot P(AD|\text{no}APOE*4)}$$

$$= \frac{RR \cdot P(APOE*4)}{RR \cdot P(APOE*4) + 1 - P(noAPOE*4)}$$

$$= \frac{1}{1 + \frac{1 - P(APOE*4)}{RR \cdot P(APOE*4)}}$$

As this relates to differential diagnosis, RR denotes the relative risk of Alzheimer's disease associated with APOE*4, in a population of demented patients. Also P(APOE*4) refers to the proportion of APOE*4 carriers among demented individuals.

If at least 50% of the Alzheimer's patients are test positive (sensitivity more than 50%), it follows that: 1-P(APOE*4) < RR·P(APOE*4).

This is true when APOE*4 is common and/or the relative risk is high. It has not yet been reported what the proportion is of persons carrying one or two APOE*4 alleles, among subjects who are refereed because of a possible dementia syndrome. Given the putative relationship between APOE*4 and the non-Alzheimer's disease dementias, it is to be expected that the relative risk of Alzheimer's disease associated with one or two APOE*4 alleles among dementia patients, will be close to one. Therefore, the sensitivity of APOE testing in the differential diagnosis of dementia is expected to be low, and as discussed above, probably the same will hold true for the specificity.

APOE genotyping has been recommended as a relatively cheap test, that could replace other diagnostic tools.[7] However, demented APOE*4 carriers can still suffer a treatable disease, although chances are limited. The devastating course and the limited therapeutic possibilities for Alzheimer's patients, justify a stately aimed to exclude (rare) types of dementia which can be cured. These diagnostic tests should therefore never be omitted.

It has further been suggested that APOE genotyping could identify a subgroup of Alzheimer patients who could benefit best from treatment. Tacrine, an acetyl-cholinesterase inhibitor,[30] was found to be less effective in demented APOE*4 carriers, as compared to patients who do not carry an APOE*4 allele.[31] As this was a small study, with several methodological weaknesses,[32] this finding needs to be confirmed by others.

As the advantages of APOE testing seem to be small, the disadvantages may be large. The disclosure of the APOE genotype may cause unnecessary concern in the offspring of Alzheimer patients, especially in those homozygous for APOE*4.[33] Currently, APOE genotyping is not a useful test in the diagnosis of Alzheimer's disease. Of course this will change when there is more evidence that the effects of new therapeutics will depend upon the APOE genotype.

References

1. Cummings JL. Dementia: the failing brain. Lancet 1995;345:1481-4.
2. McKhann G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's disease. Neurology 1984;34:39-44.
3. American Psychiatric Association. Diagnostic and statistical manual of mental disorders, third edition, revised. Washington DC, American Psychiatric Association, 1987.
4. Corey-Bloom J, Thal LJ, Galasko D, et al. Diagnosis and evaluation of dementia. Neurology 1995;45:211-8.
5. Strittmatter WJ, Saunders AM, Schmechel D, et al. Apolipoprotein E: high-avidity binding to ß-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci USA 1993;90:1977-81.
6. Farrer LA, Cupples LA, Haines JL, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. JAMA 1997;278:1349-56.
7. Roses AD. Apolipoprotein E genotyping in the differential diagnosis, not prediction, of Alzheimer's disease. Ann Neurol 1995;38:6-14.
8. Ott A, Breteler MMB, Van Harskamp F, et al. Prevalence of Alzheimer's disease and vascular dementia: association with education. The Rotterdam Study. BMJ 1995;310:970-3.
9. Gold G, Giannakopoulos P, Montes-Paixao C, et al. Sensitivity and specificity of newly proposed clinical criteria for possible vascular dementia. Neurology 1997;49:690-4.
10. Hofman A, Ott A, Breteler MMB, et al. Atherosclerosis, apolipoprotein E and the prevalence of dementia and Alzheimer's disease in a population-based study: the Rotterdam study. Lancet 1997;349:151-4.
11. Slooter AJC, Tang M-X, Van Duijn CM, et al. Apolipoprotein E ε4 and the risk of dementia with stroke. A population-based investigation. JAMA 1997;277:818-21.
12. Noguchi S, Murakami K, Yamada N. Apolipoprotein E genotype and Alzheimer's disease. Lancet 1993;342:737.
13. Frisoni GB, Calabresi L, Geroldi C, et al. Apolipoprotein E ε4 allele in Alzheimer's disease and vascular dementia. Dementia 1994;5:240-2.
14. Sakoda S, Kuriyama M, Osame M, et al. Apolipoprotein E ε4. Neurology 1994;44:2420.
15. Kawamata J, Tanaka S, Shimohama S, et al. Apolipoprotein E polymorphism in Japanese patients with Alzheimer's disease or vascular dementia. J Neurol Neurosurg Psychiatry 1994; 57:1414-6.
16. Higuchi S, Arai H, Nakagawa T, et al. The apolipoprotein E gene in Binswanger's disease and vascular dementia. Clin Genet 1996;50:459-61.
17. Lippa CF, Smith TW, Saunders AM, et al. Apolipoprotein E genotype and lewy body disease. Neurology 1995;45:97-103.
18. Benjamin R, Leake A, Edwardson JA, et al. Apolipoprotein E genes in lewy body and Parkinson's disease. Lancet 1994;343:1565.
19. Pickering-Brown SM, Mann DMA, Bourke JP, et al. Apolipoprotein E4 and Alzheimer's disease pathology in lewy body disease and in other ß-amyloid-forming diseases. Lancet 1994;343:1155.
20. Stern Y, Brandt J, Albert M, et al. The absence of an apolipoprotein ε4 allele is associated with a more aggressive form of Alzheimer's disease. Ann Neurol 1997;41:615-20.

21. Arai H, Muramatsu T, Higuchi S, et al. Apolipoprotein E gene in Parkinson's disease with or without dementia. Lancet 1994;344:889.
22. De Rijk MC, Breteler MMB, Ott A, et al. Apolipoprotein E genotype, Parkinson's disease, and dementia in Parkinson's disease: the Rotterdam study. Submitted.
23. Koller WC, Glatt SL, Hubble JP, et al. Apolipoprotein E genotypes in Parkinson's disease with and without dementia. Ann Neurol 1995;37:242-5.
24. Marder K, Maestre G, Cote L, et al. The apolipoprotein ε4 allele in Parkinson's disease with and without dementia. Neurology 1994;44:1330-1.
25. Stevens M, Van Duijn CM, De Knijff P, et al. Apolipoprotein E gene and sporadic frontal lobe dementia. Neurology 1996. In press.
26. Schneider JA, Gearing M, Robbins RS, et al. Apolipoprotein E genotype in diverse neurodegenerative disorders. Ann Neurol 1995;38:131-5.
27. Pickering-Brown SM, Siddons M, Mann DMA, et al. Apolipoprotein E allelic frequencies in patients with lobar atrophy. Neurosci Lett 1995;188:205-7.
28. Krishnan KRR, Ritchie JC, Tupler LA, et al. Apolipoprotein E ε4. Neurology 1994;44:2420-1.
29. Baron JA. The clinical utility of risk factor data. J Clin Epidemiol 1989;42:1013-20.
30. Davis KL, Powchik P. Tacrine. Lancet 1995;345:625-30.
31. Poirier J, Delisle M-C, Quirion R, et al. Apolipoprotein E4 allele as a predictor of cholinergic deficits and treatment outcome in Alzheimer disease. Proc Natl Acad Sci USA 1995;92: 12260-4.
32. Slooter AJC, Van Duijn CM. Genetic Epidemiology of Alzheimer disease. Epidemiol Rev 1997;19:107-19.
33. Bird TD, Bennett RL. Why do DNA testing? Practical and ethical implications of new neurogenetic tests. Ann Neurol 1995;38:141-6.

3.2

APOE testing in the differential diagnosis of dementia

The Rotterdam Study

Abstract

It is a matter of debate whether apolipoprotein E genotyping is useful in the differential diagnosis of Alzheimer's disease. We investigated this issue among 249 mildly to moderately demented patients derived from a population-based study. Apolipoprotein E genotyping was not associated with a statistically significant change in diagnostic certainty. We conclude that apolipoprotein E genotyping seems to be of little value in the differential diagnosis of Alzheimer's disease.

Introduction

The E4 allele of the apolipoprotein E gene (APOE*4) is a major susceptibility factor for the development of Alzheimer's disease (AD).[1,2] It is a matter of debate whether apolipoprotein E (APOE) genotyping is valuable in the differentiation of AD from other causes of dementia.[3-5] However, up till now, these statements are based on data from various case series, which may be subject to bias. In particular, test characteristics that are derived from selected series of patients may not be applicable to the disease spectrum in the general population. We present data on the performance of APOE genotyping as a differential diagnostic test of AD among patients with different subtypes of dementia in a population-based survey.

Methods

This study is based on the Rotterdam Study, a population-based cohort study of subjects aged 55 years and over, including institutionalised persons. Dementia was diagnosed as described previously.[6] Briefly, of the 10275 eligible subjects, cognitive function was assessed in 7528 (73%). Screen positive subjects were further evaluated using an interview with a close relative, neuropsychological tests, neurological examination and neuro-imaging. A panel assessed, based on all available information, whether a dementia syndrome was present according to DSM-III-R criteria.[7] A diagnosis of AD was made according to the criteria of the NINCDS-ADRDA.[8] A diagnosis of AD with cerebrovascular disease (CVD) was based on a history of stroke or neuro-imaging findings, that were considered not directly etiologically related to the dementia. Vascular dementia (VD) was diagnosed in accordance with the criteria of the NINDS-AIREN.[9] Parkinson's disease dementia (PDD) was diagnosed if the dementia started after the onset of idiopathic parkinsonism. Overall, 474 patients with dementia were detected. For this study we limited ourselves to subjects with mild or moderate impairment (i.e. clinical dementia rating scale (CDR) 2 or less and minimental state examination score (MMSE) 16 or more), since these are the people who are most likely referred to a clinic with suspicion of dementia.

Of the 347 subjects with mild or moderate dementia, a random sample of 249 patients (mean age 83.5 years; SD 7.3) was included in the present analysis. The relative frequency of different subtypes of dementia in our study population was as follows. Probable or possible AD was diagnosed in 74% (n=185), AD with CVD in 7% (n=18), VD in 16% (n=40), PDD in 6% (n=16) and other dementias in 3% (n=8). APOE genotyping was performed as described earlier[10,11] without knowledge of the diagnosis.

Test characteristics for a diagnosis of AD (probable or possible AD, or AD with CVD) were calculated, using cases with any other type of dementia as the reference group. The diagnostic work-up as described above was used as the golden standard. We calculated the sensitivity and specificity of a diagnosis of AD based on the presence of at least one APOE*4 allele. The same was done for the presence of two APOE*4 alleles. In the latter analysis, the presence of one APOE*4 allele was considered to be a non-positive/non-negative test result, i.e. heterozygotes were excluded.[12] Moreover, we calculated the predictive value and the likelihood ratio of zero, one and two APOE*4 alleles. The likelihood ratio refers to the proportion of AD patients with a given APOE genotype divided by the proportion of those with another type of dementia with the same APOE genotype. When a likelihood ratio exceeds 1, the probability of disease increases; when the likelihood ratio is less than 1, the probability of disease decreases; when the likelihood ratio approaches 1, the test

Table 1 – The presence of APOE*4 in Alzheimer's disease and other dementia types*

	Alzheimer's disease				Other dementias		
	All AD (n=185)	Probable AD† (n=141)	Possible AD (n=44)	AD + CVD (n=18)	All (n=64)	VD (n=40)	PDD (n=16)
APOE4-E4-	0.68 (0.61 to 0.74) (n=125)	0.67 (0.60 to 0.75) (n=95)	0.68 (0.54 to 0.82) (n=30)	0.56 (0.33 to 0.79) (n=10)	0.61 (0.49 to 0.73) (n=39)	0.58 (0.42 to 0.73) (n=23)	0.56 (0.32 to 0.81) (n=9)
APOE4+E4-	0.26 (0.20 to 0.32) (n=48)	0.26 (0.18 to 0.33) (n=36)	0.27 (0.14 to 0.40) (n=12)	0.44 (0.21 to 0.67) (n=8)	0.34 (0.23 to 0.46) (n=22)	0.38 (0.22 to 0.53) (n=15)	0.38 (0.14 to 0.61) (n=6)
APOE4+E4+	0.06 (0.03 to 0.10) (n=12)	0.07 (0.03 to 0.11) (n=10)	0.05 (0.00 to 0.11) (n=2)	0.00 (0.00 to 0.00) (n=0)	0.05 (0.00 to 0.10) (n=3)	0.05 (0.00 to 0.12) (n=2)	0.06 (0.00 to 0.18) (n=1)

* Values are proportions (95% CI) and numbers. AD=Alzheimer's disease; CVD=cerebrovascular disease; VD=vascular dementia; PDD=Parkinson's disease dementia.

† Including subjects with AD + CVD.

is uninformative.[12] All analyses were performed for the whole study population, and in strata of age, gender and family history of dementia.

Results

Table 1 shows that the APOE*4 alleles was equally distributed in AD patients and in those with another type of dementia (X^2=8.9; P=0.35). In all patient groups, the APOE genotype distribution was in Hardy-Weinberg equilibrium. In *table 2* test characteristics are shown for a diagnosis of AD based on the APOE genotype. We found a poor sensitivity, and a reasonable specificity. The presence of one APOE*4 allele was not informative to diagnose AD (*table 3*), since the corresponding predictive value tended to be less than the *a priori* probability (0.74, 95% CI: 0.69 to 0.80,

Table 2 – Sensitivity and specificity of a diagnosis of Alzheimer's disease based on the APOE genotype*

	Sensitivity	Specificity
APOE*4+	0.32 (0.26 to 0.39)	0.61 (0.49 to 0.73)
APOE4E4†	0.09 (0.04 to 0.13)	0.93 (0.85 to 1.0)

* Values are means (95% CI).
† Heterozygous APOE*4 carriers were excluded.

Table 3 – Test characteristics of APOE genotyping in the differential diagnosis of Alzheimer's disease*

	Predictive value	Likelihood ratio
APOE4-E4-	0.76 (0.70 to 0.83)	1.11 (0.89 to 1.38)
APOE4+E4-	0.69 (0.58 to 0.79)	0.75 (0.50 to 1.15)
APOE4+E4+	0.80 (0.60 to 1.00)	1.38 (0.40 to 4.75)

* Values are means (95% CI).

table 1). The probability of AD in case of two APOE*4 alleles slightly increased to 0.80 (95% CI: 0.60 to 1.00). However, the corresponding likelihood ratio of 1.38 was not significantly different from 1. Also, only 0.06 (95% CI: 0.03 to 0.10) of the AD patients carried this genotype.

Our findings were similar for men and women, and for subjects with and without a family history of dementia. When the study population was stratified in age tertiles, the sensitivity decreased and the specificity increased with ageing. Specifically, in the youngest age tertile (aged 82 years or less), the sensitivity associated with one APOE*4 allele or more was 0.41 (95% CI: 0.29 to 0.54) and the specificity 0.56 (95% CI: 0.37 to 0.75). In this subgroup APOE genotyping did not increase diagnostic certainty either. Our results did not change when AD patients with CVD were excluded. Moreover, further restriction to only mildly demented subjects (those with a MMSE score of at least 18 and a CDR less than 2, and within 3 years after the onset of symptoms) resulted in similar findings.

Discussion

Our study suggests that APOE genotyping is of little value in distinguishing AD patients from other demented subjects. The advantage of this study is the single-centre, population-based design. A weakness however, is the lack of neuro-pathological confirmation of the clinical diagnoses. We found a relatively low APOE*4 allele frequency in AD patients, as did other population-based studies.[13,14] Our study population is relatively old, consequently APOE*4 is less prevalent.[15] However, in our study APOE genotyping is not informative in younger patients either.

Although at this moment there is no causal treatment available for AD patients, the disease should be diagnosed with care. Treatable causes of dementia should be detected, and correct information should be provided about the prognosis. Our population-based study suggests that APOE genotyping is not useful in the differential diagnosis of AD.

References

1. Strittmatter WJ, Saunders AM, Schmechel D, et al. Apolipoprotein E: high-avidity binding to ß-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci USA 1993;90:1977-81.
2. Van Gool WA, Evenhuis HM, Van Duijn CM. A case-control study of apolipoprotein E

genotypes in Alzheimer's disease associated with Down's syndrome. Ann Neurol 1995;38:225-30.
3. Roses AD. Apolipoprotein E genotyping in the differential diagnosis, not prediction, of Alzheimer's disease. Ann Neurol 1995;38:6-14.
4. Bird TD. Apolipoprotein E genotyping in the diagnosis of Alzheimer's disease: a cautionary view. Ann Neurol 1995;38:2-4.
5. American College of Medical Genetics/American Society of Human Genetics Working Group on ApoE and Alzheimer Disease. Statement on use of apolipoprotein E testing for Alzheimer disease. JAMA 1995;274:1627-9.
6. Ott A, Breteler MMB, Van Harskamp F, et al. Prevalence of Alzheimer's disease and vascular dementia: association with education. The Rotterdam study. BMJ 1995;310:970-3.
7. American Psychiatric Association. Diagnostic and statistical manual of mental disorders, third edition, revised. Washington, DC: American Psychiatric Association, 1987.
8. McKhann G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939-44.
9. Roman GC, Tatemichi TK, Erkinjuntti T, et al. Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. Neurology 1993;43:250-60.
10. Van Duijn CM, De Knijff P, Wehnert A, et al. The apolipoprotein E ε2 allele is associated with an increased risk of early-onset Alzheimer's disease and a reduced survival. Ann Neurol 1995;37:605-10.
11. Wenham PR, Price WH, Blundell G. Apolipoprotein E genotyping by one-stage PCR. Lancet 1991;337:1158-9.
12. Simel DL, Samsa GP, Matchar DB. Likelihood ratios with confidence: sample size estimation for diagnostic test studies. J Clin Epidemiol 1991;44:763-70.
13. Lannfelt L, Lilius L, Nastase M, et al. Lack of association between apolipoprotein E allele ε4 and sporadic Alzheimer's disease. Neurosci Lett 1994;169:175-8.
14. Henderson AS, Easteal S, Jorm AF, et al. Apolipoprotein E allele ε4, dementia, and cognitive decline in a population sample. Lancet 1995;346:1387-90.
15. Corder EH, Saunders AM, Risch NJ, et al. Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. Nature Genet 1994;7:180-4.

4

APOLIPOPROTEIN E AND RISK AND PROGNOSIS OF DEMENTIA

4.1

Apolipoprotein E4 increases the risk of dementia with stroke

Abstract

Objective *To investigate the association between the apolipoprotein E (APOE) genotypes and dementia in patients with stroke, defined as either vascular dementia (VD) or Alzheimer's disease with cerebrovascular disease (AD+CVD).*

Design and Setting *Population-based, case-control study from Rotterdam and New York City.*

Participants *187 demented patients with stroke were compared with 507 controls similar in age and ethnic group.*

Main Outcome Measures *The APOE allele frequencies in patients and controls, the odds ratio (OR) of dementia with stroke, VD and AD+CVD, adjusted for age, gender, residency and education and the percent attributable risk related to the APOE*4.*

Results *Overall, demented patients with stroke had a higher APOE*4 allele frequency than controls. Compared to APOE*3 homozygote individuals, APOE*4 homozygotes had a seven-fold increased risk of dementia with stroke, while persons with the APOE3E4 genotype had a nearly two-fold increase in risk. Risks associated with APOE*4 did not vary by the subtype of dementia with stroke or by age or gender. The percent attributable risk related to the APOE*4 among demented patients with stroke was 41% overall.*

Conclusion *APOE*4 is a genetic risk factor for dementia with stroke, including VD and AD+CVD. This may imply shared genetic susceptibility to dementia associated with stroke and AD. Alternatively, dementia in patients with stroke, as currently defined, may include patients with AD.*

Introduction

The elevated risk of Alzheimer's disease (AD) among individuals with the APOE*4 allele of the apolipoprotein E gene (APOE=gene, apoE=protein) has been confirmed worldwide.[1-5] Among patients with dementia and stroke, the role of APOE remains uncertain.[6-12]. Many studies have been limited by small numbers of patients and other methodological issues. The aim of this population-based, case-control study was to examine the relationship between the APOE genotypes and dementia with stroke, or its subtypes vascular dementia (VaD) and AD with cerebrovascular disease (AD+CVD), while considering the effects of age, gender and ethnic origin.

Methods

Data were pooled from individuals participating in two population-based studies, in Rotterdam, The Netherlands and in New York City (Washington Heights). From all participants, informed consent was obtained and the study was approved by the local medical ethics committees, and the Institutional Review Board.

The Rotterdam study is a population-based cohort study of the total population in a suburb, aged 55 years and older, including institutionalised persons. The objective was to investigate determinants of chronic disabling diseases.[13] The cohort includes 7983 subjects (response rate 78%) who were examined from 1990 to 1993. In 1993 and 1994, 88% of the participants who were alive (n=6315) were re-examined. Cognitive performance was assessed by the Mini Mental State Examination and by the Geriatric Mental State schedule. Dementia was diagnosed using a three-phase design, as described elsewhere.[14] Diagnosis included an interview with a relative, neuropsychological testing, an examination by a behavioural neurologist and an MRI scan.

The Washington Heights study consists of a random sample of Medicare recipients in northern New York City provided by the Health Care Financing Administration. The objective of this investigation was to estimate the frequency of various age-related diseases of the nervous system and identify determinants of disease. The response rate was 72%, and did not differ by ethnic origin. The 2250 participants underwent an annual assessment. A physician elicited the medical history and conducted a standardised neurological examination. A standardised neuropsychological battery[15,16] and assessment of activities of daily living were used to ascertain cognitive and functional criteria for dementia.

Diagnosis

All information was reviewed at consensus conferences in either Rotterdam or New York. A panel of clinicians arrived at consensus for diagnosis of dementia according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-III-R) definition.[17] The diagnosis of stroke was implemented similarly in Rotterdam and New York using the World Health Organization (WHO) criteria.[18] At both sites, direct questioning of the next-of-kin or care-giver established the diagnosis of stroke, supplemented by neurological examination and brain imaging. The diagnosis of AD was based on the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria.[19] A diagnosis of dementia with stroke was considered for all demented patients with a history or clinical evidence of stroke, and further subdivided into: VaD, AD+CVD or dementia with stroke "unclassified". VaD was based on the criteria of the National Institute of Neurological Disorders and Stroke and the Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN).[20] A diagnosis of AD+CVD was based on a history or clinical evidence of stroke (including brain imaging), judged not to be the cause of dementia, in patients who would have otherwise met criteria for AD. VaD was distinguished from AD when the onset of dementia occurred within 3 months after the stroke or when there was an abrupt change or a stepwise decline in cognitive function. A diagnosis of dementia with stroke "unclassified" (UNC) was made when limited information was available, such as the absence of brain imaging, or in the absence of a temporal relationship between dementia and stroke. All diagnoses were made without knowledge of the APOE genotype.

Study population

Included were patients with dementia and stroke. The participants from the Washington Heights study included two patients with autopsy confirmed VaD. Excluded were patients with AD alone or dementia due to other causes. The comparison group consisted of a random sample of individuals without dementia in the Rotterdam and Washington Heights studies for whom DNA was available. For every case, approximately three controls were matched on both age (10-year interval) and ethnic group. Among Hispanics, there were too few controls above age 85 years, thus all available controls were included with adjustments made in the analysis. This resulted in a study population of 187 patients with dementia and stroke and 507 controls.

Ethnic group

There were no African-Americans or Hispanics in the Rotterdam study. In Washington Heights, individuals were classified as African-American, Caucasian (both non-Hispanic) or Hispanic groups by standardised, direct interview with the individual or a family member.[21]

Laboratory analysis

Genotypes were performed using a polymerase chain reaction, using methods modified from those previously described.[4,5,22,23]

Statistical analysis

APOE allele frequencies were determined by counting alleles and calculating sample proportions. Normally distributed, continuous data were studied using an analysis of variance (ANOVA) model, and the chi square test was used for categorical data. The relative risk of dementia with stroke, VaD or AD+CVD was estimated as an odds ratio (OR) in a multiple logistic regression analysis, and presented with a 95% confidence interval (CI) using APOE3E3 as the reference. To adjust for confounding, we added age and residency (Rotterdam or New York), gender and education to the model. Logistic regression analyses were also performed stratified by age, gender, ethnic group and residency. The proportion of dementia with stroke among APOE*4 carriers that is attributable to APOE*4 was estimated as the attributable-risk percent (AR%).

Results

Characteristics of the patients and controls are outlined in *table 1*. The distribution of the APOE alleles in the controls from Rotterdam did not differ from those in New York (Rotterdam- APOE*2 0.092, APOE*3 0.769 and APOE*4 0.138 vs. NY-APOE*2 0.081, APOE*3 0.784 and APOE*4 0.135; X^2=0.4, df=2, *P*=0.8), nor did the distribution of the APOE alleles differ among patients with dementia and stroke (Rotterdam-APOE*2 0.071, APOE*3 0.709 and APOE*4 0.220 vs. NY- APOE*2 0.078, APOE*3 0.729 and APOE*4 0.193; X^2=0.4, df=2, *P*=0.8). The distribution of subdiagnoses of dementia and stroke did not differ by location (Rotterdam-AD+CVD 33%, VaD 51%, UNC 16% vs. NY-AD+CVD 41%, VaD 46%, UNC 13%; X^2=1.7, df=2, *P*=0.4). Moreover, the APOE allele frequencies did not differ by subtype of dementia (AD+CVD- APOE*2 0.071, APOE*3 0.729 and APOE*4 0.2 vs. VaD- APOE*2 0.072, APOE*3 0.722 and APOE*4 0.206; X^2=0.5, df=4, *P*=0.9). Therefore, we pooled the data from the two sites.

The distribution of APOE genotypes were similar in patients with prevalent and incident disease (X^2=2.1, df=4, P=0.7), thus these groups were also combined. This resulted in a study population of 187 patients with dementia and stroke, subclassified as follows: 2 patients (1%) with definite VaD, 16 individuals (9%) with probable VaD, 72 subjects (39%) with possible VaD, 70 persons (37%) with AD+CVD, and 27 patients (13%) who could not be classified as either VaD or AD+CVD. The control group consisted of 507 non-demented individuals frequency matched by 10-year age interval and ethnic group (*table 1*). However, demented patients were still older and less well educated than controls. There were also more women among the patients than controls.

The distribution of the APOE genotype in patients and controls was in Hardy-Weinberg equilibrium suggesting no selective inbreeding or survival. The frequency of the APOE*4 allele was significantly higher in demented patients with stroke as compared to the controls (control APOE*2=0.086, APOE*3=0.778, APOE*4= 0.136 vs. dementia with stroke APOE*2=0.075, APOE*3=0.719, APOE*4=0.206; X^2=10.2, df=2, P=0.006). *Table 2* shows that, compared with APOE3E3, the OR for dementia with stroke associated with the APOE3E4 genotype was increased nearly seven-fold (OR=6.9; 95% CI 1.6 to 29.4) while the OR associated with APOE*4

Table 1 – Description of study population

Variables	Dementia with Stroke (n=187)*	VaD (n=90)	AD+CVD (n=70)	Controls (n=507)
Age†	80.1 (7.6)	79.1(8.1)	80.2 (7.1)	76.8 (7.1)
Sex				
men	51 (27%)	29 (32%)	16 (23%)	200 (39.8%)
women	136 (73%)	61 (68%)	54 (77%)	305 (60.2%)
Residency				
New York	96 (51%)	44 (49%)	40 (57%)	301 (59%)
Rotterdam	91 (49%)	46 (51%)	30 (43%)	206 (41%)
Ethnicity				
Caucasian	97 (51.5%)	48 (53.0%)	34 (48.6%)	292 (57.6%)
African-American	35 (19.0%)	15 (17.0%)	19 (27.0%)	77 (15.2%)
Hispanic	54 (29.0%)	27 (30.0%)	16 (23.0%)	138 (27.2%)
Asian	1 (0.5%)	0 (0%)	1 (1.4%)	0 (0%)
Education†	7.2 (3.6)	7.0 (4.0)	7.8 (3.4)	9.2 (3.9)

* Includes patients with "unclassified" dementia with stroke (n=27). VaD = vascular dementia. AD+CVD = Alzheimer's disease with cerebrovascular disease.

† mean (SD) in years.

heterozygosity was increased nearly two-fold (OR 1.8; 1.2-2.7). Adjustment for age, ethnic group or study site did not change the ORs.

The AR% or proportion of dementia with stroke among individuals with an APOE*4 that is attributable to APOE*4 was 41% (95% CI 37 to 44%), in VaD the AR% was 33% (95% CI 29% to 38%) and in AD+CVD the AR% was 44% (95% CI 39 to 48%).

APOE*4 allele frequency and relative risk associated with at least one APOE*4 allele did not change with increasing age. The APOE*4 frequency among demented patients with stroke was similar in women (0.210) and men (0.196). No effects of the APOE*2 allele were observed as compared to the APOE3E3 genotype.

The relationship between APOE*4 and dementia in patients with stroke did not vary by study site or subtype of dementia (VaD or AD+CVD). However, among African-Americans and Hispanics the OR for dementia with stroke associated with APOE*4 homozygosity was high (10.7; 95% CI 1.1 to 80.4), while the OR associated with APOE*4 heterozygosity was only slightly increased (OR=1.3; 95% CI 0.7 to 2.4). Among Caucasians, the OR for dementia with stroke associated with APOE*4 homozygosity and heterozygosity were both increased (OR=3.2 and 2.2, respectively), but only APOE*4 heterozygosity was statistically significant (OR=2.2; 95% CI 1.8 to 3.9) due to the small number of Caucasian homozygotes (OR=3.9; 95% CI 0.6 to 28.7).

Table 2 – The odds ratio for dementia with stroke associated with APOE genotype.*

APOE	Dementia with Stroke†		Vascular Dementia		AD+CVD		Controls
E3E3	n=92	OR=1 (reference)	n=48	OR=1 (reference)	n=33	OR=1 (reference)	n=302
E2E2	n=0	OR= -	n=0	OR= -	n=0	OR= -	n=2
E2E3	n=24	OR=1.1 (0.7 to 1.9)	n=10	OR=0.9 (0.4 to 1.9)	n=10	OR=1.3 (0.6 to 3.3)	n=68
E2E4	n=4	OR=0.9 (0.3 to 2.9)	n=3	OR=1.2 (0.3 to 4.5)	n=0	OR= -	n=15
E3E4	n=61	OR=1.8 (1.2 to 2.7)	n=24	OR=1.3 (0.8 to 2.2)	n=26	OR=2.2 (1.2 to 3.8)	n=117
E4E4	n=6	OR=6.9 (1.6 to 9.4)	n=5	OR=10.5 (2.4- to 6.6)	n=1	OR=3.3 (0.4 to 34.3)	n=3

* Relative risks estimated as odds ratio with adjustment for age, gender, residency and education.

† Includes 27 patients who could not be classified as either vascular dementia (VaD) or Alzheimer's disease with cerebrovascular disease (AD+CVD) as well as patients with VaD and AD+CVD.

Discussion

An increased risk of dementia with stroke, which included patients with either VaD or AD+CVD, was found to be associated with the APOE*4 allele. The association between APOE*4 and dementia with stroke was similar in women and men and did not vary with increasing age. The APOE*4 allele was associated with a higher relative risk of dementia with stroke in both the homozygous and heterozygous configuration, but among African-Americans and Hispanics the strongest effect was observed in individuals homozygous for APOE*4. No effects of the APOE*2 allele were apparent.

We pooled data from two population-based studies because prior studies had included too few patients and controls to investigate the relationship between APOE and dementia with stroke. The distribution of the APOE alleles and the subtypes of dementia with stroke were similar in two studies, lessening the possibility that the results reflect admixture of genetically distinct populations. Nevertheless, all analyses were adjusted for residency and ethnic group. Although autopsy confirmation was limited to two patients, the diagnostic work-up in both populations was relatively complete. More extensive neuropathological examination will be essential to increase the diagnostic certainty and to further clarify the association between APOE*4 and dementia with stroke.

Previous investigators[9-12] used other criteria than the NINDS-AIREN.[20] The inconsistency of the association in earlier studies may also have been related to the use of hospitalized patients and controls that did not reflect the patient population.[9-11] Alternatively, some of these differences might reflect the presence of other genetic or environmental risk factors, that modify the APOE*4 effects. APOE*2 was previously found to be associated with VaD,[10] but several studies have found no effect of this allele.[7-9,12]

Studies of stroke and APOE have also been inconsistent. Patients with ischaemic stroke have been reported to have both a higher APOE*2 frequency,[24] and APOE*4 frequency,[25] than controls, but others have found no association between the APOE genotype and stroke.[26] Although the occurrence of a stroke seems unrelated to the APOE genotype, the outcome after intracerebral haemorrhage may be worse for individuals with an APOE*4 allele.[27]

Our results imply that individuals who develop dementia with stroke and those who develop AD may share genetic susceptibility. The apoE protein may be activated in the response to cerebral ischaemia and it may function in compensatory synaptogenesis.[28,29] Alternatively, the apoE protein may be involved in the pathogenesis of VaD or AD+CVD through its effects on lipids. Compared to apoE3, apoE4 in-

creases total cholesterol and LDL-cholesterol levels which could increase the risk of atherosclerotic vascular disease; ApoE2 has the opposite effect.[30]

Cerebrovascular disease and AD are common disorders and it is not surprising that they may overlap in some individuals.[31] Thus, dementia in patients with stroke, as currently defined, may include a substantial number of individuals with AD. As many as 15% to 30% of individuals with stroke and clinically diagnosed dementia prove to have mixed diagnoses at postmortem examination which include AD and cerebrovascular disease.[32]

The APOE*4 related risk of dementia and stroke was slightly different in Caucasians than in other ethnic groups paralleling our work in AD.[5] We previously proposed that in African-Americans and Hispanics a modifying gene or environmental factor might alter the effects of one APOE*4 allele, but fails to protect against the consequences of two APOE*4 alleles.[5]

We conclude that APOE*4 is a genetic risk factor for dementia in patients with stroke, also classified as having either VaD and AD+CVD. The degree of association may vary by ethnic group. To further elucidate the pathogenesis of these types of dementia, the role of vascular risk factors and disease should be studied in relation to the APOE genotype. Furthermore, additional genetic susceptibility or modifier loci, as well as environmental factors should be investigated.

References

1. Strittmatter WJ, Saunders AM, Schmechel D, et al. Apolipoprotein E: high affinity binding to beta-amyloid and increased frequency of type 4 allele in late-onset Alzheimer's disease. Proc Natl Acad Sci USA 1993;90:1977-81.
2. Corder EH, Saunders AM, Risch NJ, et al. Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. Nature Genet 1994;7:180-4.
3. Van Duijn CM, de Knijff P, Cruts M, et al. Apolipoprotein E4 allele in a population-based study of early-onset Alzheimer's disease. Nature Genet 1994;1:74-8.
4. Henderson AS, Easteal S, Jorm AF, et al. Apolipoprotein E allele ε4, dementia, and cognitive decline in a population sample. Lancet 1995;346:1387-90.
5. Maestre G, Ottman R, Stern Y, et al. Apolipoprotein E and Alzheimer's disease: Ethnic variation in genotypic risks. Ann Neurol 1995;37:254-9.
6. Frisoni GB, Calabresi L, Geroldi C, et al. Apolipoprotein E ε4 allele in Alzheimer's disease and vascular dementia. Dementia 1994;5:240-2.
7. Pirttilä T, Lehtimäki T, Rinne J, et al. The frequency of apolipoprotein E4 allele is not increased in patients with probable vascular dementia. Acta Neurol Scand 1996;93:352-4.
8. Noguchi S, Murakami K, Yamada N. Apolipoprotein E genotype and Alzheimer's disease. Lancet 1993;342:737.
9. Sakoda S, Kuriyama M, Osame M, et al. Apolipoprotein E ε4. Neurology 1994;44:2420.

10. Bétard C, Robitaille Y, Gee M, et al. Apo E allele frequencies in Alzheimer's disease, Lewy body dementia, Alzheimer's disease with cerebrovascular disease and vascular dementia. NeuroReport 1994;5:1893-6.
11. Mahieux F, Couderc R, Moulignier A, et al. Isoforme 4 de l'apolipoprotéine E et maladie d'Alzheimer. Spécificité et étude clinique. Rev Neurol (Paris) 1995;151:231-9.
12. Stengård JH, Pekkanen J, Sulkava R, et al. Apolipoprotein E polymorphism, Alzheimer's disease and vascular dementia among elderly Finnish men. Acta Neurol Scand 1995;92:297-8.
13. Hofman A, Grobbee DE, De Jong PTVM, et al. Determinants of disease and disability in the elderly: The Rotterdam Elderly Study. Eur J Epidemiol 1991;7:403-22.
14. Ott A, Breteler MMB, Van Harskamp F, et al. Prevalence of alzheimer's disease and vascular dementia: association with education. The Rotterdam study. BMJ 1995;310:970-3.
15. Pittman J, Andrews H, Tatemichi T, et al. Diagnosis of dementia in a heterogenous population: a comparison of paradigm-based diagnosis and physician's diagnosis. Arch Neurol 1992;49:461-7.
16. Stern Y, Andrews H, Pittman J, et al. Diagnosis of dementia in a heterogenous population: development of a neuropsychological paradigm and quantified correction for education. Arch Neurol 1992;49:453-60.
17. American Psychiatric Association. Diagnostic and statistical manual of mental disorders, third edition, revised. Washington DC: American Psychiatric Association, 1987.
18. World Health Organization. International Statistical Classification of Diseases and Related Health Problems. Tenth Revision. Geneva: World Health Organization, 1992.
19. McKhann G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Workgroup under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939-44.
20. Roman GC, Tatemichi TK, Erkinjuntti T, et al. Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. Neurology 1993; 43:250-60.
21. Census of population and housing, 1990: summary tape file 1. Technical documentation prepared by bureau of census. Washington, DC: The Bureau, 1991.
22. Hixson J, Vernier D. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhAI. J Lipid Res 1991;31:545-8.
23. Wenham PR, Price WH, Blundell G. Apolipoprotein E genotyping by one-stage PCR. Lancet 1991;337:1158-9.
24. Couderc R, Mahieux F, Bailleul S, et al. Prevalence of apolipoprotein E phenotypes in ischemic cerebrovascular disease. A case-control study. Stroke 1993;24:661-4.
25. Pedro-Botet J, Sentí M, Nogués X, et al. Lipoprotein and apolipoprotein profile in men with ischemic stroke. Role of lipoprotein(a), triglyceride-rich lipoproteins, and apolipoprotein E polymorphism. Stroke 1992;23:1556-62.
26. Kuusisto J, Mykkänen L, Kervinen K, et al. Apolipoprotein E4 phenotype is not an important factor for coronary heart disease or stroke in elderly subjects. Arterioscler Thromb Vasc Biol 1995;15:1280-6.
27. Alberts MJ, Graffagnino C, McClenny C, et al. ApoE genotype and survival from intracerebral haemorrhage. Lancet 1995;346:575.

28. Kida E, Pluta R, Lossinsky AS, et al. Complete cerebral ischemia with short-term survival in rat induced by cardiac arrest. II. Extracellular and intracellular accumulation of apolipoproteins E and J in the brain. Brain Res 1995;674:341-6.
29. Poirier J. Apolipoprotein E in animal models of CNS injury and in Alzheimer's disease. Trends Neurosci 1994;17:525-30.
30. Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis 1988;8:1-21.
31. Marder K, Richards M, Bello J, et al. Clinical correlates of Alzhiemer's disease with and without silent radiographic abnormalities. Arch Neurol 1995;52:146-51.
32. Mirra SS, Markesbery WR. The neuropathology of Alzheimer's disease: Diagnostic features and standardization. In: Khachaturian ZS, Radebaugh TS (eds). Alzheimer's Disease. Causes, Diagnosis, Treatment and Care. Boca Raton: CRC Press, 1996, 111-23.

4.2

Serum apolipoprotein E and Alzheimer's disease

Abstract

*The APOE*4 allele of the apolipoprotein E gene (APOE) is an important risk factor for Alzheimer's disease (AD). It has been suggested that levels of apolipoprotein E (apoE) in plasma are increased in AD. In this population-based study, we found that serum apoE levels were lower in probable AD patients compared to non-demented controls (0.75 μmol/l (SD 0.35), versus 0.83 μmol/l (SD 0.40), P<0.05). This finding is in accordance with lower serum apoE levels as observed in carriers of the APOE*4 allele, who are over-represented in AD. After adjustment for age, sex, total protein, albumin level, body mass index and the APOE genotype, the difference in serum apoE levels largely disappeared. Our population-based study suggests that the level of apoE in serum seems not to contribute to the pathogenesis of AD, independent of the APOE genotype.*

Introduction

Alzheimer's disease (AD) is a progressive, neurodegenerative disorder characterized by neurofibrillary tangles and senile plaque deposition in the brain parenchyma. The APOE*4 allele of the apolipoprotein E gene (APOE) is a well established risk factor for AD,[1] but the mechanism of action is still incompletely understood. Up till now, the large majority of studies focused on the APOE gene, and not on the corresponding protein (apoE). The level of apoE in plasma is only partly determined by the APOE genotype.[2] Increased plasma apoE levels have been observed in AD.[3] Plasma apoE levels can not directly be compared to intracerebral levels, as these rep-

resent two distinct pools, separated by the blood-brain-barrier.[4] However, in AD, the blood-brain-barrier was found to be impaired, which may allow some plasma protein to penetrate the brain parenchyma.[5] If confirmed, the finding that apoE level is increased in plasma of AD patients is of interest, as plasma apoE level is related to vascular diseases,[6,7] and there is growing evidence that vascular factors play a role in the etiology of AD.[8] The aim of this population-based investigation is to further study the role of serum apoE level in AD.

Methods

Study population

This investigation is based on the Rotterdam Study, a population-based cohort study of subjects aged 55 years and over, including institutionalized persons.[9] Dementia was diagnosed as described previously.[10] Briefly, of the 10275 eligible subjects, 7983 participated in the study, and cognitive function was assessed in 7528 (73%). Screen positive persons were further evaluated using an interview with a close relative, neuropsychological tests, neurological examination and neuro-imaging. A panel of physicians, a neurologist and a neuropsychologist, diagnosed dementia in 474 persons based on the DSM-III-R definition.[11] AD was diagnosed according to the NINCDS-ADRDA criteria[12] in 72% of the dementia cases. The present study was restricted to probable AD patients with onset less than 3 years before examination for this study, in order to limit any selective survival.[8,13] The control group consisted of a random sample of participants without dementia. Blood samples were drawn by venipuncture from non-fasting subjects. In 129 probable AD patients and 890 controls the APOE genotype and serum apoE levels were determined on coded samples without knowledge of the diagnosis, using standard methods.[2,14] For the analysis of serum total protein and albumin level, a calorimetric method was used (Hitachi 747 Böhringer).

Statistical analysis

Differences in serum apoE level were studied using a multiple linear regression model, with adjustments for age and sex. To examine whether known determinants of serum apoE such as total protein, albumin level and body mass index, could explain an association between serum apoE level and probable AD, we included these variables in the regression model. To study the association between serum apoE and probable AD, independent of the APOE genotype, we further added the APOE genotypes to the model. The chi-square test was used to compare categorical data, and the two sample t test to study normally distributed, continuous variables.

Results

Descriptive statistics of the study population are presented in *table 1*. Probable AD patients were significantly older and included a higher proportion of females than the controls. In addition, probable AD cases had lower serum albumin levels, and a lower body mass index than the controls. Serum apoE levels were statistically significantly lower in patients with probable AD than in the non-demented controls.

Table 2 shows the relationship between serum apoE levels and the APOE genotype for probable AD patients and cognitively normal individuals. The distribution of the APOE genotype in both groups was in Hardy-Weinberg equilibrium. In both cases and controls, the APOE*2 allele was associated with higher, and the APOE*4 allele with lower serum apoE levels. For each genotype, serum apoE level was lower in probable AD cases than in the controls, although this was not statistically significant. The exception is the APOE2E2 stratum, but this contained only one patient.

As shown in *table 3*, serum apoE levels were statistically significantly lower in probable AD patients after adjustments were made for age and sex. As body mass index, protein and albumin level was related to both probable AD as well as serum apoE level (latter data not shown), we examined whether these variables could explain the association between serum apoE level and probable AD. When we adjusted for these variables, the difference in serum apoE level between cases and controls slightly decreased, and was borderline statistically significant (P=0.06). As discussed above, serum apoE levels are lower in APOE*4 carriers, who are over-represented in AD.[1] To test whether an association between serum apoE level and probable AD may be explained by the different distribution of the genotype, the APOE genotypes were entered into the model. This resulted in a further decrease of the difference in serum apoE levels between cases and controls.

Table 1 – Characteristics of the study population.*

	Alzheimer's disease n=129	Controls n=890
Age (years)	84.1 (6.5)	68.2 (7.2)
Females	73% (94)	59% (526)
Serum protein (g/l)	70.4 (5.6)	70.5 (5.8)
Serum albumin (g/l)	40.8 (2.8)	44.0 (2.7)
Body mass index (kg/m^2)	25.3 (3.8)	26.6 (3.8)
Serum apoE (µmol/l)	0.75 (0.35)	0.83 (0.40)

* Values are unadjusted means (SD) or percentages (numbers) based on all available data.

Discussion

Up until now, plasma apoE in AD has been examined in one study only, by Taddei et al., who reported increased levels.[3] However, it should be noted that this study was small, and may have been subject to selection bias. As the cases were referred to a geriatrician, and the controls were spouses or volunteers, it is more likely that the cases suffered additional disorders than the controls. For example, stroke and myocardial infarction are frequent conditions in a geriatric clinic, which are related to se-

Table 2 – Serum apoE level in demented and controls, according to APOE genotypes.*

APOE Genotype	Alzheimer's disease n=129	Controls n=890
E2E2	2.27 (-) *n*=1	1.76 (0.67) *n*=7
E2E3	1.08 (0.41) *n*=16	1.17 (0.48) *n*=130
E2E4	0.98 (0.31) *n*=3	1.11 (0.44) *n*=18
E3E3	0.72 (0.29) *n*=69	0.81 (0.34) *n*=498
E3E4	0.60 (0.18) *n*=33	0.64 (0.26) *n*=221
E4E4	0.39 (0.23) *n*=7	0.43 (0.13) *n*=16

* Values are mean serum apoE levels (SD) (µmol/l), adjusted for age, sex, body mass index, total protein and albumin level, with numbers.

Table 3 – Difference in serum apoE level between Alzheimer's patients and controls.*

Covariates	Alzheimer's disease n=129	Controls n=890
Age, sex	-0.11 (-0.21 to -0.02)	0 (reference)
Age, sex, protein, albumin, bmi†	-0.09 (-0.19 to 0.00)	0 (reference)
Age, sex, protein, albumin, bmi, APOE†‡	-0.05 (-0.14 to 0.04)	0 (reference)

* Values are mean differences (95% confidence interval) in serum apoE level (µmol/l).
† bmi indicates body mass index .
‡ APOE denotes the apolipoprotein E genotype.

rum apoE levels.[6,7] Further, in the study by Taddei et al. no adjustments were made for factors that could confound the proposed association, including body mass index, total protein and albumin level.[3] As these factors may be related to both serum apoE level and AD, they might obscure an association of apoE level with AD.

We found a close relationship between APOE genotype and serum level, confirming previous reports.[2] Therefore, it seems unlikely that the small difference in serum apoE level between cases and controls, is the result of measurement error in the laboratory tests. Unfortunately, we did not have autopsy confirmation of the diagnoses. Therefore, we can not rule out that we misclassified the dementia subtype in some instances. However, with clinical examination alone, approximately 90% of AD cases can be diagnosed correctly.[15] The restriction to patients with recent onset, limited the possibility of selective mortality.[13] A strength of our study is that it is population-based with a high response rate, which minimized the possibility of selective inclusion.

We found that probable AD patients had non-significantly lower serum apoE levels than the controls, when we adjusted for age, sex, body mass index, total protein and albumin level. Although apoE levels in serum and in the brain can not directly be compared, as these represent two separated pools,[4] it is interesting that apoE levels were found to be reduced in the hippocampus and cerebrospinal fluid of AD patients.[16,17] By contrast, APOE*4 allele expression was found to be increased in the AD brain.[18] It is however unclear whether this is cause or consequence. More research is required to determine the role of intracerebral apoE levels in the etiology of AD. This study suggests that in serum the difference in apoE level between probable AD cases and non-demented controls results largely from differences in the distribution of the APOE genotype. Therefore, the level of apoE in serum seems not to contribute to the pathogenesis of AD, independent of the APOE genotype.

References

1. Slooter AJC, Cruts M, Kalmijn S, et al. Risk estimates of dementia by apolipoprotein E genotypes from a population-based incidence study: the Rotterdam Study. Arch Neurol 1998;55:964-8.
2. Smit M, De Knijff P, Rosseneu M, et al. Apolipoprotein E polymorphism in the Netherlands and its effect on plasma lipid and apolipoprotein levels. Hum Genetics 1988;80:287-92.
3. Taddei K, Clarnette R, Gandy SE, et al. Increased plasma apolipoprotein E (apoE) levels in Alzheimer's disease. Neurosci Lett 1997;223:29-32.
4. Linton MF, Gish R, Hubl ST, et al. Phenotypes of apolipoprotein B and apolipoprotein E after liver transplantation. J Clin Invest 1991;88:270-81.

5. Duong T, Pommier EC, Scheibel AB. Immunodetection of the amyloid P component in Alzheimer's disease. Acta Neuropathol 1989;78:429-37.
6. Buring JE, O'Connor GT, Goldhaber SZ, et al. Decreased HDL_2 and HDL_3 cholesterol, apoA-I and apoA-II, and increased risk of myocardial infarction. Circulation 1992;85:22-9.
7. Couderc R, Mahieux F, Bailleul S, et al. Prevalence of apolipoprotein E phenotypes in ischemic cerebrovascular disease. A case-control study. Stroke 1993;24:661-4.
8. Hofman A, Ott A, Breteler MMB, et al. Atherosclerosis, apolipoprotein E and the prevalence of dementia and Alzheimer's disease in a population-based study: The Rotterdam Study. Lancet 1997;349:151-4.
9. Hofman A, Grobbee DE, De Jong PTVM, et al. Determinants of disease and disability in the elderly: The Rotterdam Elderly Study. Eur J Epidemiol 1991;7:403-22.
10. Ott A, Breteler MMB, Van Harskamp F, et al. Prevalence of Alzheimer's disease and vascular dementia: association with education. The Rotterdam Study. BMJ 1995;310:970-3.
11. American Psychiatric Association. Diagnostic and statistical manual of mental disorders, third edition, revised, American Psychiatric Association, Washington DC, 1987.
12. McKhann G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Workgroup under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939-44.
13. Stern Y, Brandt J, Albert M, et al. The absence of an apolipoprotein ε4 allele is associated with a more aggressive form of Alzheimer's disease. Ann Neurol 1997;41:615-20.
14. Van Vlijmen BJM, Van 't Hof HB, Mol MJTM, et al. Modulation of very low density lipoprotein production and clearance contributes to age- and gender dependent hyperlipoproteinemia in apolipoprotein E3-Leiden transgenic mice. J Clin Invest 1996;97:1184-92.
15. Galasko D, Hansen LA, Katzman R, et al. Clinical-neuropathological correlations in Alzheimer's disease and related dementias. Arch Neurol 1994;51:888-95.
16. Bertrand P, Poirier J, Oda T, et al. Association of apolipoprotein E genotype with brain levels of apolipoprotein E and apolipoprotein J (clusterin) in Alzheimer's disease. Mol Brain Res 1995;33:174-8.
17. Skoog I, Hesse C, Fredman P, et al. Apolipoprotein E in cerebrospinal fluid in 85-year-old subjects. Relation to dementia, apolipoprotein E polymorphism, cerebral atrophy, and white matter lesions. Arch Neurol 1997;54:267-72.
18. Lambert J-C, Pérez-Tur J, Dupire M-J, et al. Distortion of allelic expression of apolipoprotein E in Alzheimer's disease. Hum Mol Genet 1997;6:2151-4.

4.3

Apolipoprotein E genotype and progression of Alzheimer's disease

Abstract

*The APOE*4 allele of the apolipoprotein E gene increases the risk of Alzheimer's disease (AD), but it is controversial whether it also affects the disease course. However, all studies on this issue up until now are based on patients who may have been included at different stages of disease. In this population-based study, 97 patients were included at a similar stage, i.e. before the onset of symptoms, and followed for up to 5 years. We found that the APOE*4 allele was not a strong determinant of survival in AD. As change in cognitive function and severity of dementia were similar for AD patients with and without APOE*4, our study suggests that the progression of AD is not related to the APOE*4 allele.*

Introduction

Carriers of the APOE*4 allele of the apolipoprotein E gene (APOE) have an increased risk of Alzheimer's disease (AD).[1-3] The risk of AD is elevated, and the mean age of symptom onset reduced, as the number of APOE*4 alleles increases.[2-7] These observations may suggest that APOE*4 accelerates the neurodegenerative process underlying AD. However, findings on APOE and progression or mortality in AD have been very inconsistent.[4-12] Several groups reported that APOE*4 carrying AD patients have even a slower rate of deterioration,[4,11] and an increased survival compared to patients without APOE*4.[7,11] In addition, a decreased survival was sug-

gested for early onset AD patients who carry the APOE*2 allele.[12] Others found that APOE does not affect course,[6-10] or mortality in AD.[5] A problem in the interpretation of these findings is that patient characteristics varied considerably across studies. As all investigations up until now are based on prevalent patients, enrolment may have been at different stages of disease. The aim of this investigation was to study whether APOE*4 affects the rate of decline or survival in AD. All patients entered this population-based study at the same stage, i.e. before the onset of symptoms.

Methods

Study population

This investigation forms part of the Rotterdam Study, a population-based cohort study among persons aged 55 years or over, described in detail elsewhere.[14] The Rotterdam Study was approved by the Medical Ethics Committee of Erasmus University. Participants were recruited from 10275 eligible residents of a suburb of Rotterdam, including institutionalised persons. A total of 7983 participants (response rate 78%) were examined at baseline. Of these, 7528 persons (94%) were cognitively tested, of whom 474 were diagnosed as demented and excluded from the present study. At follow-up, after an average of 2.1 years (SD 0.9 years), 6315 persons (79%) were re-examined.

The analyses presented here are restricted to AD patients who were non-demented at baseline, of whom blood was available for APOE typing. Of these 97 incident AD patients, 9 had died before the follow-up examination. Furthermore, 2 refused to participate and 1 was inaccessible. Thus, 85 AD patients were examined at least twice. Follow-up on mortality was complete for all 97 patients.

Diagnosis

For the assessment of dementia, we used the same protocol at baseline and at follow-up.[15,16] Briefly, all subjects were screened on cognitive functioning. Screen positives underwent further neuropsychological testing, and an informant was interviewed on daily functioning of the participant. Persons who were suspected of dementia were examined by a neurologist, underwent neuropsychological testing, and, if possible, had a Magnetic Resonance Imaging brain scan made.[15,16] Of subjects who could not be re-examined in person, information was obtained from general practitioners and the regional institute for outpatient mental health care (RIAGG), which covers the entire study population. Examinations by the RIAGG include (informant) interviews, neurological and neuropsychological testing, blood biochemistry and syphilis serology. The diagnosis of AD was made by a panel of study physicians, a neurolo-

gist and a neuropsychologist, which reviewed all existing information and used criteria of the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA).[17] The age at symptom onset was taken to be the midpoint between baseline age and the age at diagnosis.

Follow-up procedures

Cognitive function was evaluated with the Dutch version of the Mini Mental State Examination (MMSE; maximum best score: 30 points).[18] The stage of AD was assessed with the clinical dementia rating scale (CDR).[19] At the first and second wave of the Rotterdam study, the MMSE was administered by specially trained research assistants. As part of the diagnostic evaluation, a CDR score was denoted by a neurologist or a study physician. All participants who were diagnosed as having AD after baseline were invited for additional follow-up examinations, at intervals of approximately 15 months. These included a neurological examination, cognitive testing, and an interview with the caregiver. The two additional follow-up examinations were performed by a neurologist or a resident in neurology, using a standardised protocol. All measurements were carried out without knowledge of the APOE genotype.

Data on mortality for the present study were available until April 1996 (mean duration 4.3 years (SD 1.1)). Information on the vital status of all participants was obtained at regular intervals from the municipal health authorities.

Apolipoprotein E genotyping

The APOE genotype was determined on coded DNA samples, as described in detail elsewhere.[3] Those who performed the genotyping were blinded to the participants' test scores. Briefly, a polymerase chain reaction was conducted, and the amplification products were digested with *Hha*I. The resulting restriction fragments were separated using precast ExcelGel gels (Pharmacia Biotech, Uppsala, Sweden) and visualised by silver staining. The results were read by three persons independently. In case of discrepancies, APOE genotyping was repeated.

Statistical analysis

Patients were assigned to one of two groups, those with and without an APOE*4 allele. Persons with the APOE2E3 and those with APOE3E3 were combined, as well as homozygotes and heterozygotes for APOE*4 because of limited statistical power for separate analyses. Simple comparisons were done with t tests and X^2 tests as appropriate.

Mortality in AD patients with and without at least one APOE*4 allele, was compared by Kaplan-Meier survival curves using the log rank statistic. To overcome possible confounding, we used Cox proportional hazard analyses. Baseline age, sex, and education level were further predictors, and the number of months from symptom onset was the time variable.

To evaluate the change in MMSE and CDR scores, a general linear mixed model was used,[20] with random intercept and random regression coefficient for the duration of follow-up. For practical reasons, the CDR staging was coded as: 0 (no dementia), 1 (questionable), 2 (mild), 3 (moderate), 4 (severe), 5 (profound) and 6 (terminal), and reported as the modified CDR (mCDR). By introducing the interaction term between duration of follow-up and carriership of APOE*4, it was assessed whether the average change in mCDR or MMSE score differed between carriers and non-carriers of APOE*4. In case of mCDR, robust standard errors were employed as this score is not normally distributed. The MMSE score however appeared to be normally distributed in this population of AD patients. In all analyses, adjustments were made for age, sex, and education level.

Results

The distribution of the APOE genotype in our study population was: APOE2E3: 6% (n=6), APOE2E4: 2% (n=2), APOE3E3: 57% (n=55), APOE3E4: 32% (n=31) and APOE4E4: 3% (n=3). The APOE genotype distribution was in Hardy-Weinberg equilibrium (X^2=0.6; df=3: P>0.2), which indicates that there was no selective survival associated with APOE. Some characteristics of the study population are shown in *table 1*. Carriers of APOE*4 were younger, included a lower pro-

Table 1 – Baseline characteristics of the study population*

	APOE*4- n=61	APOE*4+ n=36
Baseline age	82.5 (7.0)	80.0 (6.9)
Age at onset	83.8 (7.0)	81.3 (6.9)
Women	82% (50)	75% (27)
Men	18% (11)	25% (9)
Primary education only	63% (35)	34% (11)
Secondary education or more	38% (21)	67% (21)

* Values are means (SD) or proportions (numbers); data on education level were missing in 9 patients.

portion of women, and had less often primary education only, but none of these differences were statistically significant.

Among APOE*4 carriers, 17 patients (47%) had died, among non-APOE*4 carriers 28 subjects (46%). The *figure* shows that overall survival was the same in APOE*4 carriers and patients without this allele. Specifically, the median survival in APOE*4 carriers was 3.3 years (95% confidence interval (CI) 2.9 to 3.8) after symptom onset. Patients without the APOE*4 allele had a median survival of 3.4 years (95% CI 2.9 to 3.9) after onset of symptoms (log rank=0.01; df=1; P=0.93). As APOE*4 carriers were on average younger, and the survival after onset was similar, the mortality rate in carriers of the APOE*4 allele was higher when we adjusted for age, sex and education level (relative risk 1.5; 95% CI 0.8 to 2.8).

As shown in *table 2*, MMSE as well as mCDR scores were similar for carriers and non-carriers of APOE*4. The annual rate of decline in MMSE score was also equal for patients with and without APOE*4 (difference=-0.1; SE=0.6; P=0.88). Furthermore, the annual increase in mCDR score was similar in both groups (difference=0.0; SE=0.4; P=0.98).

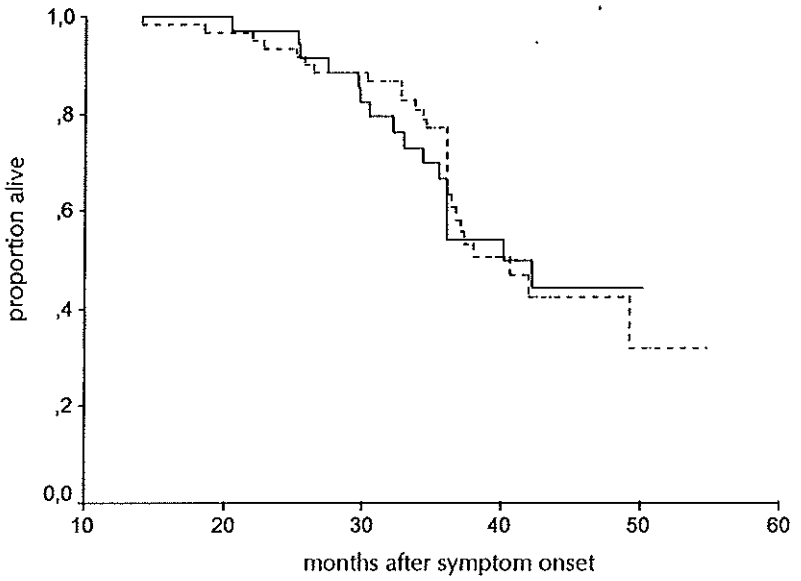

Figure – Survival curves for Alzheimer patients with (—) and without (- - -) APOE*4 from onset of symptoms.

Discussion

In summary, our study suggests that the rate of decline in AD is not related to the presence or absence of the APOE*4 allele. Furthermore, APOE*4 seems not to be a strong determinant of survival once AD has started.

This is the first study on APOE and the course and survival in AD, in which patients were enrolled before symptom onset. Of concern is the level of education, which is an important determinant of the rate of AD progression.[21] Persons with higher premorbid intelligence may be at a biologically more advanced stage of the neurodegenerative process at symptom onset, and their disease may therefore progress more rapidly.[13,21] The Rotterdam study population is however rather homogeneous with respect to education level,[15] therefore, all patients were presumably at a biologically similar stage at enrolment. Besides, adjustments were made for differences in education level. An advantage of this study is the population-based setting, which limited selection bias. In these settings, the implementation of autopsy confirmation of the AD diagnosis may be difficult, and this is obviously a limitation of the present study. However, clinical examinations were extensive, and the diagnosis may be expected to be correct in approximately 90% of AD cases with clinical examinations alone.[22] We used well recognised measures to estimate the rate of disease progression: the MMSE score for cognitive function, the CDR scale for the stage of dementia, and vital status. With up to 5 follow-up years, this is one of the studies with longest duration, reported so far.

Table 2 – MMSE and mCDR scores for Alzheimer patients with and without APOE*4*

Follow-up†		APOE*4-			APOE*4+		
		number	MMSE	mCDR	number	MMSE	mCDR
1	0	n=61	26.0 (22.0-28.0)	0.0 (0.0-0.0)	n=36	26.0 (22.0-28.1)	0.0 (0.0-0.0)
2	31	n=57	20.0 (14.0-24.2)	2.0 (1.0-2.2)	n=28	20.0 (15.8-25.1)	2.0 (1.0-3.0)
3	46	n=47	14.0 (4.0-21.0)	2.0 (1.0-4.0)	n=25	16.0 (4.0-23.0)	2.0 (1.0-4.0)
4	63	n=33	12.0 (1.4-20.0)	3.0 (2.0-5.0)	n=15	10.0 (2.2-20.8)	3.0 (1.6-5.0)

* MMSE means mini-mental state examination; mCDR denotes modified Clinical Dementia Rating scale, coded as: 0 (no dementia), 1 (questionable), 2 (mild), 3 (moderate), 4 (severe), 5 (profound) and 6 (terminal); values are medians with 10th and 90th percentile.

† Visit and mean number of follow-up months.

Inconsistent findings have emerged from studies on APOE and the course of AD.[4-12] However, all these investigations included patients in various stages of disease,[4-12] hampering comparisons of the rate of decline.[13] Furthermore, several studies included patients who were referred to specialised centres,[4,7-9,11] and may have been subject to referral bias. Important aspects regarding referral are firstly that APOE*4 was found to be related to the symptomatology of AD,[23-25] and secondly that APOE*4 carriers more often have a positive family history of dementia. Therefore, APOE*4 carriers may, on average, be referred to a memory clinic at an earlier stage of disease. This could explain why in some studies APOE*4 carrying AD patients had a less aggressive disease course,[4,7,11] as decline in AD may be slower in the earlier stages as compared to later stages.[13]

Earlier, we reported an increased mortality among APOE*2 carrying AD patients with an early onset.[12] In the present study, we found that APOE*2 carriers and patients with APOE3E3 had similar survival, and we did not observe differences across age strata. As none of the participants of this study had an onset before 65 years, we could not study whether APOE*2 has differential effects in early- versus late onset AD.

Overall, the presence of APOE*4 was not related to survival once AD had started. However, when adjustments were made for age, sex and education level, mortality was non-significantly increased in APOE*4 carriers relative to patients without this allele. This finding may be explained by the relationship of APOE*4 with cardiac co-morbidity,[26] as death in AD patients not necessarily reflects an end-stage of dementia.

The observation that APOE*4 increases the risk of AD, while it seems unrelated to its progression, suggests that the processes that initiate AD differ from those that determine its progression. APOE*4 might trigger a cascade of neurodegenerative processes, that subsequently continue independently of the APOE genotype. If this were true, it may imply that agents that could block the harmful effects of the apolipoprotein E4 isoform, or resemble the beneficial effects of apolipoprotein E2 or E3, may not retard further decline once AD has started.

In conclusion, our population-based study suggests that APOE*4 is not related to progression of AD. APOE*4 appears to increase the risk of AD, and to reduce the age at symptom onset.[1-7] However, once the disease has started, the rate of decline seems not to be influenced by APOE*4.

References

1. Strittmatter WJ, Saunders AM, Schmechel D, et al. Apolipoprotein E: high-avidity binding to ß-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease.

Proc Natl Acad Sci USA 1993;90:1977-81.
2. Farrer LA, Cupples LA, Haines JL, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein R genotype and Alzheimer's disease. A meta-analysis. JAMA 1997; 278:1349-56.
3. Slooter AJC, Cruts M, Kalmijn S, et al. Risk estimates of dementia by apolipoprotein E genotypes from a population-based incidence study: the Rotterdam Study. Arch Neurol 1998;55:964-8.
4. Frisoni GB, Govoni S, Geroldi C, et al. Gene dose of the ε4 allele of apolipoprotein E and disease progression in sporadic late-onset Alzheimer's disease. Ann Neurol 1995;37:596-604.
5. Gomez-Isla T, West HL, Rebeck GW, et al. Clinical and pathological correlates of apolipoprotein E ε4 in Alzheimer's disease. Ann Neurol 1996;39:62-70.
6. Basun H, Grut M, Winblad B, et al. Apolipoprotein ε4 allele and disease progression in patients with late-onset Alzheimer's disease. Neurosci Lett 1995;183:32-4.
7. Corder EH, Saunders AM, Strittmatter WJ, et al. Apolipoprotein E, survival in Alzheimer's disease patients, and the competing risks of death and Alzheimer's disease. Neurology 1995; 45:1323-8.
8. Dal Forno G, Rasmusson X, Brandt J, et al. Apolipoprotein E genotype and rate of decline in probable Alzheimer's disease. Arch Neurol 1996;53:345-50.
9. Kurz A, Egensperger R, Haupt M, et al. Apolipoprotein E ε4 allele, cognitive decline, and deterioration of everyday performance in Alzheimer's disease. Neurology 1996;47:440-3.
10. Growdon JH, Locascio JJ, Corkin S, et al. Apolipoprotein E genotype does not influence rates of cognitive decline in Alzheimer's disease. Neurology 1996;47:444-8.
11. Stern Y, Brandt J, Albert M, et al. The absence of an apolipoprotein ε4 allele is associated with a more aggressive form of Alzheimer's disease. Ann Neurol 1997;41:615-20.
12. Van Duijn CM, De Knijff P, Wehnert A, et al. The apolipoprotein E ε2 allele is associated with an increased risk of early-onset Alzheimer's disease and a reduced survival. Ann Neurol 1995; 37:605-10.
13. Plassman BL, Breitner JCS. Apolipoprotein E and cognitive decline in Alzheimer's disease. Neurology 1996;47:317-20.
14. Hofman A, Grobbee DE, De Jong PTVM, et al. Determinants of disease and disability in the elderly. The Rotterdam Elderly Study. Eur J Epidemiol 1991;7:403-22.
15. Ott A, Breteler MMB, Van Harskamp F, et al. Prevalence of Alzheimer's disease and vascular dementia: association with education: the Rotterdam study. BMJ 1995;310:970-3.
16. Ott A, Breteler MMB, Van Harskamp F, et al. The incidence and risk of dementia. The Rotterdam Study. Am J Epidemiol 1998;147:574-80.
17. McKhann G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939-44.
18. Folstein MS, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189-98.
19. Hughes CP, Berg L, Danziger WL, Coben LA, Martin RL. A new clinical scale for the staging of dementia. Br J Psychiatry 1982;140:566-72.
20. Laird NM, Ware JH. Random-effects models for longitudinal data. Biometrics 1982;38:963-74.

21. Stern Y, Tang M-X, Denaro J, Mayeux R. Increased risk of mortality in Alzheimer's disease patients with more advanced educational and occupational attainment. Ann Neurol 1995; 37:590-5.
22. Galasko D, Hansen LA, Katzman R, et al. Clinical-neuropathological correlations in Alzheimer's disease and related dementias. Arch Neurol 1994;51:888-95.
23. Ramachandran G, Marder K, Tang M, et al. A preliminary study of apolipoprotein E genotype and psychiatric manifestations of Alzheimer's disease. Neurology 1996;47:256-9.
24. Lehtovirta M, Soininen H, Helisalmi S, et al. Clinical and neuropsychological characteristics in familial and sporadic Alzheimer's disease. Relation to apolipoprotein E polymorphism. Neurology 1996;46:413-9.
25. Bronzova J, Van Duijn CM, Havekes LM, et al. Apolipoprotein E genotype and concomitant clinical features in early-onset Alzheimer's disease. J Neurol 1996;243:465-8.
26. Van der Cammen TJM, Verschoor CJ, Van Loon CPM, et al. Risk of left ventricular dysfunction in patients with probable Alzheimer's disease with APOE*4 allele. J Am Geriatr Soc 1998. In press.

5

APOLIPOPROTEIN E AND ATHEROSCLEROSIS

5.1

Apolipoprotein E and atherosclerosis

Introduction

Lipoproteins and apolipoproteins

Extraction of plasma lipids yields triacylglycerols (triglycerides), phospholipids, cholesterol and cholesteryl esters as well as a small fraction of free fatty acids. The transport of these hydrophobic lipids in aqueous plasma is made possible by associating the more insoluble lipids (triglycerides and cholesteryl esters) with more polar ones (phospholipids and cholesterol), and then combining them with protein to form a hydrophilic lipoprotein. Several groups of lipoproteins have been identified. These are chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL). Besides, there are two other lipoprotein classes: chylomicron remnants and VLDL-remnants or intermediate density lipoproteins (IDL). The density of lipoproteins increases as the protein content rises and the lipid content falls. Triglycerides are the predominant lipids in chylomicrons and VLDL, whereas cholesterol and phospholipid are the predominant lipids in LDL and HDL, respectively. The protein part of lipoproteins is known as apolipoproteins or apoproteins, constituting nearly 60% of some HDL and as little as 1% of chylomicrons. Fourteen distinct apolipoproteins have been identified, each associated with a specific class of lipoproteins. Apolipoprotein E (apoE) is found in chylomicrons and its remnants, in VLDL and in HDL.[1]

Apolipoprotein E

ApoE is a single polypeptide with a molecular weight of 34200 D. After post-translational cleavage, it has 299 amino acids. There are three common isoforms: apoE2,

apoE3 and apoE4, which differ in one or two amino acids. The most common form, apoE3, contains a cysteine residue at position 112 and an arginine residue at position 158. ApoE2 has cysteine at both positions, and apoE4 has there arginine. The biosynthesis of apoE is under control of a single genetic locus on region q12-q13.2 of chromosome 19. The apoE2, apoE3 and apoE4 isoforms are coded by three codominant alleles: APOE*2, APOE*3 and APOE*4, respectively. The allele frequencies in Caucasians are 0.08, 0.77 and 0.15 respectively. In Japanese and Chinese the APOE*4 frequency is lower, in Africans and Finns higher.[2,3] Besides, more than 20 other apoE isoforms have been identified, with allele frequencies below 0.001. The liver is the major site of apoE production, but apoE is also synthesised in the brain. These pools are strictly separated as circulating apoE can not pass the blood-brain-barrier.[4,5] ApoE plays a role in tissue regeneration, immunoregulation, cell differentiation and growth.[6] Interestingly, in persons who can not synthesise apoE, neurologic, endocrine, and immune functions are normal; the only apparent abnormality is the metabolism of lipoproteins.[7]

Metabolism of lipoproteins

Dietary triglycerides and cholesterol are packed in the intestinal mucosal cells together with several apolipoproteins into chylomicrons. After entry in the circulation, the apolipoprotein composition of these particles changes, partly because of acquisition of apoE. During subsequent lipolysis the chylomicrons become smaller, and the remnants are cleared from the circulation by the LDL receptor related protein (LRP). ApoE serves as a high affinity ligand for this receptor.

VLDL carries triglycerides from the liver to the extrahepatic tissues. Newly secreted VLDL contains several apolipoproteins, but little apoE. The apoE content in VLDL increases after release in the circulation. VLDL is cleared from the bloodstream by LRP or the LDL-receptor, using apoE as a high avinity ligand. The remnant particles (IDL) are further hydrolysed and converted into LDL, thereby loosing apolipoproteins. The resulting LDL particles contain 60-70% of total plasma cholesterol, which are cleared from the circulation by the liver and by cells from the reticulo-endothelial system, such as macrophages in the subendothelial space.

HDL, another lipoprotein that contains apoE, is the major lipoprotein involved in the transport of cholesterol from the peripheral tissues to the liver. Esterified cholesterol can be transferred from HDL to chylomicrons, VLDL, and LDL. Other HDL particles are removed from the circulation, using apoE as a ligand for receptor binding.

Atherosclerosis

The transport of lipoproteins into the artery wall is the first stage of an atherosclerotic lesion. This transport is a concentration-dependent process that does not require receptor-mediated endocytosis.[8,9] Lipoproteins that have been trapped in the subendothelial space are oxidised. Mildly oxidised LDL induces monocyte adherence, migration and conversion into macrophages. It further stimulates apoE secretion by macrophages. Oxidised lipoproteins accumulate with cholesterol in these macrophages to form foam cells, the hallmark of the fatty streak.[10] HDL on the other hand, protects against oxidation of LDL. Foam cells secrete products that also promote the further progression of the atherosclerotic plaque.[10] When LDL becomes highly oxidised, it gets toxic to macrophages,[11] which may contribute to the formation of the necrotic core found in the second stage of an atherosclerotic lesion, the fibrous plaque. This is a central acellular area of lipid from necrotic foam cells, covered by a fibrous cap containing smooth muscle cells and collagen. The growing lesion then encroaches the arterial lumen, by the migration of new mononuclear cells, the proliferation of macrophages and smooth muscle cells, the production of an extracellular matrix and the accumulation of lipid in the necrotic core.[10] The final stage is a complex lesion, with thrombus formation by deposition of fibrin and platelets. Mildly oxidised LDL further contributes to arterial occlusion by stimulating the release of coagulant agents, and by inhibiting reactive vasodilatation. The plaque is thought to weaken as a result of cell death and the secretion of proteases from foam cells.[12] Besides, the atherosclerotic lesion is subject to calcification. A weakened plaque over an area of calcification predisposes to plaque rupture, because of different physical properties which are subjected to the pulses of arterial blood pressure.

Apolipoprotein E, lipids and atherosclerosis

Lipid levels

The APOE polymorphism partly determines the level of plasma cholesterol, and this effect further depends on diet and gender.[13] There seems not to be a consistent relationship between the APOE genotype and triglycerides or HDL cholesterol levels.[2] APOE*2 carriers have lower levels of total cholesterol, LDL-cholesterol and apolipoprotein B, and higher levels of apoE.[14-16] APOE*4 carriers show the opposite in most populations studied.[14,15] The "cholesterol-lowering" effect of APOE*2 is two or three times the 'cholesterol-raising' effect of the APOE*4 allele, depending on the population studied.[2] The APOE*2 allele can however not be regarded as entirely benign, as it is also associated with type III hyperlipoproteinemia.

Lipid metabolism

APOE*4 carriers have a more efficient catabolism of chylomicron remnants and IDL, compared to persons with other APOE genotypes.[2] Therefore, the rate of conversion of VLDL remnants to LDL is higher, resulting in a downregulation of hepatic LDL receptors. These APOE*4-mediated alterations lead to lower concentrations of cholesterol-containing remnant particles, and higher levels of cholesterol-rich LDL particles. The net alteration is an increase in total cholesterol concentration. The apoE isoforms also differ in their preference to various lipoprotein classes, which may lead to different clearance rates.[2]

ApoE is also produced by macrophages in atherosclerotic lesions.[17] Loading of macrophages with cholesterol stimulates apoE synthesis and secretion, which regulates the storage and efflux of cholesterol.[17] ApoE secretion by macrophages may further inhibit the aggregability of platelets and modulate lymphocyte function. In addition, apoE interacts with the extracellular matrix, thereby influencing the retention of lipoproteins.[18]

Atherosclerosis and vascular disease

Among healthy young adults who died of external causes, APOE*4 carriers had more atherosclerotic lesions at autopsy than those without this allele.[19] In patients with diabetes mellitus, APOE*2 tended to protect from macro- and microangiopathy, while APOE*4 had a predisposing effect.[20]

Conflicting results have emerged on the association between APOE and coronary heart disease (CHD).[21-24] In a meta-analysis on the risk of CHD, it was found that APOE*4 carriers had a 1.3 fold increased risk, and APOE*2 carriers have a similar risk, as compared to homozygotes for APOE*3.[25] However, most of these studies were cross-sectional and may be subject to bias. In prospective studies, an increased risk of fatal CHD,[26,27] as well as a similar risk of all CHD[28] have been reported for carriers of APOE*4.

It is not yet clear whether there is an association between the APOE genotypes and cerebrovascular disease. In cross-sectional studies, no relationship was found between APOE and stroke,[29-31] as well as an increased risk for APOE*2 carriers,[32] and an elevated risk for carriers of APOE*4.[33] In follow-up studies on APOE and stroke no association was observed in two studies, [28,34] but others reported a decreased risk for APOE*2 carriers younger than 80 years.[35]

Atherosclerosis of the carotid arteries, as estimated by ultrasonography, was found to be weakly associated with the APOE*2 allele.[36] However, this study may have been subject to bias, as any participant with clinical evidence of atherosclerosis had been excluded.[36] Another investigation reported the opposite association be-

tween the APOE genotype and carotid artery atherosclerosis.[37] In this study, APOE*2 carriers had less carotid artery intima-media thickness than APOE*3 homozygotes. APOE*4 carriers had the thickest carotid artery intima-media.[37]

Transgenic mice

ApoE deficient mice have delayed clearance of lipoproteins, exhibit very severe hypercholesterolemia and develop atherosclerotic lesions that resemble those in humans, especially when fed a high-fat diet.[38,39] In apoE deficient mice there was a normalisation of plasma lipid levels, and regression of atherosclerotic lesions after introducing apoE expression.[40,41] Local production of apoE by macrophages was found to diminish atherosclerosis, independent of its cholesterol lowering effect.[42]

ApoE levels in human atherosclerosis

Findings on apoE levels in humans with atherosclerotic disease are not straightforward. ApoE concentrations were found to be higher,[43-45] or no different[46] in CHD patients as compared to control subjects. In persons with a previous stroke, apoE levels were found to be lower compared to a control group free of cerebrovascular disease.[32] A problem with the latter study is that the APOE genotype distribution in controls was very unusual.

Conclusion

ApoE plays an important role in lipid metabolism. The association between the APOE genotype and atherosclerotic disease is not yet clear, and the mechanism of action of this putative relationship is incompletely understood. Further studies are needed to clarify the role of serum apoE levels in the association between the APOE genotype and the development of atherosclerotic lesions.

References

1. Harper's review of biochemistry. Martin DW, Mayes PA, Rodwell VW, et al. (eds.). Twentieth edition, Lange medical publications. Los Altos, 1985.
2. Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis 1988;8:1-21.
3. Osuntokun BO, Sahota A, Ogunniyi, AO, et al. Lack of association between apolipoprotein E ε4 and Alzheimer's disease in elderly Nigerians. Ann Neurol 1995;38:463-5.
4. Linton MF, Gish R, Hubl ST, et al. Phenotypes of apolipoprotein B and apolipoprotein E

after liver transplantation. J Clin Invest 1991;88:270-81.
5. Rösler N, Wichart I, Jellinger KA. Intra vitam lumbar cerebrospinal fluid and serum and postmortem vertricular immunoreactive apolipoprotein E in patients with Alzheimer's disease. J Neurol Neurosurg Psychiatry 1996;60:452-4.
6. Mahley RW. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science 1988;240:622-30.
7. Schaefer EJ, Gregg RE, Ghiselli G, et al. Familial apolipoprotein E deficiency. J Clin Invest 1986;78:1206-19.
8. Steinberg D, Parthasarathy S, Carew TE, et al. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med 1989;320:915-24.
9. Young SG, Parthasarathy S. Why are low density lipoproteins atherogenic? West J Med 1994;160:153-64.
10. Berliner JA, Navab M, Fogelman AM, et al. Atherosclerosis: Basic mechanisms. Oxidation, inflammation, and genetics. Circulation 1995;91:2488-96.
11. Reid VC, Mitchinson MJ. Toxicity of oxidised low density lipoprotein towards mouse peritoneal macrophages in vitro. Atherosclerosis 1993;98:17-24.
12. Beisiegel U, St. Clair RW. An emerging understanding of the interactions of plasma lipoproteins with the arterial wall that leads to the development of atherosclerosis. Curr Opin Lipidol 1996;7:265-8.
13. De Knijff P, Havekes LM. Apolipoprotein E as a risk factor for coronary heart disease: a genetic and molecular biology approach. Curr Opin Lipidol 1996;7:59-63.
14. Braeckman L, De Bacquer D, Rosseneu M, et al. Apolipoprotein E polymorphism in middle-aged Belgian men: phenotype distribution and relation to serum lipids and lipoproteins. Atherosclerosis 1996;120:67-73.
15. Smit M, De Knijff P, Rosseneu M, et al. Apolipoprotein E polymorphism in the Netherlands and its effect on plasma lipid and apolipoprotein levels. Hum Genet 1988;80:287-92.
16. Demant T, Bedford D, Packard CJ, et al. Influence of apolipoprotein E polymorphism on apolipoprotein B-100 metabolism in normolipemic subjects. J Clin Invest 1991;88:1490-1501.
17. Rosenfeld ME, Butler S, Ord VA, et al. Abundant expression of apoprotein E by macrophages in human and rabbit atherosclerotic lesions. Arterioscler Thromb 1993;13:1382-9.
18. Mazzone T. Apolipoprotein E secretion by macrophages: its potential physiological functions. Curr Opin Lipidol 1996;7:303-7.
19. Hixson JE. Apolipoprotein E polymorphisms affect atherosclerosis in young males. Arterioscler Tromb 1991;11:1237-44.
20. Ukkola O, Kervinen K, Salmela PI, et al. Apolipoprotein E phenotype is related to macro- and microangiopathy in patients with non-insulin-dependent diabetes mellitus. Atherosclerosis 1993;101:9-15.
21. Damaraju S, Qun-Tao Y, Safavi F, et al. Apolipoprotein e4 is not a genetic risk factor for coronary artery disease or restenosis after percutaneous transluminal coronary angioplasty. Am J Cardiol 1995;75:1181-3.
22. Wang XL, McCredie RM, Wilcken DEL. Polymorphisms of the apolipoprotein E gene and severity of coronary artery disease defined by angiography. Arterioscler Thromb Vasc Biol 1995;15:1030-4.

23. Van Bockxmeer FM, Mamotte CDS, Gibbons FA, et al. Angiotensin-converting enzyme and apolipoprotein E genotypes and restenosis after coronary angioplasty. Circulation 1995;92:2066-71.
24. De Knijff P, Jansen H, Lie KI, et al. Apolipoprotein ε4 and coronary artery disease. Lancet 1992;340:1351.
25. Wilson PWF, Schaefer EJ, Larson MG, et al. Apolipoprotein E alleles and risk of coronary disease. A meta-analysis. Arterioscler Tromb Vasc Biol 1996;16:1250-5.
26. Stengård JH, Zerba KE, Pekkanen J, et al. Apolipoprotein E polymorphism predicts death from coronary heart disease in a longitudinal study of elderly Finnish men. Circulation 1995;91:265-9.
27. Eichner JE, Kuller LH, Orchard TJ, et al. Relation of apolipoprotein E phenotype to myocardial infarction and mortality from coronary artery disease. Am J Cardiol 1993;71:160-5.
28. Kuusisto J, Mykkänen L, Kervinen K, et al. Apolipoprotein E4 phenotype is not an important risk factor for coronary heart disease or stroke in elderly subjects. Arterioscler Tromb Vasc Biol 1995;15:1280-6.
29. Coria F, Rubio I, Nuñez E, et al. Apolipoprotein E variants in ischemic stroke. Stroke 1995;26:2375-6.
30. Hachinski V, Graffagnino C, Beaudry M, et al. Lipids and stroke. A paradox resolved. Arch Neurol 1996;53:303-8.
31. Kuller LH, Shemanski L, Manolio T, et al. Relationship between apoE, MRI findings, and cognitive function in the cardiovascular health study. Stroke 1998;29:388-98.
32. Couderc R, Mahieux F, Bailleul S, et al. Prevalence of apolipoprotein E phenotypes in ischemic cerebrovascular disease. A case-control study. Stroke 1993;24:661-4.
33. Pedro-Botet J, Sentí M, Nogués X, et al. Lipoprotein and apolipoprotein profile in men with ischemic stroke. Role of lipoprotein(a), triglyceride-rich lipoproteins, and apolipoprotein E polymorphism. Stroke 1992;23:1556-62.
34. Basun H, Corder EH, Guo Z, et al. Apolipoprotein E polymorphism and stroke in a population sample aged 75 years or more. Stroke 1996;27:1310-5.
35. Ferrucci L, Guralnik JM, Pahor M, et al. Apolipoprotein E2 allele and risk of stroke in the older population. Stroke 1997;28:2410-6.
36. De Andrade M, Thandi I, Brown S, et al. Relationship of the apolipoprotein E polymorphism with carotid artery atherosclerosis. Am J Hum Genet 1995;56:1379-90.
37. Terry JG, Howard G, Mercuri M, et al. Apolipoprotein E polymorphism is associated with segment-specific extracranial carotid artery intima-media thickening. Stroke 1996;27:1755-9.
38. Nakashima Y, Plump AS, Raines EW, et al. ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. Arterioscler Thromb 1994;14:133-40.
39. Reddick RL, Zhang SH, Maeda N. Atherosclerosis in mice lacling apoE. Evaluation of lesional development and progression. Arterioscler Thromb 1994;14:141-7.
40. Boisvert WA, Spangenberg J, Curtiss LK. Treatment of severe hypercholesterolemia in apolipoprotein E-deficient mice by bone marrow transplantation. J Clin Invest 1995;96:1118-24.
41. Kashyap VS, Santamarina-Fojo S, Brown DR, et al. Apolipoprotein E deficiency in mice: gene replacement and prevention of atherosclerosis using adenovirus vectors. J Clin Invest 1995;96:1612-20.

42. Bellosta S, Mahley RW, Sanan DA, et al. Macrophage-specific expression of human apolipoprotein E reduces atherosclerosis in hypercholesterolemic apolipoprotein E-null mice. J Clin Invest 1995;96:2170-9.
43. Chivot L, Mainard F, Bigot E, et al. Logistic discriminant analysis of lipids and apolipoproteins in a population of coronary bypass patients and the significance of apolipoproteins C-III and E. Atherosclerosis 1990;82:205-11.
44. Buring JE, O'Connor GT, Goldhaber SZ, et al. Decreased HDL_2 and HDL_3 cholesterol, apoA-I and apoA-II, and increased risk of myocardial infarction. Circulation 1992;85:22-9.
45. Syvänne M, Rosseneu M, Labeur C, et al. Enrichment with apolipoprotein E characterizes postprandial TG-rich lipoproteins in patients with non-insulin-dependent diabetes mellitus and coronary artery disease: a preliminary report. Atherosclerosis 1994;105:25-34.
46. Bittolo Bon G, Cazzolato G, Saccardi M, et al. Total plasma apoE and high density lipoprotein apoE in survivors of myocardial infarction. Atherosclerosis 1984;53:69-75.

5.2

Apolipoprotein E and atherosclerosis

The Rotterdam Study

Abstract

*The apolipoprotein E (APOE) polymorphism fully defines the apoE isoforms, and partly determines the level of apoE in serum. Findings on the APOE genotype, serum apoE level and atherosclerosis have been inconsistent. A population-based cross-sectional study was performed on 934 subjects to investigate the association between the APOE genotype, serum apoE levels and atherosclerosis. Atherosclerosis was non-invasively assessed by the presence of plaques in the common carotid arteries (CCAs), calcifications of the abdominal aorta, the CCA intima-media wall thickness, and the ankle to arm blood pressure index. Associations were adjusted for age and sex. Carriers of the APOE*2 allele had a lower prevalence of CCA plaques, a non-significantly decreased prevalence of aorta calcifications, a thinner CCA intima-media and a non-significantly higher ankle-arm index, relative to the APOE3E3 group. Overall, the APOE*4 allele was not associated with the atherosclerosis indicators, although the APOE4E4 genotype was weakly related to atherosclerosis in the abdominal aorta and peripheral arteries. Adjusting for serum apoE, total or high density lipoprotein (HDL) cholesterol levels did not essentially alter these findings. Serum apoE levels were not related to atherosclerosis in the common carotid arteries, but increasing serum apoE levels were related to aorta calcifications and lower ankle-arm indexes. These associations diminished when adjustments were made for total and HDL cholesterol. Our results suggest that the APOE*4 allele is not a strong risk factor for atherosclerosis. The consistent trend for an inverse association between APOE*2 and atherosclerosis was independent of serum apoE, total and HDL cholesterol level, and may therefore result from differences in apoE isoforms.*

Introduction

Atherosclerosis is the most important cause of morbidity and mortality in western countries. High levels of total and low density lipoprotein (LDL) cholesterol and low levels of high density lipoprotein (HDL) cholesterol predispose to the development of atherosclerosis.[1] Serum levels of these lipids are partly determined by the apolipoprotein E genotype (APOE). The APOE gene has three common alleles, APOE*2, APOE*3 and APOE*4, which fully determine the protein isoforms apoE2, apoE3 and apoE4, respectively.[2-4] Compared to homozygotes for APOE*3, the most common genotype, APOE*2 is associated with lower levels of total and LDL cholesterol and with higher levels of HDL cholesterol, while APOE*4 has opposite effects.[2-4] ApoE plays a pivotal role in the transport of lipoproteins,[4] and is involved in numerous processes in the arterial wall.[5]

Studies on the APOE polymorphism and clinical vascular disease have been inconsistent.[6] In a meta-analysis on APOE and coronary heart disease (CHD), APOE*4 appeared to be a weak risk factor (summary odds ratio 1.3), while no association with APOE*2 was observed.[6] However, almost all of these investigations are based on hospital-based patient-series in which selection bias could have played a role. In one prospective population-based survey, APOE*4 was found to be a risk factor for fatal CHD.[7] In a similar study however, no association of APOE*4 was found with either CHD or stroke.[8] No effects of APOE*2 were observed in these two studies.[7,8]

There is a limited number of investigations on the APOE genotype and atherosclerosis. An autopsy study suggested a predisposing role for APOE*4, and a protective effect of APOE*2, in the development of atherosclerosis.[9] In studies on atherosclerosis of the carotid arteries, inconsistent associations with APOE have been reported.[10-13]

The APOE genotype partly determines serum apoE levels.[2,3] Also, the role of serum apoE level in atherosclerosis is unclear. In patients with atherosclerotic disease, apoE concentrations were found to be higher,[14] and lower[15] than in controls.

As serum apoE levels are partly determined by the APOE genotype,[2,3] and the APOE alleles fully define the apoE isoforms,[4] it is unclear whether a putative relation between APOE and atherosclerosis is the result of either different serum levels or different isoforms. The aim of this population-based study was to investigate the association between the APOE genotype, serum apoE level and atherosclerosis.

Methods

Study population

This study is part of the Rotterdam Study, a population-based, single-centre cohort study on chronic and disabling diseases in the elderly. All inhabitants of a suburb of Rotterdam, aged at least 55 years, including people living in homes for the elderly, were invited to participate. The design of the study has been described previously.[16] From all subjects informed consent was obtained and the study was approved by the medical ethics committee of the Erasmus University Medical School. In total 7983 participants (response rate 78%) were examined. Here we describe our observations on a random sample of the first 1000 subjects who visited the research centre. The analyses presented here are restricted to 934 persons of whom both the APOE genotype and serum apoE level were successfully determined.

Clinical investigations

To assess the presence of atherosclerosis in the common carotid arteries (CCAs), ultrasonography was performed with a 7.5 MHz linear-array transducer and a Duplex scanner (ATL UltraMark IV). Both CCAs were evaluated for the presence of atherosclerotic plaques, defined as a focal widening relative to adjacent segments with protrusion into the lumen, as described previously.[17]

In addition, the intima-media thickness of the distal part of both CCAs were measured and averaged, as described before.[17] The presence of plaques as well as an increased CCAs intima-media wall thickness are considered to be indicators of generalised atherosclerosis.[18]

Atherosclerosis of the arteries of the lower extremities was assessed by the ratio of the ankle to brachial systolic blood pressure (ankle-arm index). A decreased ankle-arm index is a measure for peripheral atherosclerosis.[19] Arm blood pressure was measured in sitting position at the right upper arm with a random-zero sphygmomanometer, and the average of two measurements obtained at one occasion was used. Ankle systolic blood pressure was determined with the subject in supine position at both the right and left posterior tibial artery using also a random-zero sphygmomanometer and a Doppler ultrasound transducer. The analyses were based on the lowest of the ankle-arm index on the left and the right side.

Aortic atherosclerosis was diagnosed by radiographic detection of calcific deposits in the abdominal aorta.[20] These calcifications are associated with cardiovascular disease, and correlate well with atherosclerotic plaques, as observed at autopsy.[20,21] Lateral abdominal X-rays were made from a fixed distance with the subject seated. Calcifications were judged to be present when linear densities were seen in an area

parallel and anterior to the lumbar spine (L1-L4). In the analyses, aorta calcifications were classified as absent or present.

Information on health status, drug use and smoking behaviour was obtained with a computerised questionnaire. Height and weight were measured, and body mass index was calculated.

Laboratory investigations

APOE genotyping and the determination of the serum apoE level were performed on coded samples from non-fasting subjects, without knowledge of the other measurements. Genotyping was performed using a polymerase chain reaction, as described elsewhere.[22] Serum apoE levels were determined by enzyme-linked immunosorbent assay.[23] Serum total and HDL cholesterol were determined with an automated enzymatic procedure.[24]

Statistical analysis

The association between the APOE genotype and atherosclerosis was studied in the following multivariate models. The relative risks of plaques in the CCAs and aorta calcifications were estimated as odds ratios (OR) in a multiple logistic regression model, and presented with a 95% confidence interval (95% CI). Differences in CCA intima-media wall thickness and the ankle-arm index were studied in a multiple linear regression model. As determinants we studied firstly the APOE alleles, which were entered as dummy variables, using the most common APOE genotype (APOE3E3) as the reference. Persons with APOE2E2 or APOE2E3 were grouped, as well as those with APOE3E4 or APOE4E4; persons carrying both the APOE*2 and the APOE*4 allele were excluded form these models. Secondly, we used models with the APOE alleles and serum apoE levels. Serum apoE levels were entered in the model as dummy variables, which assumes no a priori distribution. Categories were made according to quintiles of the distribution of serum apoE level, and the lowest quintile was used as a reference. Thirdly, to explore whether the putative effects of APOE are independent of total and HDL cholesterol, we constructed models with the APOE genotypes, total cholesterol and HDL cholesterol. Fourthly, we built models with the APOE genotypes and the following cardiovascular risk factors: systolic blood pressure, diastolic blood pressure, body mass index, smoking, total and HDL cholesterol, in order to study effects of APOE irrespective of these variables. To study the effects of serum apoE level, we used models with quintiles of serum apoE level as the determinant. Further, we built models with serum apoE quintiles and total and HDL cholesterol, to investigate the role of serum apoE independent of total and HDL cholesterol. Finally, differences in total and HDL cholesterol were studied in a linear regression model with quintiles of serum apoE level as the deter-

minant. To adjust for confounding, age and sex were included in all the above models.

The percentage of explained variance was estimated by the squared adjusted multiple correlation coefficient.[25] The Pearson's chi-square statistic was used for categorical data, and the two sample t-test for continuous, normally distributed variables. In regression models with total and HDL cholesterol or the above cardiovascular risk factors, the indicator method for missing data was used.[26] As no major differences between men and women, or between age categories were observed in the associations between the APOE genotype and atherosclerosis, or between serum apoE and atherosclerosis, findings are presented for the whole study-population.

Results

The distribution of the APOE polymorphism in our study population was in Hardy-Weinberg equilibrium (APOE2E2 0.7% (n=7), APOE2E3 14.7% (n=137), APOE2E4 2.0% (n=19), APOE3E3 55.7% (n=520), APOE3E4 24.9% (n=233) and APOE4E4 1.9% (n=18)). Descriptive statistics are presented in *table 1*. APOE*2 carriers had, on average, a higher body mass index, lower total cholesterol levels, and higher levels of HDL cholesterol, as compared to carriers of APOE3E3 or APOE*4. Serum apoE level was highest in APOE*2 carriers, intermediate in subjects with APOE3E3 and lowest in carriers of the APOE*4 allele. For each APOE genotype group separately, unadjusted mean serum apoE levels were 1.72 µmol/L (SD 0.66)

Table 1 – Characteristics of the study population according to APOE alleles*

Characteristic	APOE*2 + n=144	APOE3E3 n=520	APOE*4 + n=251
Age in years	69.1 (7.6)	68.9 (8.0)	69.1 (8.2)
Men	33% (47)	41% (212)	45% (112)
Systolic blood pressure in mmHg	135.5 (20.8)	136.0 (19.8)	134.5 (20.0)
Diastolic blood pressure in mmHg	71.8 (11.1)	70.5 (10.5)	71.0 (11.1)
Body mass index in kg/m^2	27.1 (3.5)	26.4 (3.8)	26.6 (4.0)
Current smoking	23% (32)	23% (116)	23% (56)
Total cholesterol in mmol/L	6.5 (1.2)	6.7 (1.2)	6.8 (1.2)
HDL cholesterol in mmol/L	1.4 (0.4)	1.3 (0.4)	1.3 (0.3)
Serum apoE level inµmol/L	1.19 (0.50)	0.80 (0.33)	0.62 (0.25)

* Values are unadjusted means (SD) or percentages (numbers), based on all available information; APOE*2+ denotes the APOE2E2 or the APOE2E3 genotype; APOE*4+ denotes APOE3E4 or APOE4E4.

for APOE2E2, 1.16 µmol/L (SD 0.48) for APOE2E3, 1.08 µmol/L (SD 0.45) for APOE2E4, 0.80 µmol/L (SD 0.33) for APOE3E3, 0.63 µmol/L (SD 0.25) for APOE3E4 and 0.42 µmol/L (SD 0.12) for APOE4E4. These mean apoE concentrations did not change when adjustments were made for age or sex. The APOE genotype explained 24% of the variance in serum apoE levels.

As shown in *table 2*, we found a strong association between serum apoE level and total cholesterol in the whole study-population and in strata of APOE3E3 and APOE*4 carriers. By contrast, among APOE*2 carriers, serum apoE level was only weakly related to serum total cholesterol.

Table 3 shows that carriers of the APOE*2 allele had a lower prevalence of plaques in the CCAs. This inverse association held when adjustments were made for serum apoE level. In addition, APOE*2 carriers had a lower prevalence of aorta calcifications, which reached statistical significance when adjustments were made for serum apoE level. Overall, APOE*4 was not associated with an increased prevalence of CCAs plaques or aorta calcifications. However, the APOE4E4 genotype was non-significantly related to aorta calcifications (OR=1.6; 95% CI 0.6 to 4.8). Adjusting for total and HDL cholesterol yielded similar results as the models with adjustments for age and sex only.

The median CCA intima-media wall thickness in the APOE3E3 group was 0.78 mm (10th centile 0.62, 90th centile 0.97 mm). As shown in *table 4*, APOE*2 carriers had a thinner CCA intima-media compared to this reference group. The CCA intima-media was similar for persons with the APOE3E3 genotype and for APOE*4 carriers. The median ankle-arm index in the APOE3E3 group was 1.12 (10th centile

Table 2 – Differences in serum total cholesterol level according to quintiles of serum apoE level*

	<0.53	0.53-0.68	0.68-0.85	0.85-1.09	≥1.09
Total Cholesterol in mmol/L					
All	0 (reference)	0.30 (0.07 to 0.52)	0.37 (0.15 to 0.60)	0.85 (0.63 to 1.08)	0.91 (0.69 to 1.14)
APOE*2 +	0 (reference)	0.17 (-0.87 to 1.21)	-0.18 (-1.07 to 0.72)	-0.01 (-0.84 to 0.82)	0.71 (-0.02 to 1.44)
APOE3E3	0 (reference)	0.28 (-0.01 to 0.57)	0.45 (0.16 to 0.74)	1.09 (0.81 to 1.37)	1.36 (1.04 to 1.68)
APOE*4 +	0 (reference)	0.46 (0.12 to 0.80)	0.65 (0.29 to 1.01)	1.43 (0.96 to 1.90)	1.11 (0.49 to 1.72)

* Values are differences with 95% confidence interval; APOE*2+ denotes the APOE2E2 or the APOE2E3 genotype; APOE*4+ means APOE3E4 or APOE4E4; serum apoE levels are in µmol/L.

Table 3 – Odds ratios for common carotid artery plaques and aorta calcifications associated with APOE alleles*

	APOE*2 +	APOE3E3	APOE*4 +
CCA plaques†			
Events (observations)	n=14 (n=142)	n=93 (n=510)	n=34 (n=245)
Model 1‡	0.5 (0.3 to 0.9)	1 (reference)	0.7 (0.4 to 1.1)
Model 2§	0.5 (0.3 to 0.9)	1 (reference)	0.7 (0.5 to 1.2)
Model 3‖	0.5 (0.3 to 0.9)	1 (reference)	0.7 (0.4 to 1.1)
Aorta Calcifications			
Events (observations)	n=59 (n=128)	n=186 (n=474)	n=94 (n=226)
Model 1‡	0.7 (0.5 to 1.1)	1 (reference)	0.9 (0.6 to 1.2)
Model 2§	0.5 (0.3 to 0.8)	1 (reference)	1.0 (0.7 to 1.4)
Model 3‖	0.8 (0.5 to 1.2)	1 (reference)	0.8 (0.6 to 1.2)

* Values are odds ratios with 95% confidence interval; APOE*2+ denotes the APOE2E2 or the APOE2E3 genotype; APOE*4+ means APOE3E4 or APOE4E4;
† CCA denotes common carotid arteries;
‡ Model 1: adjusted for age and sex;
§ Model 2: adjusted for age, sex and serum apoE level;
‖ Model 3: adjusted for age, sex, serum total cholesterol and HDL cholesterol.

Table 4 – Differences in common carotid artery intima-media thickness and ankle-arm index according to APOE alleles*

	APOE*2 +	APOE3E3	APOE*4 +
CCA intima-media (mm)†			
Observations	n=142	n=513	n=247
Model 1‡	-0.04 (-0.06 to -0.01)	0 (reference)	-0.02 (-0.03 to 0.00)
Model 2§	-0.03 (-0.06 to -0.01)	0 (reference)	-0.01 (-0.03 to 0.01)
Model 3‖	-0.03 (-0.06 to -.001)	0 (reference)	-0.02 (-0.04 to 0.00)
Ankle-arm index¶			
Observations	n=138	n=496	n=243
Model 1‡	0.03 (-0.01 to 0.07)	0 (reference)	0.00 (-0.03 to 0.03)
Model 2§	0.06 (0.02 to 0.10)	0 (reference)	-0.02 (0.05 to 0.01)
Model 3‖	0.02 (-0.01 to 0.06)	0 (reference)	0.00 (-0.03 to 0.03)

* Values are mean differences with 95% confidence interval; APOE*2+ denotes APOE2E2 or APOE2E3; APOE*4+ means APOE3E4 or APOE4E4;
† CCA denotes common carotid arteries;
‡ Model 1: adjusted for age and sex;
§ Model 2: adjusted for age, sex and serum apoE level;
‖ Model 3: adjusted for age, sex, serum total cholesterol and HDL cholesterol;
¶ The ankle-arm index is inversely associated with atherosclerosis.[19]

0.84, 90th centile 1.31). APOE*2 carriers had, on average, a higher index than the APOE3E3 group. The association between APOE*2 and the ankle-arm index became stronger and reached statistical significance when adjustments were made for serum apoE level, suggesting an independent effect of the APOE*2 allele. Persons with the APOE4E4 genotype had a slightly lower ankle-arm index than homozygotes for APOE*3 (difference: -0.01; 95% CI -0.10 to 0.08). However, carriers of APOE*4 had overall a similar ankle-arm index as the APOE*3 homozygotes, and a slightly lower ankle-arm index when adjustments were for serum apoE level, but this was not statistically significant. When total and HDL cholesterol were added to the models on CCA intima-media and the ankle-arm index, the associations slightly weakened as compared to the models with adjustments for age and sex only.

Table 5 shows that the prevalence of CCA plaques was not related to serum apoE levels. By contrast, the prevalence of aorta calcifications was found to increase with increasing apoE level, and this was observed both unadjusted (test for trend: p=0.05), and adjusted for the APOE genotypes (test for trend: p=0.005). When we adjusted for serum total and HDL cholesterol, the association between serum apoE and aorta calcifications diminished (test for trend: p=0.50).

The median CCA intima-media wall thickness in the lowest quintile of the serum apoE distribution was 0.75 mm (10th centile 0.60, 90th centile 0.96 mm). As shown in *table 6,* serum apoE level was not associated with the CCA intima-media wall thickness. The median ankle-arm index in the lowest quintile of the serum apoE distribution was 1.15 (10th centile 0.89, 90th centile 1.32 mm). Increasing serum apoE levels were found to be related to decreasing ankle-arm indexes, both unadjusted (test for trend: p=0.03), and adjusted for the APOE genotypes (test for trend: p=0.001). When adjustments were made for total and HDL cholesterol, the association between serum apoE and the ankle-arm index diminished (test for trend: p=0.39).

Discussion

In this population-based study, we found overall no association between the APOE*4 allele and various indicators of atherosclerosis, although the APOE4E4 genotype was weakly associated with atherosclerosis in the abdominal aorta and peripheral arteries. By contrast, we found a consistent trend for an inverse relationship between APOE*2 and various indicators of atherosclerosis, including plaques in the CCAs, aorta calcifications, the CCA intima-media wall thickness and the ankle to arm systolic blood pressure index. Adjusting for serum apoE levels strengthened the association between APOE*2 and aorta calcifications and the ankle-arm index, whereas adjusting for total and HDL cholesterol only slightly changed the estimates. Serum

Table 5 – Odds ratios for common carotid artery plaques and aorta calcifications according to quintiles of serum apoE level*

	<0.53	0.53-0.68	0.68-0.85	0.85-1.09	≥1.09
CCA plaques†					
Events (observations)	n=26 (n=206)	n=28 (n=178)	n=33 (n=175)	n=30 (n=180)	n=26 (n=176)
Model 1‡	1 (reference)	1.4 (0.7 to 2.4)	1.7 (1.0 to 3.0)	1.6 (0.9 to 2.8)	1.4 (0.7 to 2.5)
Model 2§	1 (reference)	1.3 (0.7 to 2.4)	1.7 (1.0 to 3.1)	1.6 (0.8 to 2.9)	1.7 (0.9 to 3.2)
Model 3‖	1 (reference)	1.3 (0.7 to 2.4)	1.7 (0.9 to 3.0)	1.5 (0.8 to 2.8)	1.3 (0.7 to 2.5)
Aorta Calcifications					
Events (observations)	n=104 (n=193)	n=95 (n=163)	n=99 (n=166)	n=88 (n=159)	n=111 (n=165)
Model 1‡	1 (reference)	1.2 (0.8 to 1.8)	1.2 (0.8 to 1.9)	1.1 (0.7 to 1.6)	1.7 (1.1 to 2.7)
Model 2§	1 (reference)	1.2 (0.8 to 1.9)	1.3 (0.8 to 2.0)	1.2 (0.7 to 1.9)	2.4 (1.4 to 4.0)
Model 3‖	1 (reference)	1.1 (0.7 to 1.7)	1.1 (0.7 to 1.7)	0.8 (0.5 to 1.3)	1.4 (0.8 to 2.2)

* Values are odds ratios with 95% confidence interval; serum apoE levels are in μmol/l;

† CCA denotes common carotid arteries;

‡ Model 1: adjusted for age and sex;

§ Model 2: adjusted for age, sex and APOE genotypes;

‖ Model 3: adjusted for age, sex, total and HDL cholesterol.

Table 6 – Differences in common carotid artery intima-media and ankle-arm index according to quintiles of serum apoE level*

	<0.53	0.53-0.68	0.68-0.85	0.85-1.09	≥1.09
CCA intima-media (mm)†					
Observations	n=208	n=181	n=175	n=180	n=177
Model 1‡	0 (reference)	0.02 (-0.01 to 0.04)	0.02 (0.00 to 0.05)	0.01 (-0.02 to 0.04)	0.00 (-0.03 to 0.03)
Model 2§	0 (reference)	0.01 (-0.01 to 0.04)	0.02 (-0.01 to 0.05)	0.01 (-0.02 to 0.04)	0.01 (-0.02 to 0.03)
Model 3‖	0 (reference)	0.01 (-0.01 to 0.03)	0.02 (-0.01 to 0.04)	0.00 (-0.03 to 0.03)	-0.01 (-0.04 to 0.01)
Ankle-arm index¶					
Observations	n=203	n=175	n=173	n=174	n=171
Model 1‡	0 (reference)	-0.01 (-0.05 to 0.03)	0.01 (-0.03 to 0.05)	-0.03 (-0.07 to 0.01)	-0.04 (-0.08 to 0.00)
Model 2§	0 (reference)	-0.01 (-0.05 to 0.03)	0.01 (-0.03 to 0.05)	-0.04 (-0.09 to 0.00)	-0.07 (-0.12 to -0.03)
Model 3‖	0 (reference)	0.00 (-0.04 to 0.04)	0.02 (-0.02 to 0.06)	-0.01 (-0.05 to 0.03)	-0.02 (-0.06 to 0.02)

* Values are differences with 95% confidence interval; serum apoE levels are in μmol/l;
† CCA denotes common carotid arteries;
‡ Model 1: adjusted for age and sex;
§ Model 2: adjusted for age, sex and APOE genotypes;
‖ Model 3: adjusted for age, sex, total and HDL cholesterol;
¶ The ankle-arm index is inversely associated with atherosclerosis.[19]

apoE levels were not related to atherosclerosis in the CCAs, but increasing serum apoE levels were associated with lower ankle-arm indexes and the presence of aorta calcifications. These two associations diminished when adjustments were made for total and HDL cholesterol. APOE*2 carriers had higher levels of serum apoE, but in these persons, apoE was only weakly associated with total cholesterol.

The limitations of the present study should not be ignored. As this is a cross-sectional survey, we can not exclude that selective survival may have occurred. However, when we restricted the study-population to persons in whom mortality may be less important (i.e. non-diabetic persons younger than 65, without CHD or stroke, who have never smoked), similar findings were obtained (data not shown). A follow-up study, in which APOE genotyping is performed on material collected relatively early in life, could characterise participants who died before assessment of atherosclerosis. As far as we know, such a study has not been done.

An advantage of this study is the population-based approach with a high response rate. We estimated the presence of atherosclerosis using established, validated techniques.[18,19,21] However, some measurement error may have occurred. Since all laboratory analyses were performed without knowledge of the other measurements, such misclassification is most likely non-differential and will therefore most likely result in an underestimation of the true effect.

Previous studies on the APOE genotype and atherosclerosis are limited, and results have been inconclusive.[9-13] In an autopsy study in young males who died of external causes, the extent of atherosclerosis was greatest in APOE*4 carriers, and least in carriers of APOE*2.[9] By contrast, a report from the Atherosclerosis Risk In Communities (ARIC) study among subjects free from symptomatic cardiovascular disease showed opposite results; i.e., carriers of APOE*2 were had a borderline statistically significant increased risk of carotid atherosclerosis relative to the APOE3E3 genotype, while no association with APOE*4 was observed.[12] In a study among patients referred for coronary angiography, APOE*4 carriers had an increased thickness of the internal carotid artery intima-media, while APOE*2 carriers had a decreased intima-media wall thickness.[10] The latter findings were confirmed in a recent study among asymptomatic individuals for CHD.[11] By contrast, in non-diabetic Japanese who were asymptomatic for CHD, no association between the APOE polymorphism and carotid or femoral arterial wall thickness was found.[13] This is the first population-based investigation on the APOE genotype and atherosclerosis of the aorta and the arteries of the lower extremities, and the first study on serum apoE levels and non-invasively measured atherosclerosis.

The observation that APOE*2 carriers have in serum higher levels of apoE and HDL cholesterol and lower levels of total cholesterol, and that APOE*4 has opposite effects, is in line with earlier reports.[2-4] Compared to the APOE3E3 genotype,

catabolism of chylomicron and VLDL remnants occurs at a lower rate in APOE*2 carriers, while a similar though opposite mechanism occurs in APOE*4 carriers.[4] Therefore, in APOE*2 carriers, the rate of conversion of VLDL remnants to LDL is lower, resulting in an up-regulation of hepatic LDL receptors.[4] This leads to higher levels of cholesterol-containing remnant particles, and lower levels of cholesterol-rich LDL particles. Since remnant particles are present in normal subjects in small quantities, the net APOE*2 alteration is a decrease in total cholesterol level.[4] As apoE is found primarily in chylomicron and VLDL remnants, a delayed catabolism will result in higher apoE levels. This is in line with our observation that APOE*2 carriers had higher apoE levels and lower levels of total cholesterol than the other APOE groups. Furthermore, in contrast to the other APOE groups, serum apoE levels were in APOE*2 carriers only weakly related to total cholesterol.

In the present study, APOE*4 was overall not associated with the atherosclerosis indicators, although the APOE4E4 genotype was weakly related to atherosclerosis in the abdominal aorta and peripheral arteries. By contrast, APOE*2 was consistently, inversely related to atherosclerosis. Adjustment for serum apoE level strengthened these findings, whereas adjusting for total and HDL cholesterol, or cardiovascular risk factors only slightly changed the inverse association. As the effects of the APOE polymorphism on atherosclerosis seem therefore not to result from its effects on serum apoE or cholesterol levels, the APOE genotype may be involved in atherosclerosis through alternative pathways. As the APOE alleles fully determine the apoE protein isoforms, the APOE*2 effects may result from structural differences in apoE isoforms. Atherosclerosis is associated with increased oxidative stress, as the oxidative modification of LDL seems to be a crucial step in its development.[27] The various apoE isoforms have been found to exert different antioxidant effects.[28] Furthermore, as apoE seems to have several other properties with relevance to vessel wall homeostasis, including the modulation of platelets aggregability and the proliferation and migration of smooth muscle cells and lymphocytes,[5,29] it is possible that these properties may differ across the apoE isoforms.

Although serum apoE levels were found to be related to the ankle-arm index, and aorta calcifications, no association was observed between serum apoE levels and CCA atherosclerosis. This might suggest differences in the aetiology of atherosclerosis across sites. However, it is possible that these differences are due to chance. If serum apoE levels are truly increased in atherosclerosis, this could be cause or consequence. The observation that increases in cholesterol level and oxidised LDL may stimulate apoE secretion by macrophages,[5,30] may suggest that apoE secretion increases after the development of atherosclerosis. However, it should be noted that the large majority of apoE, as detected in serum, is produced by the liver.[29] Any compensatory apoE secretion by macrophages will probably not

result in a substantial difference in serum apoE level. Serum apoE level seems therefore not to increase substantially as a consequence of atherosclerosis. On the other hand, most apoE is a constituent of VLDL and chylomicron remnants, particles with atherogenic properties, while relatively small amounts of apoE are found in a subclass of HDL cholesterol.[4,29] This is in line with our observation that apoE levels were positively related to total cholesterol levels. When we adjusted for total and HDL cholesterol, the association between serum apoE level and atherosclerosis diminished, in agreement with previous observations.[14] These findings support the hypothesis that apoE level is positively related to atherosclerosis, because apoE is a component of atherogenic lipoproteins. This is also consistent with our finding that the inverse relation between APOE*2 and atherosclerosis strengthened when adjustments were made for serum apoE level.

In conclusion, we found that the APOE*4 allele was not strongly related to various indicators of atherosclerosis. By contrast, a consistent trend for an inverse relationship between APOE*2 and atherosclerosis was observed, which was independent of serum apoE, total and HDL cholesterol level, and may therefore result from differences in the apoE isoforms.

References

1. Pooling Project Research Group. Relationship of blood pressure, serum cholesterol, smoking habit, relative weight and ECG abnormalities to incidence of major coronary events: final report of the pooling project. J Chron Dis 1978;31:201-306.
2. Smit M, De Knijff P, Rosseneu M, et al. Apolipoprotein E polymorphism in the Netherlands and its effect on plasma lipid and apolipoprotein levels. Hum Genetics 1988;80:287-292.
3. Braeckman L, De Bacquer D, Rosseneu M, et al. Apolipoprotein E polymorphism in middle-aged Belgian men: phenotype distribution and relation to serum lipids and lipoproteins. Atherosclerosis 1996;120:67-73.
4. Davignon J, Gregg RE, Sing F. Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis 1988;8:1-21.
5. Mazzone T. Apolipoprotein E secretion by macrophages: its potential physiological functions. Curr Opinion Lipidol 1996;7:303-307.
6. Wilson PWF, Schaefer EJ, Larson MG, et al. Apolipoprotein E alleles and risk of coronary disease. A meta-analysis. Arterioscler Thromb Vasc Biol 1996;16:1250-1255.
7. Stengård JH, Zerba KE, Pekkanen J, et al. Apolipoprotein E polymorphism predicts death from coronary heart disease in a longitudinal study of elderly Finnish men. Circulation 1995;91:265-269.
8. Kuusisto J, Mykkänen L, Kervinen K, et al. Apolipoprotein E4 phenotype is not an important risk factor for coronary heart disease or stroke in elderly subjects. Arterioscler Thromb Vasc Biol 1995;15:1280-1286.

9. Hixson JE and the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. Apolipoprotein E polymorphism affect atherosclerosis in young males. Arterioscler Thromb 1991;11:1237-1244.
10. Terry JG, Howard G, Mercuri M, et al. Apolipoprotein E polymorphism is associated with segment-specific extracranial carotid artery intima-media thickening. Stroke 1996;27:1755-1759.
11. Cattin L, Fisicaro M, Tonizzo M, et al. Polymorphism of the apolipoprotein E gene and early carotid artery atherosclerosis defined by ultrasonography in asymptomatic adults. Arterioscler Thromb Vasc Biol 1997;17:91-94.
12. De Andrade M, Thandi I, Brown S, et al. Relationship of the apolipoprotein E polymorphism with carotid artery atherosclerosis. Am J Hum Genet 1995;56:1379-1390.
13. Kogawa K, Nishizawa Y, Hosoi M, et al. Effect of polymorphism of apolipoprotein E and angiotensin-converting enzyme genes on arterial wall thickness. Diabetes 1997;46:682-687.
14. Buring JE, O'Connor GT, Goldhaber SZ, et al. Decreased HDL_2 and HDL_3 cholesterol, apoA-I and apoA-II, and increased risk of myocardial infarction. Circulation 1992;85:22-29.
15. Couderc R, Mahieux F, Bailleul S, et al. Prevalence of apolipoprotein E phenotypes in ischemic cerebrovascular disease. A case-control study. Stroke 1993;24:661-664.
16. Hofman A, Grobbee DE, De Jong PTVM, et al. Determinants of disease and disability in the elderly: the Rotterdam elderly study. Eur J Epidemiol 1991;7:403-422.
17. Bots ML, Hofman A, De Jong PTVM, et al. Common carotid artery intima-media thickness as an indicator of atherosclerosis at other sides of the carotid artery. The Rotterdam Study. Ann Epidemiol 1996;6:147-153.
18. Grobbee DE, Bots ML. Carotid artery intima-media thickness as an indicator of generalized atherosclerosis. J Int Med 1994;236:567-73.
19. Vogt MT, Wolfson SK, Kuller LH. Lower extremity arterial disease and the aging process: a review. J Clin Epidemiol 1992;45:529-542.
20. Witteman JCM, Grobbee DE, Valkenburg HA, et al. Cigarette smoking and the development and progression of aortic atherosclerosis: a 9-year population-based follow-up study in women. Circulation 1993;88:2156-2162.
21. Hyman JB, Epstein FH. A study of the correlation between roentgenographic and postmortem calcification of the aorta. Am Heart J 1954;47:540-543.
22. Slooter AJC, Cruts M, Kalmijn S, et al. Risk estimates of dementia by apolipoprotein E genotypes from a population-based incidence study: the Rotterdam Study. Arch Neurol 1998;55:964-8.
23. Van Vlijmen BJM, Van 't Hof HB, Mol MJTM, et al. Modulation of very low density lipoprotein production and clearance contributes to age- and gender dependent hyperlipoproteinemia in apolipoprotein E3-Leiden transgenic mice. J Clin Invest 1996;97:1184-1192.
24. Van Gent CM, Vandervoort HA, De Bruyn AM, et al. Cholesterol determinations. A comparative study of methods with special reference to enzymatic procedures. Clin Chem Acta 1977;75:243-251.
25. Altman DG. Multiple regression. Practical statistics for medical research. London, Chapman & Hall, 1991;345-346.
26. Miettinen OS. Theoretical epidemiology: principles of occurrence research in medicine. New York: John Wiley & Sons, 1985;231-3.

27. Witztum JL. The oxidation hypothesis of atherosclerosis. Lancet 1994;344:793-795.
28. Miyata M, Smith JD. Apolipoprotein E allele-specific antioxidant activity and effects on cytotoxicity by oxidative insults and ß-amyloid peptides. Nature Genet 1996;14:55-61.
29. Mahley RW. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science 1988;240:622-630.
30. Cader AA, Steinberg FM, Mazzone T, et al. Mechanisms of enhanced macrophage apoE secretion by oxidized LDL. J Lipid Res 1997;38:981-991.

6

APOLIPOPROTEIN E, ATHEROSCLEROSIS AND DEMENTIA

6.1

Apolipoprotein E, atherosclerosis and cognitive decline

Abstract

*The APOE*4 allele of the apolipoprotein E gene (APOE) and atherosclerosis are risk factors for cognitive decline. We investigated whether the effects of the APOE genotype and atherosclerosis on cognitive decline are independent. A population-based follow-up study was performed on 838 subjects who were non-demented at baseline. The Mini Mental State Examination (MMSE) score at follow-up was studied as a function of APOE and atherosclerosis, assessed by five indicators based on non-invasive measurements and clinical endpoints. Mild, non-significant effects on the MMSE score were found for atherosclerosis in the absence of APOE*4 and for APOE*4 in the absence of atherosclerosis. APOE*4 carriers with two or more indicators of atherosclerosis positive, had a significantly lower MMSE score at follow-up (mean difference -0.7 points; 95 percent confidence interval -1.1 to -0.2) relative to non-APOE*4 carriers with no evidence of atherosclerosis. Our findings suggest that the consequences of APOE and atherosclerosis are not independent, and that particularly APOE*4 carriers with atherosclerosis are at increased risk of cognitive decline.*

Introduction

Cognitive decline in elderly persons is an important predictor of dementia and, from a public health point of view, of growing significance in our aging society. Several genetic and environmental factors may lead to age-related cognitive deterioration and eventually dementia.[1] An important genetic risk factor for cognitive decline and dementia is the APOE*4 allele of the apolipoprotein E gene (APOE).[2-6] It is controversial whether APOE*2, another APOE allele, decreases the risk of dementia and

cognitive decline.[5-9] In Caucasians, APOE*4 has a frequency of approximately 15 percent, APOE*2 a frequency of 8 percent, and the most common APOE allele, APOE*3 has a frequency of 77 percent.[10]

Apart from its role in cognitive function, apolipoprotein E is known to play a central role in lipid metabolism.[10] APOE*4 carriers have higher total and LDL cholesterol levels, and have an increased risk of atherosclerosis, compared to persons without this allele,[10-12] although not in all studies.[13]

Atherosclerosis is also a risk factor for dementia[14] and cognitive decline.[15] Therefore, one may hypothesize that atherosclerosis is an intermediate factor in the association between APOE*4 and cognitive decline. Up to date, few studies investigated the relation between APOE, atherosclerosis and cognitive function. In a case-control study of prevalent patients with dementia, we found evidence that APOE*4 potentiates the effects of atherosclerosis.[14] However, this study may have been subject to bias, since differences in survival have been observed for demented patients related to the APOE genotype.[9,16]

Here we present a follow-up study on cognitive decline in 838 subjects who were non-demented at baseline. The aim of this population-based investigation was to examine whether the effects of the APOE genotype and atherosclerosis on cognitive decline are independent.

Methods

Study population

This study is part of the Rotterdam Study, a population-based follow-up study of determinants of chronic diseases in the elderly. All inhabitants of a suburb of Rotterdam, aged 55 years or over, including those living in homes for the elderly, were invited to participate. The design of the study has been described previously.[17] From all subjects informed consent was obtained and the study was approved by the medical ethics committee of the Erasmus University Medical School. In total 7983 participants (response rate 78 percent) were examined from 1990 to 1993 (baseline). At follow-up in 1993 and 1994, 6315 persons (79.1 percent) were re-examined; 768 subjects (9.6 percent) had died, 106 (1.3 percent) were inaccessible and 794 (9.9 percent) refused to participate. The mean period of follow-up was 2.9 years (SD 0.4).

At the time of the present analyses, a random sample of 1585 subjects was invited to participate in an ongoing study on atherosclerosis of the carotid arteries. The analyses presented here are restricted to persons for whom data on cognitive function were available both at baseline and at follow-up (n=1197). In individuals of whom blood was available, APOE typing was performed (n=899). Subjects who

were demented at baseline (n=10), and those who were suspected of dementia at baseline (i.e. Mini Mental State Examination (MMSE) score of 25 points or less, n=51) were excluded. This resulted in a study population of 838 subjects.

Measurements

Cognitive function The Dutch version of the MMSE[18] was used to assess global cognitive performance. The MMSE is a test that covers several cognitive functions, and yields a maximum best score of 30 points. The MMSE was administered by specially trained research assistants. Dementia at baseline was diagnosed by a three phase approach, as described in detail elsewhere.[14] A panel of physicians and neuropsychologists assessed whether a dementia syndrome was present according to criteria of the American Psychiatric Association (DSM-III-R).[19]

Apolipoprotein E We used coded blood samples for APOE genotyping. This was performed without knowledge of the other measurements, using the primers and amplification conditions as described by Wenham and colleagues.[20] The polymerase chain reaction product was digested with the restriction enzyme *Hha*I and fragments were separated by electrophoresis on a 5% agarose gel. APOE alleles were visualized by ethidium bromide staining.[9]

Atherosclerosis At baseline, we examined the presence of atherosclerosis by clinical vascular disease (history of stroke or previous myocardial infarction) and non-invasive measurements (atherosclerosis of the arteries of the lower extremities and of the carotid arteries). A history of stroke and a history of myocardial infarction were assessed by direct questioning and considered positive when the diagnosis had been made by a treating physician. To estimate the presence of atherosclerosis in the carotid arteries, ultrasonography of both carotid arteries was performed with a 7.5 MHz linear-array transducer and a duplex scanner (ATL UltraMark IV, Advanced Technology Laboratories, Bethel, WA). Both carotid bifurcations were evaluated for the presence of atherosclerotic plaques, defined as a focal widening relative to adjacent segments with protrusion into the lumen, as described previously.[21] In addition, the intima-media thickness of the distal part of both common carotid arteries (CCA) were measured and averaged, as described before.[22] The presence of plaques as well as an increased CCA intima-media thickness are reliable indicators of generalized atherosclerosis.[23,24] Atherosclerosis of the arteries of the lower extremities was assessed by the ratio of the ankle to brachial systolic blood pressure (ankle-arm index). A decreased ankle-arm index has been shown to be a reliable indicator of generalized atherosclerosis.[25-27] To calculate the ankle-arm index, blood pressure was measured in the sitting position at the right upper arm with a random-zero sphygmomanometer, and the average of two measurements obtained at one occasion was used. Ankle

systolic blood pressure was determined with the subject in supine position at both the right and left posterior tibial arteries using also a random-zero sphygmomanometer and a Doppler ultrasound transducer. The analyses were based on the average of the ankle-arm index on the left and the right side.

Possible confounders Information on a family history of dementia, and lifetime formal schooling was obtained using a computerized questionnaire. The family history of dementia was considered positive if one or more first degree relatives suffered from dementia. Attained level of education was assessed according to the international standard classification of education (Unesco, Paris, 1976).[28] For the present analyses participants were grouped into five categories.

Classification

Firstly, subjects were grouped according to the six possible APOE genotypes (APOE2E2, APOE2E3, APOE2E4, APOE3E3, APOE3E4 and APOE4E4). Participants were also classified into APOE*2 carriers (subjects with APOE2E2 or APOE2E3), homozygotes for APOE*3, and APOE*4 carriers (individuals with the APOE3E4 or the APOE4E4 genotype), whereby persons with the APOE2E4 genotype were excluded. Persons with the most common genotype (APOE3E3) were the reference group.

Secondly, subjects were grouped according to the presence or absence of the above five indicators of atherosclerosis: a history of stroke, a previous myocardial infarction, the ankle-arm index, the CCA intima-media thickness and the presence of one or more plaques in both carotid bifurcations. Continuous variables were split at the median. Specifically, the indicator ankle-arm index was positive for those with an index beneath the median, while the indicator CCA intima-media thickness was positive in those with a wall thickness above the median.

A problem in the analysis on the separate indicators is that these are not always positive simultaneously. For instance, persons without a history of stroke may have a history of myocardial infarction. To optimize the contrast, we constructed a more rigorous atherosclerosis score to identify those with evidence of atherosclerosis at multiple sites (i.e. two or more indicators, mentioned above, positive). These persons were assigned an atherosclerosis score of two. The atherosclerosis score was one in those with only one indicator positive. Persons with no indicators positive had an atherosclerosis score of zero, and were used as the reference group.

Thirdly, the combined effects of the APOE genotype and atherosclerosis were studied according to a strategy described by Ottman.[29] We classified subjects into 4 groups: (1) non-APOE*4 carriers with no evidence of atherosclerosis (reference group), (2) APOE*4 carriers with no evidence of atherosclerosis, (3) non-APOE*4 carriers with evidence of atherosclerosis, and (4) APOE*4 carriers with evidence of

atherosclerosis. These analyses were performed for each of the five indicators of atherosclerosis separately, as well as for the atherosclerosis score. Since the atherosclerosis score had three values, this resulted in six subgroups. The effects of both APOE*2 and atherosclerosis were studied similarly.

Statistical analysis

The chi-square statistic was used to compare categorical data, and the two sample t test to study normally distributed, continuous variables. MMSE scores were studied in a multiple linear regression model, with the study groups entered as dummy variables, using the above classification. Differences in MMSE scores are presented with a 95 percent confidence interval (CI). All analyses on the MMSE score were adjusted for age, gender and level of education. All reported p values are based on two sided tests using a significance level of 5%.

Table 1 – Baseline characteristics of the study population*

Characteristic	APOE*2 + n=136	APOE3E3 n=459	APOE*4 + n=224
Age at baseline (years)	67.8 (6.8)	68.1 (7.4)	67.9 (7.3)
Men	30.9% (42)	41.2% (189)	45.5% (102)
Family history of dementia	21.5% (29)	21.5% (98)	32.3% (72)
Educational level: primary education only	13.3% (18)	21.3% (97)	19.2% (43)
History of stroke	1.5% (2)	4.2% (19)	3.6% (8)
History of myocardial infarction	3.0% (4)	8.3% (37)	7.3% (16)
CCA intima-media (mm)†	0.75 (0.12)	0.78 (0.15)	0.78 (0.15)
Plaque in carotid bifurcations	43.2% (51)	54.7% (208)	49.7% (89)
Ankle-arm index	1.12 (0.19)	1.10 (0.21)	1.10 (0.19)
Atherosclerosis score zero‡	27.3% (36)	20.7% (93)	22.0% (48)
Atherosclerosis score one‡	34.1% (45)	28.7% (129)	29.8% (65)
Atherosclerosis score two‡	38.6% (51)	50.7% (228)	48.2% (105)

* Values are unadjusted means (SD) or percentages (numbers), based on all available information; APOE*2+ denotes the APOE2E2 or the APOE2E3 genotype; APOE*4+ denotes APOE3E4 or APOE4E4.

† CCA indicates common carotid arteries.

‡ Atherosclerosis score is zero when none of the indicators of atherosclerosis are positive, one when only one indicator is positive, and two when two or more indicators are positive.

Results

Descriptive statistics

The distribution of the APOE genotype in our study population was: APOE2E2: 1.3 percent (n=11), APOE2E3: 14.9 percent (n=125), APOE2E4: 2.3 percent (n=19), APOE3E3: 54.8 percent (n=459), APOE3E4: 24.8 percent (n=208) and APOE4E4: 1.9 percent (n=16). The APOE genotype distribution was in Hardy-Weinberg equilibrium (X^2=5.1; degrees of freedom=3: P>0.2). Descriptive statistics of the study population are presented in *table 1*. Significantly more persons with APOE*4 had a positive family history of dementia in first degree relatives, compared to subjects without this allele. There were significantly more men among APOE*4 carriers than among APOE*2 carriers. APOE*2 carriers had less often primary education only, as compared to the APOE3E3 group. In persons with APOE*2, we found a significantly thinner CCA intima-media wall thickness, less often plaques in the carotid bifurcations and a larger proportion of subjects with an atherosclerosis score of zero, as compared to the APOE3E3 group. The inverse association between APOE*2 and indicators of atherosclerosis could also be observed in a multivariate model with adjustments for age and gender (data not shown) .

Apolipoprotein E and MMSE

The median MMSE score at follow-up in the APOE3E3 group was 28 (10th centile 25, 90th centile 29). As shown in *table 2*, APOE*4 carriers performed significantly worse compared to this reference group. APOE*2 carriers had a similar MMSE score as the APOE3E3 group. The MMSE score at follow-up was comparable for subjects with APOE3E4 relative to those with APOE4E4, and also for persons with APOE2E2 as compared to those with APOE2E3. However, the statistical power for these comparisons was limited, since there were only 16 homozygotes for APOE*4 and 11 for APOE*2.

Table 2 – Difference in MMSE score at follow-up according to the APOE genotype*

APOE*2+	APOE3E3	APOE*4+
-0.1 (-0.5 to 0.2)	reference	-0.4 (-0.7 to -0.1)
n=136	n=459	n=224

* Values are means (95% confidence interval) adjusted for age, gender and education level; APOE*2+ denotes the APOE2E2 or the APOE2E3 genotype; APOE*4+ denotes APOE3E4 or APOE4E4.

Atherosclerosis and MMSE

As shown in *table 3*, subjects with evidence of atherosclerosis as estimated by a history of stroke, the CCA intima-media thickness, the presence of plaques in the carotid bifurcations and the ankle-arm index, performed worse on the MMSE at follow-up, compared to those for whom that indicator was negative, although this did only reach statistical significance in analyses on the CCA intima-media thickness.

Table 4 shows that the MMSE score at follow-up is lowest in those with two or more indicators positive, intermediate in persons with one indicator positive, and highest in those with no indicator positive. The median MMSE score at follow-up in persons with no indicator positive was 28 (10th centile 26, 90th centile 29).

Table 3 – Difference in MMSE score at follow-up according to the presence or absence of various indicators of atherosclerosis at baseline*

indicator	indicator –	indicator +
History of stroke	reference n=804	-0.2 (-0.8 to 0.4) n=29
History of myocardial infarction	reference n=758	0.1 (-0.4 to 0.5) n=59
CCA intima-media† (Median: 0.75 mm)	reference n=412	-0.3 (-0.6 to 0.0) n=414
Plaque in carotid bifurcations	reference n=339	0.0 (-0.3 to 0.3) n=355
Ankle-arm index (Median: 1.13)	reference n=403	-0.2 (-0.4 to 0.1) n=402

* Values are means (95% confidence interval), adjusted for age, gender and education level.

† CCA indicates common carotid arteries.

Table 4 – Difference in MMSE score at follow-up according to the atherosclerosis score*

Atherosclerosis score zero	Atherosclerosis score one	Atherosclerosis score two
reference	-0.1 (-0.4 to 0.3)	-0.3 (-0.6 to 0.1)
n=184	n=242	n=393

* Atherosclerosis score is zero in those with no indicator of atherosclerosis positive, one in persons with one indicator positive, and two in those with two or more indicators positive; values are means (95% confidence interval) adjusted for age, gender and education level.

Apolipoprotein E, atherosclerosis and MMSE

The combined effects of APOE*4 and atherosclerosis on the MMSE score at follow-up, are shown in *table 5*. Among APOE*4 carriers, those with evidence of atherosclerosis (column 5) performed worse compared to those without (column 3). In the absence of APOE*4, none of the indicators of atherosclerosis were associated with a statistically significantly lower MMSE score at follow-up (compare column 4 versus column 2).

The various indicators were studied simultaneously using the atherosclerosis score. In this analysis, non-APOE*4 carriers with an atherosclerosis score of zero were the reference group (median MMSE at follow-up 28, 10th centile 26, 90th centile 29). As shown in *table 6*, APOE*4 carriers performed worse compared to persons without this allele, in all strata of the atherosclerosis score. The performance on the MMSE at follow-up decreased with increasing atherosclerosis scores, for those with and without APOE*4. With this classification we could study the effects of APOE*4 and atherosclerosis separately. In the absence of evidence of atherosclerosis (score

Table 5 – Difference in MMSE score at follow-up according to the presence or absence of APOE*4 and various indicators of atherosclerosis at baseline*

atherosclerosis indicator	APOE*4 – atherosclerosis indicator –	APOE*4 + atherosclerosis indicator –	APOE*4 – atherosclerosis indicator +	APOE*4 + atherosclerosis indicator +
History of stroke	reference	-0.4 (-0.6 to -0.1)	-0.3 (-1.1 to 0.5)	-0.6 (-1.8 to 0.6)
	n=571	n=233	n=21	n=8
History of myocardial infarction	reference	-0.3 (-0.6 to 0.0)	0.2 (-0.3 to 0.8)	-0.7 (-1.6 to -0.1)
	n=538	n=220	n=42	n=17
CCA intima-media† (Median: 0.75 mm)	reference	-0.1 (-0.5 to 0.3)	0.0 (-0.3 to 0.3)	-0.6 (-1.0 to -0.3)
	n=298	n=114	n=292	n=122
Plaque in carotid bifurcations	reference	-0.2 (-0.6 to 0.2)	-0.1 (-0.4 to 0.2)	-0.6 (-1.0 to -0.2)
	n=240	n=99	n=259	n=96
Ankle-arm index (Median: 1.13)	reference	-0.3 (-0.7 to 0.1)	-0.1 (-0.3 to 0.2)	-0.4 (-0.8 to -0.1)
	n=282	n=121	n=286	n=116

* Values are mean differences (95% confidence interval), adjusted for age, gender and education level.

† CCA indicates common carotid arteries.

zero), the mean difference in MMSE score between persons with and without APOE*4 was -0.2 (95 percent CI -0.7 to 0.4). In the absence of APOE*4, those with an atherosclerosis score of two differed on average -0.2 MMSE points (95 percent CI -0.5 to 0.2) from those with a score of zero. However, the MMSE score for APOE*4 carriers with an atherosclerosis score of two was on average -0.7 MMSE points (95 percent CI -1.1 to -0.2) lower compared to non-APOE*4 carriers with a score of zero. This difference was larger than the sum of the separate effects (-0.2 and -0.2), and could not be explained by an asymmetric distribution of homozygotes for APOE*4. Among APOE*4 carriers with an atherosclerosis score of zero, 8 percent were homozygote. In APOE*4 carriers with a score of one, 5 percent were homozygote, and among APOE*4 carriers with an atherosclerosis score of two, 8 percent were homozygote (X^2=0.7; degrees of freedom=2: P=0.7).

There was no evidence that the APOE*2 allele modified the effects of atherosclerosis with regard to the MMSE score at follow-up (data not shown).

Possible confounders and modifiers

When, in addition to age and gender, adjustments were made for a positive family history of dementia, the findings were very similar for all analyses described above. Finally, in all analyses effect modification by gender was studied. No major differences were observed between men and women.

Discussion

We investigated the association of APOE, atherosclerosis and cognitive decline in a population-based follow-up study of 838 subjects. Both APOE*4 carriers and per-

Table 6 – Difference in MMSE score at follow-up according to the presence or absence of APOE*4 and the atherosclerosis score*

	Atherosclerosis score zero	Atherosclerosis score one	Atherosclerosis score two
APOE*4-	reference	0 (-0.4 to 0.4)	-0.2 (-0.5 to 0.2)
	n=129	n=173	n=280
APOE*4+	-0.2 (-0.7 to 0.4)	-0.3 (-0.9 to 0.2)	-0.7 (-1.1 to -0.2)
	n=55	n=69	n=113

* Atherosclerosis score is zero in those with no indicator of atherosclerosis positive, one in persons with one indicator positive, and two in those with two or more indicators positive; values are means (95% confidence interval) adjusted for age, gender and education level.

sons with evidence of atherosclerosis had a lower MMSE score at follow-up. When studied simultaneously, we found mild, non-significant effects for atherosclerosis in the absence of APOE*4 and for APOE*4 in the absence of atherosclerosis. Persons with both APOE*4 and evidence of atherosclerosis at several sites performed significantly worse on the MMSE as compared to those with neither risk factors. Furthermore, the effects of both atherosclerosis and APOE*4 were found to be stronger than expected from the sum of the separate effects.

In this study, cognitive function was assessed by the MMSE. Although originally developed as a screening tool for dementia, this test has been shown to be suitable also for studies on cognition in non-demented community residents.[30] The differences in MMSE score that we observed were relatively small and may not be relevant for an individual, but can be important on a population level.[31] To estimate the presence of atherosclerosis, we used indicators which have been well validated.[23-27] Nevertheless, some misclassification may have occurred. Since all examinations were performed without knowledge of the other measurements, any misclassification is most likely nondifferential and will therefore result in an underestimation of the true effect. An advantage of our study is the prospective design. Since APOE may be related to the survival of patients with dementia,[9,16] we included only subjects who were not demented at baseline. In this study the follow-up period was relatively short, on average 2.9 years. Hence, loss to follow-up due to mortality, which may be related to the APOE genotype,[10-12] was limited.

Similar to our findings, others found that APOE*4 may be a risk factor for cognitive decline.[5,6] Diverse findings have emerged on the effects of APOE*2.[5,6] We found no difference between APOE*2 and APOE3E3 with regard to cognitive deterioration. Furthermore, our findings suggest that atherosclerosis is not more prevalent in carriers of the APOE*4 allele, relative to persons with other APOE genotypes, as has been suggested before.[10-12] In addition, we found that atherosclerosis may be less common in carriers of the APOE*2 allele. However, analyses on the association between the APOE genotype and atherosclerosis were based on cross-sectional data, and should be confirmed in follow-up studies.

One may hypothesize that atherosclerosis is an intermediate factor in the association between APOE and cognitive decline. That is, APOE may be a determinant of atherosclerosis,[10-12] and atherosclerosis on its turn may be a determinant of impaired cognitive function.[15] We found no evidence for an increased prevalence of atherosclerosis in APOE*4 carriers compared to persons with other APOE genotypes. Besides, in the absence of APOE*4, cognitive performance was only mildly decreased in those with atherosclerosis compared to those without. Furthermore, we found an inverse association between APOE*2 and atherosclerosis, but not a better cognitive performance of APOE*2 carriers. These findings make it unlikely that

atherosclerosis is an intermediate factor in the association between APOE and cognitive decline.

Earlier, we reported that APOE*4 potentiates the relation between atherosclerosis in a series of prevalent patients with dementia.[14] In this prospective study we addressed the question whether the effects of APOE*4 and atherosclerosis on cognitive decline are independent. We found small, non-significant effects on the MMSE score for atherosclerosis in the absence of APOE*4 and for APOE*4 in the absence of atherosclerosis. The observation that the consequences of both APOE*4 and atherosclerosis were worse than the sum of the effects of these two factors separately, suggests a synergistic effect of the APOE*4 allele and atherosclerosis.

The presence of atherosclerosis in the brain itself is difficult to investigate during life. However, the indicators we used are strongly associated with cerebral white matter lesions, as has been shown in a study using Magnetic Resonance Imaging of the brain.[22] These white matter lesions are associated with cerebral arteriosclerosis, hypoperfusion, and ischaemia.[32,33] Our study may indicate that the cognitive consequences of these cerebral lesions may be more pronounced in carriers of the APOE*4 allele, compared to persons without APOE*4. In line with our findings, there is evidence from studies in rats that apolipoprotein E plays a role in the response to cerebral ischaemia.[34] Furthermore, APOE*4 carriers may have a less favorable outcome after intracerebral haemorrhage, as compared to subjects without APOE*4.[35] Moreover, APOE may play a role in reinnervation after neuronal loss.[36,37]

In conclusion, this follow-up study suggests that in particular APOE*4 carriers with atherosclerosis are at increased risk of cognitive decline. We found mild, non-significant effects on cognitive performance for atherosclerosis in the absence of APOE*4 and for APOE*4 in the absence of atherosclerosis. Our findings are not compatible with the view that atherosclerosis is an intermediate factor in the association between APOE*4 and cognitive decline. An important clinical and public health issue will be whether the development of dementia of APOE*4 carriers may be prevented by intervention on vascular risk factors.

References

1. Van Duijn CM. Epidemiology of the dementias: recent developments and new approaches. J Neurol Neurosurg Psychiatry 1996;60:478-88.
2. Strittmatter WJ, Saunders AM, Schmechel D, et al. Apolipoprotein E: high-avidity binding to ß-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci USA 1993;90:1977-81.
3. Kuusisto J, Koivisto K, Kervinen K, et al. Association of apolipoprotein E phenotypes with late onset Alzheimer's disease: population based study. BMJ 1994;309:636-8.

4. Slooter AJC, Tang M-X, Van Duijn CM, et al. Apolipoprotein E4 increases the risk of dementia with stroke. A population-based investigation. JAMA 1997;277:818-21.
5. Henderson AS, Easteal S, Jorm AF, et al. Apolipoprotein E allele ε4, dementia, and cognitive decline in a population sample. Lancet 1995;346:1387-90.
6. Hyman BT, Gomez-Isla T, Briggs M, et al. Apolipoprotein E and cognitive change in an elderly population. Ann Neurol 1996;40:55-60.
7. Corder EH, Saunders AM, Risch NJ, et al. Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. Nature Genet 1994;7:180-4.
8. Talbot C, Lendon C, Craddock N, et al. Protection against Alzheimer's disease with apoE ε2. Lancet 1994;343:1432-3.
9. Van Duijn CM, De Knijff P, Wehnert A, et al. The apolipoprotein E ε2 allele is associated with an increased risk of early-onset Alzheimer's disease and a reduced survival. Ann Neurol 1995;37:605-10.
10. Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis 1988;8:1-21.
11. Pedro-Botet J, Sentí M, Nogués X, et al. Lipoprotein and apolipoprotein profile in men with ischemic stroke. Role of lipoprotein(a), triglyceride-rich lipoproteins, and apolipoprotein E polymorphism. Stroke 1992;23:1556-62.
12. Van Bockxmeer FM, Mamotte CDS. Apolipoprotein ε4 homozygosity in young men with coronary heart disease. Lancet 1992;340:879-80.
13. Kuusisto J, Mykkänen L, Kervinen K, et al. Apolipoprotein E4 phenotype is not an important risk factor for coronary heart disease or stroke in elderly patients. Arterioscler Tromb Vasc Biol 1995;15:1280-6
14. Hofman A, Ott A, Breteler MMB, et al. Atherosclerosis, apolipoprotein E, and the prevalence of dementia and Alzheimer's disease in the Rotterdam study. Lancet 1997;349:151-4.
15. Breteler MMB, Claus JJ, Grobbee DE et al. Cardiovascular disease and distribution of cognitive function in elderly people: the Rotterdam study. BMJ 1994;308:1604-8.
16. Stern Y, Brandt J, Albert M, et al. The absence of an apolipoprotein ε4 allele is associated with a more aggressive form of Alzheimer's disease. Ann Neurol 1997 (in press).
17. Hofman A, Grobbee DE, De Jong PTVM, et al. Determinants of disease and disability in the elderly: the Rotterdam elderly study. Eur J Epidemiol 1991;7:403-22.
18. Folstein MS, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189-98.
19. American Psychiatric Association. Diagnostic and statistical manual of mental disorders, third edition, revised. DSM-III-R. Washington DC: American Psychiatric Association, 1987.
20. Wenham PR, Price WH, Blundell G. Apolipoprotein E genotyping by one-stage PCR. Lancet 1991;337:1158-9.
21. Bots ML, Hofman A, De Jong PTVM, et al. Common carotid artery intima-media thickness as an indicator of atherosclerosis at other sides of the carotid artery. The Rotterdam Study. Ann Epidemiol 1996;6:147-53.
22. Bots ML, Van Swieten JC, Breteler MMB, et al. Cerebral white matter lesions and atherosclerosis in the Rotterdam study. Lancet 1993;341:1232-7.
23. Grobbee DE, Bots ML. Carotid artery intima-media thickness as an indicator of generalized atherosclerosis. J Int Med 1994;236:567-73.

24. Wong M, Edelstein J, Wollman J, et al. Ultrasonic-pathological comparison of the human arterial wall. Verification of intima-media thickness. Arterioscler Tromb 1993;13:482-6.
25. Vogt MT, Wolfson SK, Kuller LH. Lower extremity arterial disease and the aging process: a review. J Clin Epidemiol 1992;45:529-42.
26. Ouriel K, McDonnell AE, Metz CE, et al. A critical evaluation of stress testing in the diagnosis of peripheral vascular disease. Surgery 1982;91:686-93.
27. Fowkes FGR, Housley E, Cawood EHH, et al. Edinburgh artery study: prevalence of asymptomatic and symptomatic peripheral arterial disease in the general population. Int J Epidemiol 1991;20:384-92.
28. Netherlands Central Bureau of Statistics. Standard classification of education SOI-1978. Voorburg: Netherlands Central Bureau of Statistics, 1987.
29. Ottman R. An epidemiologic approach to gene-environment interaction. Genetic Epidemiology 1990;7:177-85.
30. Tombaugh TN, McIntyre NJ. The mini-mental state examination: a comprehensive review. J Am Geriatr Soc 1992;40:922-35.
31. Rose G. Sick individuals and sick populations. Int J Epidemiol 1985;14:32-8.
32. Meyer JS, Kawamura J, Terayama Y. White matter lesions in the elderly. J Neurol Sci 1992;110:1-7.
33. Van Swieten JC, Van den Hout JH, Van Ketel BA, et al. Periventricular lesions in the white matter on magnetic resonance imaging in the elderly: a morphometric correlation with arteriolosclerosis and dilated perivascular spaces. Brain 1991;114:761-74.
34. Kida E, Pluta R, Lossinsky AS, et al. Complete cerebral ischemia with short-term survival in rat induced by cardiac arrest. II. Extracellular and intracellular accumulation of apolipoproteins E and J in the brain. Brain Res 1995;674:341-6.
35. Alberts MJ, Graffagnino C, McClenny C, et al. ApoE genotype and survival from intracerebral haemorrhage. Lancet 1995;346:575.
36. Poirier J. Apolipoprotein E in animal models of CNS injury and in Alzheimer's disease. Trends Neurosci 1994;17:525-30.
37. Mahley RW, Nathan BP, Bellosta S, et al. Apolipoprotein E: impact of cytoskeletal stability in neurons and the relationship to Alzheimer's disease. Curr Opin Lipidol 1995;6:86-91.

6.2

The role of apolipoprotein E and atherosclerosis in dementia

Abstract

*The APOE*4 allele of the apolipoprotein E gene (APOE) may increase the risk of both dementia and atherosclerosis, whereas the APOE*2 allele seems to have opposite effects. Atherosclerosis was suggested to be a risk factor for dementia, particularly in APOE*4 carriers. We investigated the combined effects of the APOE genotype and atherosclerosis in dementia with recent onset. In a population-based setting, 244 dementia cases with recent onset were compared to 1002 non-demented controls; atherosclerosis was assessed using non-invasive measurements. We found that the association between the APOE genotypes and dementia, did not essentially alter when adjustments were made for atherosclerosis or other vascular factors. Atherosclerosis seems therefore not to be an intermediate factor in the association of APOE with dementia. In addition, we observed a synergistic effect of APOE*4 and atherosclerosis on the risk of dementia. Our findings contribute to the growing evidence that APOE is involved in the response to cerebral damage.*

Introduction

It is now well known that the apolipoprotein E gene (APOE) plays a role in the development of dementia. While the APOE*4 allele increases the risk, the APOE*2 allele may exert a protective effect.[1,2] Atherosclerosis is another common risk factor for dementia,[3] in which the APOE genotype seems to be involved as well.[4,5] The APOE*4 allele was found to increase the risk of atherosclerosis, although not in all studies.[4,5] APOE*2 on the other hand appeared to be inversely associated with athe-

rosclerosis.[4,5] Therefore, it can be hypothesised that the effects of the APOE polymorphism on dementia (partly) result from its effects on atherosclerosis. A synergistic interaction has been observed for APOE*4 and atherosclerosis on the prevalence of dementia.[3] However, this cross-sectional study may have been subject to selective mortality, as the APOE genotype was observed to be related to survival in AD.[6]

The aim of this study is to investigate whether APOE is related to dementia, independent of its association with atherosclerosis. In addition, we wanted to further investigate the combined effects of atherosclerosis and APOE*4 in dementia. These issues were explored in a population-based setting on dementia patients with recent onset.

Methods

Study population

This investigation forms part of the Rotterdam Study, a population-based prospective follow-up study among elderly aged at least 55 years.[7] Approval was given by the local Medical Ethics Committee, and all subjects gave informed consent. Participants were recruited from 10275 eligible residents of a suburb of Rotterdam, including institutionalised persons. A total of 7983 participants (response rate 78%) were examined at baseline. Of these, 7528 persons (94%) were cognitively tested, and 474 appeared to be demented.[8] At follow-up, after an average of 2.1 years (SD 0.9 years), 6315 persons (79%) were re-examined; 768 subjects (10%) had died, 106 (1%) were inaccessible and 794 (10%) refused to participate. Follow-up of all subjects who were not examined in person was completed by evaluating medical files. Of the 7054 participants who were non-demented at baseline, 162 subjects were found to be demented at follow-up.[9]

The analyses presented here are restricted to dementia cases with onset within 3 years before examinations for this study, in order to limit any selective survival, associated with APOE or atherosclerosis.[3,6] The controls were a random sample of participants in the Rotterdam Study, who were screen-negative for dementia at follow-up. As persons with missing data on atherosclerosis or the APOE genotype were excluded, this resulted in a study population of 244 dementia cases (157 prevalent and 87 incident patients) and 1002 non-demented controls.

Dementia diagnosis

For the assessment of dementia, the same protocol at baseline and at follow-up was used.[8,9] Briefly, all subjects were screened on cognitive functioning. Screen positives underwent further neuropsychological testing, and an informant was interviewed on

daily functioning of the participant. Persons who were suspected of dementia, were examined by a neurologist, underwent neuropsychological testing, and, if possible, had a Magnetic Resonance Imaging (MRI) brain scan made.[8,9] Of subjects who could not be re-examined in person, information was obtained from general practitioners and the regional institute for outpatient mental health care (RIAGG), which covers the entire study population. In the Netherlands, the RIAGG is consulted for social and psychiatric problems and is responsible for dementia-care-facility indications. Examinations by the RIAGG include (informant) interviews, neurological and neuropsychological testing, blood biochemistry and syphilis serology.

The diagnosis of dementia was made by a panel of study physicians, a neurologist and a neuropsychologist, which reviewed all existing information and used Diagnostic and Statistical Manual of mental disorders (DSM-III-R) criteria.[10] A diagnosis of AD and vascular dementia (VaD) was based on criteria of the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA),[11] and of the National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN).[12] Cerebrovascular disease was recognised by a history of stroke, evident transient ischaemic attack, or evidence of vascular damage on brain scans.

Measurements

At study entry, people were interviewed about attained level of education and health status, using a computerised questionnaire. Height and weight were measured, and body mass index was calculated.

At baseline, the presence of atherosclerosis in the common carotid arteries (CCAs) was assessed ultrasonographically with a 7.5 MHz linear-array transducer and a Duplex scanner (ATL UltraMark IV). Both CCAs were evaluated for the presence of atherosclerotic plaques, defined as a focal widening relative to adjacent segments with protrusion into the lumen, as described previously.[13] In addition, the intima-media thickness of the distal part of both CCAs were measured and averaged, as described before.[13] The presence of plaques as well as an increased CCAs intima-media wall thickness are indicators of generalised atherosclerosis.[14] At baseline, atherosclerosis of the arteries of the lower extremities was assessed by the ratio of the ankle to brachial systolic blood pressure (ankle-arm index). A decreased ankle-arm index is a measure for peripheral atherosclerosis.[15] Arm blood pressure was measured in sitting position at the right upper arm with a random-zero sphygmomanometer, and the average of two measurements obtained at one occasion was used. Ankle systolic blood pressure was determined with the subject in supine position at both the right and left posterior tibial artery using also a random-zero sphygmomanometer and a

Doppler ultrasound transducer. The analyses were based on the lowest of the ankle-arm index on the left and the right side.

APOE genotyping was performed on coded DNA samples without knowledge of the diagnosis, as described elsewhere.[1] The results were read by three persons independently. In case of discrepancies, APOE genotyping was repeated. Serum total and high density lipoprotein (HDL) cholesterol were determined with an automated enzymatic procedure.[16]

Statistical analysis

The relative risk of dementia or its subtypes was studied as an odds ratio (OR) in a multiple logistic regression model, and presented with a 95% confidence interval (95% CI). To overcome confounding, in all analyses adjustments were made for age, sex and education level. Education was dichotomised as primary school or less, and more than primary school. To study atherosclerosis, a composite measure was used, as defined before.[3] Briefly, a point was added to this score if the following characteristics were present: plaques in at least one of the CCAs, an average CCA intima-media wall thickness in the highest quartile of the distribution in the Rotterdam Study, or an ankle-arm index less than 0.9.[3] This atherosclerosis score was analysed in four categories, corresponding to score values of 0-3, with a zero score as the reference.

To study whether the APOE polymorphism is associated with dementia, independent of atherosclerosis, we put both the APOE genotypes and the atherosclerosis score in the model. This model was extended with the following vascular factors: systolic blood pressure, diastolic blood pressure, body mass index, total and HDL cholesterol, in order to study effects of APOE irrespective of possible intermediate vascular factors.

To investigate the combined effect of APOE and atherosclerosis, the study population was dichotomised according to the atherosclerosis score, to yield maximum statistical power, and classified into 4 groups: (1) APOE3E3 carriers with an atherosclerosis of 0 or 1 (reference group); (2) Carriers of APOE*4 with an atherosclerosis score of 0 or 1; (3) APOE*3 homozygotes with an atherosclerosis score of 2 or 3; and (4) APOE*4 carriers with an atherosclerosis of 2 or 3. Possible interaction was studied according to the principles as described by Rothman.[17,18] Briefly, interaction of factors A and B was considered to be a departure from additivity, and judged to be present if:

$$(OR_{A+B+} - OR_{A+B-}) - (OR_{A-B+} - 1) > 0.$$

The combined effects of APOE2E2 or APOE2E3 and atherosclerosis were studied similarly. As no major differences were observed between men and women, our observations are presented for all.

Results

The distribution of the APOE genotype was in all subgroups in Hardy-Weinberg equilibrium. Some characteristics of the study population are presented in *table 1*. Dementia patients were older, included a higher proportion of women and were lower educated than the controls. Besides, demented cases had, on average, a higher systolic blood pressure, a lower body mass index, a lower serum cholesterol level, and a higher atherosclerosis score.

Table 2 shows the association between the APOE genotypes and dementia, as well as its main subtypes, AD and VaD (model 1). As expected, carriers of the APOE*4 allele were at increased risk of dementia. The APOE2E3 genotype was inversely related to dementia, AD and VaD, although not statistically significant. The addition of the atherosclerosis score to the model, did not essentially alter these observations (model 2). Further adjustment for other vascular factors, i.e. systolic blood pressure, diastolic blood pressure, body mass index, total and HDL cholesterol, did not change risk estimates either (not shown).

Table 3 shows the combined effects of APOE*2 and atherosclerosis on dementia, AD and VaD. We did not observe a clear interaction between APOE*2 and atherosclerosis. Among persons with an atherosclerosis score of 2 or 3, the odds ratio for AD in APOE*2 was lower than in persons with APOE3E3 (1.2 versus 2.0). However, the estimate in APOE*2 carriers is rather imprecise, as the confidence interval is wide.

Table 1 – Baseline characteristics of the study population*

	All dementia n=244	Controls n=1002
Age (SD)	82.4 (7.5)	68.7 (7.9)
Women	67% (164)	60% (604)
Primary education only	58% (129)	23% (225)
Systolic blood pressure in mmHg	142.3 (24.1)	136.3 (20.7)
Diastolic blood pressure in mmHg	70.8 (14.2)	71.1 (10.5)
Body mass index in kg/m^2	25.6 (3.9)	26.6 (4.0)
Total cholesterol in mmmol/L	6.26 (1.16)	6.69 (1.22)
HDL cholesterol in mmmol/L	1.34 (0.38)	1.34 (0.38)
Atherosclerosis score 0	30% (74)	67% (670)
Atherosclerosis score 1	24% (58)	21% (215)
Atherosclerosis score 2	29% (71)	9% (87)
Atherosclerosis score 3	17% (41)	3% (30)

* Values are unadjusted means (SD) or percentages (numbers), based on all available information.

Table 2 – Odds ratios for dementia associated with apolipoprotein E genotypes: adjustment for atherosclerosis*

	Dementia		Alzheimer's disease		Vascular dementia	
Apolipoprotein E	Model 1†	Model 2‡	Model 1†	Model 2‡	Model 1†	Model 2‡
APOE2E2	1.7 (0.3 to 10.1)	2.1 (0.3 to 13.4)	1.0 (0.1 to 11.9)	1.2 (0.1 to 14.4)	-	-
APOE2E3	0.6 (0.4 to 1.2)	0.7 (0.4 to 1.4)	0.6 (0.3 to 1.1)	0.6 (0.3 to 1.3)	0.5 (0.1 to 1.9)	0.6 (0.2 to 2.5)
APOE2E4	3.6 (1.1 to 1.2)	3.9 (1.2-12.6)	1.5 (0.3 to 6.4)	1.6 (0.4 to 7.0)	7.2 (1.5 to 3.5)	16.9 (3.4 to 83.2)
APOE3E3	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
APOE3E4	1.6 (1.0 to 2.5)	1.7 (1.1 to 2.7)	1.5 (0.9 to 2.5)	1.5 (0.9 to 2.6)	1.8 (0.8 to 3.9)	2.2 (1.0 to 5.1)
APOE4E4	15.0 (5.9 to 8.6)	15.2 (5.9 to 39.4)	17.2 (6.2 to 8.0)	17.1 (6.1 to 48.4)	-	-

* Values are odds ratios (95% confidence intervals), and number of patients/controls;

† Model 1: adjusted for age, sex and education level;

‡ Model 2 includes in addition the atherosclerosis score, systolic blood pressure, diastolic blood pressure, body mass index, total and HDL cholesterol.

Table 3 – Odds ratios for dementia and Alzheimer's disease associated with both atherosclerosis and APOE*2*

	Dementia		Alzheimer's disease		Vascular dementia	
Atherosclerosis	APOE3E3	APOE*2+	APOE3E3	APOE*2+	APOE3E3	APOE*2+
Score 0 or 1	1 (reference 61/490	0.7 (0.4 to 1.2) 18/148	1 (reference) 47/490	0.8 (0.4 to 1.5) 15/148	1 (reference 3/490	- 0/148
Score 2 or 3	2.1 (1.3 to 3.2) 60/76	2.1 (0.8 to 5.5) 11/13	2.0 (1.3 to 3.3) 44/76	1.2 (0.3 to 4.1) 5/13	4.1 (1.3 to 13.4) 4/76	5.7 (0.7 to 49.5) 1/13

* Values are odds ratios (95% confidence intervals) adjusted for age, sex and education level, and number of patients/controls.

Table 4 – Odds ratios for dementia and Alzheimer's disease associated with both atherosclerosis and APOE*4*

	Dementia		Alzheimer's disease		Vascular dementia	
Atherosclerosis	APOE3E3	APOE*4+	APOE3E3	APOE*4+	APOE3E3	APOE*4+
Score 0 or 1	1 (reference 61/490	2.1 (1.4 to 3.1) 53/247	1 (reference) 51/490	2.2 (1.5 to 3.4) 46/247	1 (reference 51/490	2.3 (1.1 to 5.3) 0/148
Score 2 or 3	2.6 (1.7 to 4.0) 60/76	7.3 (3.9 to 13.4) 41/28	2.5 (1.6 to 4.0) 50/76	6.8 (3.6 to 12.9) 31/28	5.9 (2.8 to 12.4) 50/76	12.4 (4.9 to 31.1) 31/28

* Values are odds ratios (95% confidence intervals) adjusted for age, sex and education level, and number of patients/controls.

As shown in *table 4*, the effects of both APOE*4 and atherosclerosis on dementia were stronger than the separate effects. APOE*4 with an atherosclerosis score of 0 or 1, was related to an increased risk of all dementia, AD and VaD. The same held true for APOE3E3 with an atherosclerosis score of 2 or 3. However, the highest odds ratio for all three outcomes was observed in APOE*4 carriers with a score of 2 or 3. This odds ratio could not be explained by an asymmetric distribution of homozygotes for APOE*4 across the strata of the atherosclerosis score. For dementia, the interaction effect was (7.3 - 2.1) - (2.6 - 1) = 3.6 (95% CI -1.3 to 8.5). Similarly, the interaction effect for AD was 3.1 (95% CI -1.9 to 8.1), and for VaD 5.2 (95% CI -8.5 to 18.9). Restriction of the cases to incident patients only, did not essentially change our observations.

Discussion

We found that the association between the APOE genotypes and dementia, did not essentially alter when adjustments were made for atherosclerosis or other vascular factors. In addition, the combined effects of atherosclerosis and APOE*4 were stronger than the sum of the separate effects, although this did not reach statistical significance.

A drawback of this study is the lack of autopsy confirmation of the diagnoses of dementia subtypes. However, with clinical examination alone, approximately 90% of AD cases are diagnosed correctly.[19] Another issue is the assessment of atherosclerosis, which may have been subject to misclassification. It should be noted that all examinations were performed without knowledge of the other measurements. Therefore, any measurement error that occurred is random, and most likely resulted in an underestimation of the true relationship. All the indicators of atherosclerosis are reliable estimates of generalised athersclerosis.[14,15] During life, it is difficult to assess the presence of atherosclerosis in the brain. However, the methods that were used in this study are strongly associated with cerebral white matter lesions, as observed with MRI.[20] These lesions are believed to reflect arteriosclerosis, hypoperfusion, and ischaemia.[21] A further advantage of our study is that it is population-based with high response rate and complete follow-up. Survival bias was limited by including subjects who deceased during follow-up, and because the study population was restricted to patients with recent dementia onset.

Our estimates of the odds ratio for dementia associated with the APOE genotypes are in line with previous reports.[2] We confirmed the elevated risk of AD for persons with the APOE2E4 genotype;[2] the high odds ratio for VaD is inaccurate, as the confidence interval is wide. The APOE2E3 genotype was found to be inversely related to all dementia, AD and VaD, although this did not reach statistical signifi-

cance. The putative protective effect on atherosclerosis by the APOE*2 allele,[4,5] did not explain this inverse association between APOE2E3 and dementia, as adjusting for atherosclerosis or other vascular factors did not make a difference. Adjustments for vascular factors did not alter the APOE*4 related odds ratios for dementia either. These observations show that atherosclerosis seems not to be intermediate in the association between the APOE genotypes and dementia.

We did not observe an obvious interaction between APOE*2 and atherosclerosis in dementia. By contrast, we could extend the previously reported interaction between APOE*4 and atherosclerosis in dementia,[3] to a larger group of demented patients with recent onset. A synergistic effect on cognitive decline has also been reported between APOE*4 and atherosclerosis,[22] and between APOE*4 and cerebrovascular disease.[23] Furthermore, there may be a synergistic effect of white matter lesions and APOE*4 in the development of dementia.[24] These findings are in line with post-mortem studies, which showed that APOE*4 is related to neuropathological characteristics of AD,[25] and that the AD neuropathology is especially related to clinical dementia in the presence of cerebral infarcts.[26]

Therefore, the cognitive consequences of cerebrovascular damage seem to be more severe in carriers of the APOE*4 allele than in persons without this allele. This hypothesis is supported by a growing body of literature that APOE is involved in the response to cerebral injury.[27-29] The effects of APOE may be through various mechanisms, which are not necessarily exclusive. These include the reinnervation after neuronal injury, protection against oxidative insults and the modulation of inflammation.[28] Interestingly, transgenic mice carrying human APOE*4 were shown to have larger infarct volumes after cerebral ischaemia than mice with human APOE*3.[28] With the exception of *in vitro* experiments on apoE in oxidative stress,[30] we are not aware of other reports that could explain the inverse association of APOE*2 with dementia.

In conclusion, our study suggests that atherosclerosis is not an intermediate factor in the association between the APOE genotype and dementia. The putative synergistic effect of APOE*4 and atherosclerosis on the risk of dementia, contributes to the growing evidence that APOE plays a role in the response to cerebral damage.

References

1. Slooter AJC, Cruts M, Kalmijn S, et al. Risk estimates of dementia by apolipoprotein E genotypes from a population-based incidence study: the Rotterdam Study. Arch Neurol 1998;55:964-8.
2. Farrer LA, Cupples LA, Haines JL, et al. Effects of age, sex, and ethnicity on the association

of apolipoprotein E genotype with Alzheimer disease. A meta-analysis. JAMA 1997;278: 1349-56.
3. Hofman A, Ott A, Breteler MMB, et al. Atherosclerosis, apolipoprotein E and the prevalence of dementia and Alzheimer's disease in a population-based study: The Rotterdam Study. Lancet 1997;349:151-4.
4. Hixson JE and the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. Apolipoprotein E polymorphism affect atherosclerosis in young males. Arterioscler Thromb 1991;11:1237-44.
5. Slooter AJC, Havekes LM, Bots ML, et al. Apolipoprotein E and atherosclerosis: The Rotterdam Study. Submitted.
6. Stern Y, Brandt J, Albert M, et al. The absence of an apolipoprotein ε4 allele is associated with a more aggressive form of Alzheimer's disease. Ann Neurol 1997;41:615-20.
7. Hofman A, Grobbee DE, De Jong PTVM, et al. Determinants of disease and disability in the elderly: The Rotterdam Elderly Study. Eur J Epidemiol 1991;7:403-22.
8. Ott A, Breteler MMB, Van Harskamp F, et al. Prevalence of Alzheimer's disease and vascular dementia: association with education. The Rotterdam Study. BMJ 1995;310:970-3.
9. Ott A, Breteler MMB, Van Harskamp F, Stijnen T, Hofman A. The incidence and risk of dementia. The Rotterdam Study. Am J Epidemiol 1998;147:574-80.
10. American Psychiatric Association. Diagnostic and statistical manual of mental disorders, third edition, revised. Washington DC, American Psychiatric Association, 1987.
11. McKhann G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939-44.
12. Roman GC, Tatemichi TK, Erkinjuntti T, et al. Vascular dementia: Diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. Neurology 1993;43:250-60.
13. Bots ML, Hofman A, De Jong PTVM, et al. Common carotid artery intima-media thickness as an indicator of atherosclerosis at other sides of the carotid artery. The Rotterdam Study. Ann Epidemiol 1996;6:147-53.
14. Grobbee DE, Bots ML. Carotid artery intima-media thickness as an indicator of generalized atherosclerosis. J Int Med 1994;236:567-73.
15. Vogt MT, Wolfson SK, Kuller LH. Lower extremity arterial disease and the aging process: a review. J Clin Epidemiol 1992;45:529-42.
16. Van Gent CM, Vandervoort HA, De Bruyn AM, Klein F. Cholesterol determinations. A comparative study of methods with special reference to enzymatic procedures Clin Chem Acta 1977;75:243-51.
17. Rothman KJ. Modern epidemiology. Boston, Litle, Brown, 1986.
18. Hosmer DW, Lemeshow S. Confidence interval estimation of interaction. Epidemiology 1992;3:452-6.
19. Galasko D, Hansen LA, Katzman R, et al. Clinical-neuropathological correlations in Alzheimer's disease and related dementias. Arch Neurol 1994;51:888-95.
20. Bots ML, Van Swieten JC, Breteler MMB, et al. Cerebral white matter lesions and atherosclerosis in The Rotterdam Study. Lancet 1993;341:1232-7.
21. Van Swieten JC, Van den Hout JH, Van Ketel BA, et al. Periventricular lesions in the white

matter on magnetic resonance imaging in the elderly: a morphometric correlation with arteriolosclerosis and dilated perivascular spaces. Brain 1991;114:761-74.
22. Slooter AJC, Van Duijn CM, Bots ML, et al. Apolipoprotein E genotype, atherosclerosis, and cognitive decline: The Rotterdam Study. J Neural Transm. 1998;553:17-29.
23. Kalmijn S, Feskens EJM, Launer LJ, et al. Cerebrovascular disease, the apolipoprotein e4 allele, and cognitive decline in a community-based study of elderly men. Stroke 1996;27: 2230-5.
24. Skoog I, Hesse C, Aevarsson Ó, et al. A population study of apoE genotype at the age of 85: relation to dementia, cerebrovascular disease, and mortality. J Neurol Neurosurg Psychiatry 1998;64:37-43.
25. Polvikoski T, Sulkava R, Haltia M, et al. Apolipoprotein E, dementia, and cortical deposition of ß-amyloid protein. N Engl J Med 1995;333:1242-7.
26. Snowdon DA, Greiner LH, Mortimer JA, et al. Brain infarction and the clinical expression of Alzheimer disease. The nun study. JAMA 1997;277:813-7.
27. Poirier J. Apolipoprotein E in animal models of CNS injury and in Alzheimer's disease. Trends Neurosci 1994;17:525-30.
28. Laskowitz DT, Roses AD. Apolipoprotein E: an expanding role in the neurobiology of disease. Alz Rep 1998;1:5-12.
29. Ali SM, Dunn E, Oostveen JA, et al. Induction of apolipoprotein E mRNA in the hippocampus of the gerbil after transient global ischemia. Mol Brain Res 1996;38:37-44.
30. Miyata M, Smith J. Apolipoprotein E allele-specific antioxidant activity and effects on cytotoxicity by oxidative insults and ß-amyloid peptides. Nat Genet 1996;14:55-61.

6.3

Smoking, apolipoprotein E and the risk of dementia

Abstract

Background *Previous studies suggested a protective effect of smoking on Alzheimer's disease. These studies were mostly case-control studies based on prevalent cases. Prospective studies on the risk of dementia associated with smoking are scarce and inconclusive.*

Methods *We performed a population-based follow-up study of 6870 elderly who were initially free of dementia. Smoking history was obtained at baseline and subjects were classified as never smokers, former smokers, and current smokers. Complete ascertainment of cases with incident dementia was pursued. Using never smokers as the reference, relative risks were calculated with Cox proportional hazards regression, adjusting for age, sex, education, and alcohol intake. We examined modification by age, sex and apolipoprotein E (APOE) genotype.*

Findings *During an average follow-up of 2.1 years, 146 incident cases of dementia were detected, of whom 105 had Alzheimer's disease. Smokers had a more than twofold increased risk of dementia (relative risk (RR) 2.2; 95% CI 1.3 to 3.6), and Alzheimer's disease (RR=2.3; 95% CI 1.3 to 4.1). Smoking appeared a strong risk factor of Alzheimer's disease in those without the APOE*4 allele (RR=4.6; 95% CI 1.5 to 14.2), whereas no effect was observed in subjects with the APOE*4 allele (RR=0.6; 95% CI 0.1 to 4.8).*

Interpretation *This prospective study did not confirm results from previous studies, which suggested a general protective effect of smoking on dementia and Alzheimer's disease. Smoking was associated with a doubling of the risk of dementia and Alzheimer's disease. Carriers of the APOE*4 allele had no increased risk, suggesting interaction between smoking and the APOE genotype in the aetiology of Alzheimer's disease.*

Introduction

Dementia is a frequent disorder with immense impact on quality of life. Its prevalence increases exponentially with age to at least 30% in people aged 85 years and over.[1] Alzheimer's disease and vascular dementia are the major subtypes of dementia, and account for about 90% of patients.[1] Smoking has both been related to vascular dementia and Alzheimer's disease. As an important risk factor for cardiovascular diseases,[2,3] smoking is supposed to increase the risk of vascular dementia.[4] In contrast, an inverse association has been observed between smoking and Alzheimer's disease.[5] In a study on early onset Alzheimer's disease this possible protective effect of smoking was found to be limited to carriers of the apolipoprotein E4 allele (APOE*4).[6] Although there are several possible explanations for a protective effect of smoking on Alzheimer's disease,[5,6] it is also conceivable that findings in previous studies were the result of bias.[7] Also, smoking strongly increases mortality and smokers who survive till later age may be a more selected group than contemporaneous non-smokers.[8]

We performed a prospective population-based study of the association of cigarette smoking with dementia and Alzheimer's disease, while considering the effects of age, sex and the APOE genotype.

Methods

Study population

The Rotterdam Study is a community-based prospective cohort study among persons aged 55 years and over,[9] for which approval was given by the local Medical Ethics Committee. All 10275 residents of a suburb of Rotterdam over the age of 55 years were eligible and of those 7983 (78%) participated and gave informed consent and permission to obtain medical information from treating physicians. Baseline examinations took place from 1990 to 1993 and comprised a home interview and medical examinations at the study centre or, for institutionalised persons, at their institution. At baseline, 7528 persons (94%) were evaluated for dementia,[1] of whom 474 were diagnosed with dementia and excluded from the present study. Of those without dementia an additional 184 were excluded because of missing data on smoking history. The remaining 6870 subjects were followed-up for an average of 2.1 years (range 1.5-3.4), until the second round of examinations. During this follow-up period 429 participants (6.2%) died. Another 962 subjects (14.0%) were not re-examined because of refusal or severe illness. Follow-up of all subjects who were not examined in person was completed by evaluating their medical files.

Dementia diagnosis and measurements

For the assessment of dementia the same protocol was used at baseline and at follow-up, as described in more detail elsewhere.[1,10] Briefly, all subjects were screened with a brief test of cognition (Mini Mental State Examination[11] and the Geriatric Mental State schedule, organic level[12]). Screen positives underwent further cognitive testing, and an informant was interviewed on daily functioning of the participant. Persons who were suspected of dementia, were examined by a behavioural neurologist, underwent neuropsychological testing, and if possible had a brain scan made by magnetic resonance imaging.[1,10] Of subjects who could not be re-examined in person, information was obtained from general practitioners and the regional institute for outpatient mental health care (RIAGG), which covers the entire study population. In the Netherlands, the RIAGG is responsible for dementia-care-facility indications. Examinations by the RIAGG include (informant) interviews, neurologic and neuropsychologic testing, blood biochemistry and syphilis serology. We avoided misdiagnosing terminal decline for dementia by not diagnosing dementia in subjects with unexplained mental deterioration within half a year before death.

The diagnosis of dementia was based on Diagnostic and Statistical Manual of mental disorders (DSM-III-R) criteria,[13] and made by a panel of study physicians, a neurologist and a neuropsychologist, which reviewed all existing information. A diagnosis of Alzheimer's disease and vascular dementia was based on criteria of the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA),[14] and of the National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN).[15] Alzheimer patients were further classified into those with and without cerebrovascular disease.[14] For other dementias, DSM-III-R criteria were used.[13] Cerebrovascular disease was recognised by history of stroke or transient ischaemic attack, or by evidence for cerebrovascular lesions on brain scans.

At baseline, participants were asked about their present and former smoking habits. People who smoked cigarettes were asked for the age at first smoking, for the duration of interval periods without smoking, and for average daily number of cigarettes smoked. Former smokers were asked about starting age, interval periods without smoking, age at quitting, and average daily number of cigarettes smoked. Subjects were also asked about attained level of education and, as part of a detailed food frequency questionnaire, about their average daily alcohol consumption.[16]

APOE genotyping was performed on coded DNA samples without knowledge of the diagnosis for subjects who developed dementia (DNA available for 122 subjects) and a random sample of 1428 non-demented participants. The polymerase chain reaction product was digested with the restriction enzyme *HhaI*, and fragments

were separated by electrophoresis.[17] The results of the APOE typing were read by three persons independently. In case of discrepancies, APOE genotyping was repeated.

Data analysis

Cigarette smoking at baseline was categorised into never, former and current smoking. The number of pack-year exposure was calculated by the average daily number of cigarettes divided by 20 and multiplied with the number of years smoked. In all analyses never smokers were used as reference category. The relative risk of dementia by smoking was calculated with Cox proportional hazards regression, and presented with a 95% confidence interval (95% CI). Age at onset of dementia was taken to be the midpoint between baseline age and age at diagnosis. For non re-examined cases without known age at diagnosis, onset age was defined as the midpoint between baseline age and age at the end of the follow-up period. For non re-examined surviving subjects the follow-up ended at December 31, 1994.[10] Since age is a major determinant of dementia and age-distributions differed by smoking status, age was adjusted for by including linear and squared baseline age in the models. Sex, education and alcohol consumption were judged to possibly confound the association and therefore added to all models. Because in our data only primary education was associated with incident dementia, education was dichotomised into primary school or less, and more than primary school. Daily alcohol intake, expressed in grams of pure alcohol per day, was added as continuous variable.

We also investigated whether the smoking related risk of dementia was modified by the APOE genotype. The APOE genotype was available for 122 of those who developed dementia and a random sample of 1428 non-demented participants. Data were analysed as a nested case-control study using logistic regression analysis. Corrections for age, sex, education and alcohol intake were made similarly to the above models. Length of follow-up was considered to possibly affect the association because the probability of developing dementia increases with duration of follow-up, whereas smoking may increase mortality. Therefore, we added duration of follow-up as a continuous variable to the logistic regression models.

Results

Table 1 summarises characteristics of the study population. More than half of all women had never smoked, whereas more than half of the men had stopped smoking cigarettes. Smokers were on average younger than never smokers. Those who never

Table 1 – Baseline characteristics of the study population by gender and status of cigarette smoking.

	Total (n=6870)			Men (n=2776)			Women (n=4094)		
	Never smokers	Former smokers	Current smokers	Never smokers	Former smokers	Current smokers	Never smokers	Former smokers	Current smokers
Proportion	39.1%	40.1%	20.7%	16.4%	58.6%	25.0%	54.6%	27.6%	17.8%
Age (years) (SD)	71.9 (9.9)	68.3 (8.0)	66.4 (7.6)	69.8 (9.8)	68.2 (7.7)	67.1 (7.9)	72.3 (9.9)	68.5 (8.5)	65.7 (7.3)
Primary education only	29.9%	19.4%	22.4%	15.0%	15.5%	18.5%	32.9%	25.0%	26.1%
Alcohol intake (gram/day)	5.6 (9.8)	12.2 (15.3)	15.1 (19.8)	12.3 (15.2)	15.8 (17.2)	21.0 (23.6)	4.2 (7.6)	7.1 (10.2)	9.9 (13.7)

Table 2 – Relative risk of dementia by baseline cigarette smoking, for the entire study population, women, and men*

	Total study population			Men			Women		
	Never smokers	Former smokers	Current smokers	Never smokers	Former smokers	Current smokers	Never smokers	Former smokers	Current smokers
Adjusted for age (and sex)	1 (reference)	1.3 (0.9 to 2.0)	2.2 (1.4 to 3.6)	1 (reference)	3.7 (1.1 to 12.7)	5.9 (1.7 to 21.3)	1 (reference)	1.1 (0.7 to 1.8)	2.0 (1.0 to 3.7)
Adjusted for age, alcohol intake, education (and sex)	1 (reference)	1.4 (0.9 to 2.0)	2.2 (1.3 to 3.6)	1 (reference)	3.9 (1.1 to 13.5)	5.8 (1.6 to 20.9)	1 (reference)	1.1 (0.7 to 1.8)	2.0 (1.0 to 3.7)

* Relative risks with 95% confidence intervals.

smoked more often had primary education only. Average daily alcohol intake was higher among smokers than non-smokers.

The mean follow-up period was 2.1 years (range 1.5-3.4), and included 14761 person-years of observation. We detected 146 incident cases of dementia, of whom 105 (72%) had Alzheimer's disease and 19 (13%) vascular dementia. The incidence rates of dementia per 1000 personyears for the agegroups 55-64 years, 65-74 years, 75-84 years, and 85 years and over were 0.9, 2.7, 20.1, and 52.0. For men these figures were 1.1, 2.5, 18.6, 28.0 and for women 0.8, 2.7, 20.9, and 58.8, respectively.

Table 2 shows that smokers were at increased risk of dementia. Smoking increased the risk of dementia for both men and women. In men, the relative small number of cases in the reference category resulted in high relative risks, but these were rather unstable. Smoking both increased the risk of Alzheimer's disease and vascular dementia (*table 3*). The relative risk of dementia for current smokers at baseline was higher in persons who died or were not re-examined than in participants who were re-examined in person: 2.7 (95% CI 1.2 to 6.0) versus 2.0 (95% CI 1.0 to 3.7). The mean age at onset of dementia for never smokers was 85.5 years. Both former and current smokers were on average younger at dementia onset than never smokers (respectively 4.1 years, 95% CI 1.7 to 6.6; and 8.6 years, 95% CI 5.2 to 12.1).

The total amount of pack-years smoked was available for 1368 (96%) of current smokers and for 2527 (92%) of former smokers. For current smokers at baseline, the relative risk of dementia was 2.5 (95% CI 1.1 to 5.5) for those who smoked less than 20 pack-years and 3.0 (95% CI 1.6 to 5.4) for those who smoked 20 pack-years or more. For former smokers these figures were 1.5 (95% CI 1.0 to 2.5) and 2.1 (95% CI 1.2 to 3.7), respectively.

The APOE*4 allele modified the association between smoking and dementia (*table 4*). In subjects without the APOE*4 allele, smoking increased the risk of dementia substantially (RR=3.2; 95% CI 1.2 to 8.5). However, among carriers of

Table 3 – Relative risk of dementia subtypes by baseline cigarette smoking.*

	Never smokers	Former smokers	Current smokers
All Alzheimer's disease (n=105)	1 (reference)	1.3 (0.8 to 2.1)	2.3 (1.3 to 4.1)
Alzheimer's disease without CVD (n=88)	1 (reference)	1.4 (0.8 to 2.3)	2.1 (1.1 to 4.0)
Alzheimer's disease with CVD (n=17)	1 (reference)	1.2 (0.4 to 4.2)	3.9 (1.0 to 15.2)
Vascular dementia (n=19)	1 (reference)	1.4 (0.5 to 4.4)	2.2 (0.6 to 8.4)
Other dementia (n=22)	1 (reference)	1.5 (0.5 to 4.2)	2.1 (0.6 to 6.8)

* Relative risks with 95% confidence intervals, adjusted for age, sex, alcohol intake, and education.

APOE*4, there appeared to be no association between smoking and dementia (RR=1.4; 95% CI 0.3 to 5.6). This effect modification was more marked in Alzheimer patients with an increased relative risk of 4.6 (95% CI 1.5 to 14.2) for subjects without APOE*4 and a non-significantly decreased relative risk of 0.6 (95% CI 0.1 to 4.8) in those with APOE*4.

Discussion

In this large prospective population-based study, people who smoked cigarettes were at increased risk of dementia and Alzheimer's disease. An elevated relative risk was particularly present for smokers without the APOE*4 allele.

The present investigation has several advantages over previous studies on smoking and dementia.[5] Selection bias was avoided because the study was prospective and population-based with a high response rate and complete follow-up. As smoking habits were assessed before onset of dementia, major recall bias was avoided. It is conceivable though, in particular because of our relatively short follow-up period, that some persons who developed clinical dementia during the follow-up period had already slight impairment of memory or judgement at baseline. This may have resulted in some misclassification of exposure status among cases. If this has occurred, we consider it more likely that it concerned underreporting rather than overreporting of smoking habits. Consequently, if any recall bias occurred, this most likely resulted in an underestimation of the true effect. Survival bias was avoided by including subjects who died during follow-up. However, case finding methods differed for subjects that could be re-examined in person and those who could not, and as a result we may have missed some very early cases in the latter group. Relative risks by

Table 4 – Relative risk of dementia and Alzheimer's disease by baseline cigarette smoking and apolipoprotein E4.*

	Never smokers	Former smokers	Current smokers
All dementia			
APOE*4 absent (n=73)	1 (reference)	1.9 (0.8 to 4.6)	3.2 (1.2 to 8.5)
APOE*4 present (n=49)	1 (reference)	1.0 (0.3 to 3.3)	1.4 (0.3 to 5.6)
Alzheimer's disease			
APOE*4 absent (n=57)	1 (reference)	2.4 (0.9 to 6.5)	4.6 (1.5 to 14.2)
APOE*4 present (n=33)	1 (reference)	0.9 (0.2 to 3.6)	0.6 (0.1 to 4.8)

* Relative risks estimated as odds ratios with 95% confidence intervals, adjusted for age, sex, alcohol intake, and education.

smoking were slightly higher in those who died, refused, or were too ill to be re-examined, than in the re-examined group (2.7 versus 2.0). Because we possibly slightly underdiagnosed dementia in the non-re-examined group, the actual difference may even be larger. The higher relative risk of dementia in subjects who died or could not be re-examined illustrates that survival bias may indeed have diluted the association of smoking and dementia in previous, cross-sectional studies.

Educational level and alcohol intake are well-known correlates of smoking habits, and both have been invoked in relation to dementia as well.[1,18] When we adjusted for these variables in the analyses, the smoking associated risk of dementia remained virtually unchanged.

The follow-up in this study was relatively short. The main disadvantage of this is the limited number of incident dementia cases, which restricted the power in some of our subanalyses. On the other hand, it facilitated both the complete follow-up of the cohort and the use of identical screening and diagnostic procedures at baseline and follow-up.

A limitation of the present study is the lack of autopsy confirmation of our Alzheimer's disease diagnoses. Another problem is that Alzheimer's disease pathology and cerebrovascular disease are often concurrently present and it may be difficult, both clinically and pathologically, to estimate their contribution to the development of dementia.[15] Although our clinical work-up was extensive and we adhered to commonly accepted and reliable criteria, we can not rule out that we misclassified the dementia subtype in some cases.

Smoking increased the risk of dementia for both sexes. The relative risks seemed higher for men than for women. However, the number of never smoking men (the reference group) was small, and among them only a few became demented. This led to greater variability in relative risk estimates in men and the gender differences may be due to chance. Possible effect modification between gender and smoking should be looked at more closely in further studies.

Most previous studies on smoking and dementia used prevalent cases and a case-control design.[5] As reviewed by Lee, 4 out of 17 studies reported a significant inverse relation between smoking and Alzheimer's disease.[5] Some earlier results may differ from the present findings due to differing methods.[7] Of particular concern is the definition of Alzheimer's disease in the earlier studies. Patients with coexisting vascular disease may have been classified as 'mixed' dementia. By excluding these from the Alzheimer category, these studies may have selectively cleared cigarette smokers from the cases, as smokers are more prone to vascular diseases.[2,3] However, we found that smokers had also an increased risk of Alzheimer's disease without cerebrovascular disease. The few studies which estimated the relation with vascular dementia found, as we did, a positive association with smoking.[19,20] A few studies have

used incident cases to assess the risk of Alzheimer's disease by smoking, with varying results.[21-24] However, none of these looked at a possible effect-modification by APOE genotype.

Smoking is a risk factor for vascular disease, including atherosclerosis and thrombosis.[2,3] Reduction of cerebral perfusion is an acute effect of cigarette smoking, and this may be harmful in elderly with a compromised cerebral circulation.[25] In the pathogenesis of Alzheimer's disease, by far the most common dementing illness, vascular involvement is probably more important than previously thought.[26,27]

Van Duijn et al previously reported that the inverse association between smoking and Alzheimer's disease was limited to carriers of the APOE*4 allele.[6] In line with that observation we found that the increased risk of dementia and Alzheimer's disease associated with smoking was restricted to persons without this allele. Before considering possible explanations for this apparent effect modification by APOE*4, one should consider that elderly smoking APOE*4 carriers may be a selected group. The APOE*4 allele not only increases the risk of Alzheimer's disease but also of cardiovascular disease.[29] Therefore, mortality may be disproportionately increased among smoking APOE*4 carriers.[7] If atherosclerosis is indeed the intermediate factor in the association between smoking and dementia, various explanations could be hypothesised for a modifying effect of APOE*4. Firstly, the presence of the APOE*4 allele might alter the association between smoking and atherosclerosis. However, in the Rotterdam Study the strength of the relation between smoking and various indicators of atherosclerosis was similar for subjects with or without an APOE*4 allele. Secondly, the APOE*4 allele may inhibit the association between vascular damage and dementia. This is however in sharp contrast to the synergistic effect that we observed between APOE*4 and atherosclerosis on the risk of dementia.[27] Thirdly, smoking may exert different and opposite effects on the risk of Alzheimer's disease, being generally harmful through for example a vascular mechanism, but also partly beneficial in selected individuals, especially in those who carry an APOE*4 allele. This hypothesis may be supported by the observation that in Alzheimer patients carriers of APOE*4 have fewer nicotinic receptor binding sites and decreased activity of choline acetyltransferase, as compared to non-carriers of this allele.[28] Smoking could have some effects on the cholinergic system, which might counterbalance the selective impairment that was found to be associated with APOE*4, including increasing the density of nicotine receptors or facilitating the release of acetylcholine.[6,22]

In conclusion, we found that for the majority of the population, namely those not carrying the APOE*4 allele, smoking increased the risk of dementia and Alzheimer's disease. For subjects with the APOE*4 allele however, smoking was not associated with an increased risk of dementia.

References

1. Ott A, Breteler MMB, Van Harskamp F, et al. Prevalence of Alzheimer's disease and vascular dementia: association with education. The Rotterdam study. BMJ 1995;310:970-3.
2. Bartecchi CE, MacKenzie TD, Schrier RW. The human costs of tobacco use (1). N Engl J Med 1994;330:907-12.
3. Shinton R, Beevers G. Meta-analysis of the relation between cigarette smoking and stroke. BMJ 1989;298:789-94.
4. Hachinski VC. Preventable senility: a call for action against the vascular dementias. Lancet 1992;340:645-8.
5.. Lee PN. Smoking and Alzheimer's disease: A review of the epidemiological evidence. Neuroepidemiology 1994;13:131-44.
6. Van Duijn CM, Havekes LM, Van Broeckhoven C, De Knijff P, Hofman A. Apolipoprotein E genotype and association between smoking and early onset Alzheimer's disease. BMJ 1995;310:627-31.
7. Riggs JE. Smoking and Alzheimer's disease: protective effect or differential survival bias? Lancet 1993;342:793-4.
8. Doll R, Peto R, Wheatly K, Gray R, Sutherland I. Mortality in relation to smoking: 40 years' observations on male British doctors. BMJ 1994;309:901-11.
9. Hofman A, Grobbee DE, De Jong PTVM, Van den Ouweland FA. Determinants of disease and disability in the elderly. The Rotterdam Elderly Study. Eur J Epidemiol 1991;7:403-22.
10. Ott A, Breteler MMB, van Harskamp F, Stijnen T, Hofman A. The incidence and risk of dementia. The Rotterdam Study. Am J Epidemiol 1998;147:574-80.
11. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiat Res 1975;12:189-98.
12. Copeland JRM, Kelcher MJ, Kellet JM, et al. A semi-structured clinical interview for the assessment of diagnosis and mental state in the elderly. The Geriatric Mental State Schedule I. Development and reliability. Psychol Med 1976;6:439-49.
13. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. Third edition, revised. American Psychiatric Association, Washington DC, 1987.
14. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939-44.
15. Roman GC, Tatemichi TK, Erkinjuntti T, et al. Vascular dementia: Diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. Neurology 1993; 43:250-60.
16. Geleijnse JM, Witteman JC, den Breeijen JH, et al. Dietary electrolyte intake and blood pressure in older subjects: the Rotterdam Study. J Hypertens 1996;14:737-41.
17. Wenham PR, Price WH, Blundell G. Apolipoprotein E genotyping by one-stage PCR. Lancet 1991;337:1158-9.
18. Orgogozo JM, Dartigues JF, Lafont S, et al. Wine consumption and dementia in the elderly: a prospective community study in the Bordeaux area. Rev Neurol (Paris) 1997;153:185-92.
19. Meyer JS, McClintic KL, Rogers RL, Sims P, Mortel KF. Aetiological considerations and risk factors for multi-infarct dementia. J Neurol Neurosurg Psychiatry 1988;51:1489-97.

20. Gorelick PB, Brody J, Cohen D, et al. Risk factors for dementia associated with multiple cerebral infarcts. A case-control analysis in predominantly African-American hospital-based patients. Arch Neurol 1993;50:714-20.
21. Hebert LE, Scherr PA, Beckett LA, et al. Relation of smoking and alcohol consumption to incident Alzheimer's disease. Am J Epidemiol 1992;135:347-55.
22. Brenner DE, Kukull WA, van Belle G, et al. Relationship between cigarette smoking and Alzheimer's disease in a population-based case-control study. Neurology 1993;43:293-300.
23. Prince M, Cullen M, Mann A. Risk factors for Alzheimer's disease and dementia: A case-control study based on the MRC elderly hypertension trial. Neurology 1994;44:97-104.
24. Yoshitake T, Kiyohara Y, Kato I, et al. Incidence and risk factors of vascular dementia and Alzheimer's disease in a defined elderly Japanese population: the Hisayama Study. Neurology 1995;45:1161-8.
25. Cruickshank JM, Neil-Dwyer G, Dorrance DE, Hayes Y, Patel S. Acute effects of smoking on blood pressure and cerebral blood flow. J Hum Hypertens 1989;3:443-9.
26. Thomas T, Thomas G, McLendon C, et al. β-Amyloid-mediated vasoactivity and vascular endothelial damage. Nature 1996;380:168-71.
27. Hofman A, Ott A, Breteler MMB, et al. Atherosclerosis, apolipoprotein E, and the prevalence of dementia and Alzheimer's disease in a population-based study: The Rotterdam Study. Lancet 1997;349:151-4.
28. Poirier J, Delisle M-C, Quirion R, et al. Apolipoprotein E4 allele as a predictor of cholinergic deficits and treatment outcome in Alzheimer disease. Proc Natl Acad Sci USA 1995;92: 12260-4.
29. Wilson PWF, Schaefer EJ, Larson MG, Ordovas JM. Apolipoprotein E alleles and risk of coronary disease. A meta-analysis. Arterioscler Thromb Vasc Biol 1996;16:1250-5.

7

IMPACT OF APOLIPOPROTEIN E ON MYOCARDIAL INFARCTION, STROKE, DEMENTIA AND MORTALITY

7

The impact of the apolipoprotein E genotype on myocardial infarction, stroke, dementia and mortality

Abstract

*The impact of the apolipoprotein E (APOE) genotype in vascular diseases, dementia and mortality was assessed in 5290 elderly participants in a population-based study who were followed for, on average, 4.0 years. Subjects with the APOE4E4 genotype had a higher risk of myocardial infarction than persons with APOE3E3, in particular before 65 years of age. The incidence of stroke was not strongly related to the APOE polymorphism. Although carriers of the APOE*4 allele were at increased risk of dementia, the contribution of APOE*4 to the incidence of dementia on a population level was modest. This reflects the relatively low frequency of this allele. Mortality was overall not related to APOE, but increased in persons with either the APOE2E4 or the APOE4E4 genotype who were younger than 65 years. Our study suggests that the APOE genotype does not play an important role in mortality or the risk of vascular diseases in the elderly.*

Introduction

The rapid developments in genetics have led to the discovery of non-traditional risk factors for disease. One genetic variation (polymorphism) with possibly large public health implications is the apolipoprotein E genotype (APOE). The APOE gene has three common alleles, APOE*2, APOE*3 and APOE*4. As compared to APOE*3, the most common allele, APOE*2 is associated with lower serum levels of athero-

genic lipoproteins, while APOE*4 has opposite effects.[1] Yet, studies on APOE and vascular diseases have been contradictory, particularly in older populations.[2-6]

The APOE genotype was found to be involved in Alzheimer's disease,[7,8] and other, less common types of dementia.[9] Almost all studies on APOE and dementia are based on prevalent, clinic-based patient-series, which are subject to selection bias. Reliable estimates of the risk of dementia, associated with the APOE genotypes, are currently not available.

The APOE polymorphism has been suggested to be a determinant of longevity, but the few reports on this issue are all based on case-control studies on the oldest old.[1,10,11] It is thus unclear how APOE allele frequencies change with ageing, and follow-up studies on the association of APOE with mortality are lacking.

The impact of the APOE genotype on major causes of morbidity and mortality has never been studied simultaneously. The aim of this population-based single-centre follow-up study was to investigate the role of the APOE genotype in myocardial infarction, stroke, dementia and mortality.

Methods

Study population

The Rotterdam Study is a population-based prospective cohort study among persons aged 55 years or over,[12] for which approval was given by the Erasmus University Medical School Ethics Committee. Participants were recruited from 10275 eligible residents of a suburb of Rotterdam, including institutionalised persons. A total of 7983 participants (response rate 78%) were examined at baseline. During follow-up, information on vital status was complete for all participants. At the time of the present study, follow-up on the occurrence of myocardial infarction or stroke was complete for 7053 subjects (88% of the cohort). At the second wave, after an average of 2.1 years (SD 0.9 years), 6315 persons (79%) were re-examined; 768 subjects (10%) had died, 106 (1%) were inaccessible and 794 (10%) refused to participate. As persons with a yet undetermined APOE genotype were excluded (n=2693, 34% of the cohort), our study population comprised 5290 individuals.

Baseline measurements

At baseline, people were interviewed about their health status, drug use, and attained level of education. Cognitive function was assessed using the mini-mental state examination (MMSE) and the geriatric mental state schedule (GMS).[13,14] An electrocardiogram (ECG) was made, and digitally stored in 4854 participants of the present study (92%). These ECGs were analysed by the Modular ECG Analysis System

(MEANS), which has been evaluated extensively.[15] Missing ECGs were mainly due to temporary technical problems. Blood was collected for laboratory analyses.

Follow-up procedures

The follow-up period for the present study ended in April 1996 (mean duration 4.0 years (SD 1.2)). The occurrence of myocardial infarction or stroke was recorded in 4805 participants of the present study (91%). These events were reported by general practitioners with a computerised surgery (85% of the participants). All reported information was verified by study physicians who inspected all available information. In addition, the complete medical records of all participants in non-computerised practices (other 15%) were regularly checked by research physicians.

All events were classified independently by two study physicians. In case of disagreement, a consensus was reached in a separate session. All events were verified by an expert in the field of vascular or neurologic diseases. If the expert disagreed with the research physician, the expert's opinion was considered final.

Myocardial infarction

A myocardial infarction before baseline was diagnosed on the basis of ECG findings and self-report. Besides, of subjects with self-reported myocardial infarction without ECG evidence, and of persons with no self-report of myocardial infarction but with ECG evidence, additional information was collected from the general practitioner or cardiologist.[16] Definite myocardial infarction was defined according to the general practitioner, cardiologist or ECG (n=631 participants in the present study). Possible myocardial infarction was based on self-report without verification by general practitioner, cardiologist or ECG (n=32). As the first occurrence of a myocardial infarction was utilised, we excluded from the longitudinal analyses subjects with myocardial infarction before baseline (n=663), as well as those with missing data on a previous myocardial infarction (n=345). Myocardial infarction during follow-up was diagnosed in 87 of the remaining 3840 participants with complete follow-up, using the definitions of the 10th revision of the International Classification of Diseases (ICD-10).[17]

Stroke

A stroke before baseline was determined on the basis of the question 'did you ever suffer from a stroke, diagnosed by a physician?' Signs and symptoms had to last more than 24 hours. Of the persons with an affirmative answer, the general practitioner was asked for supplementary information. To optimise prevalence data, medical records of subjects living in homes for the elderly, who were cognitively impaired, were systematically reviewed.[18] In total, 221 participants in the present study were

identified with a stroke before baseline, who were excluded from the longitudinal analyses. Persons with missing data on a stroke before baseline (n=207), were included in the group of 'no previous stroke', as these groups appeared not to differ in risk of stroke during follow-up. As there was no complete follow-up in another 471 participants, this resulted in a cohort of 4598 subjects at risk of stroke, of whom 154 cases were detected. Stroke diagnosis was based on all available information,[19] using ICD-10 criteria.[17]

Dementia

Besides the above described reports during follow-up, dementia cases were identified in the Rotterdam Study examinations. The same protocol was used by the same investigators at the first and second wave.[18,20] Briefly, all subjects were screened with the MMSE and the GMS. Those suspected of dementia, were examined by a neurologist, underwent neuropsychological testing, and if possible, neuro-imaging. Besides, an informant was interviewed on daily functioning. Of subjects who could not be re-examined in person, information was obtained from the regional institute for outpatient mental health care (RIAGG).[18,20] The diagnosis of dementia was made by a panel of study physicians, a neurologist and a neuropsychologist, which reviewed all information and used Diagnostic and Statistical Manual of mental disorders (DSM-III-R) criteria.[21] In the present study, 348 participants were demented at baseline, and excluded from the longitudinal analyses, together with subjects whose dementia status at baseline was uncertain (n=16). This resulted in a cohort of 4926 participants in the present study at risk for dementia, of whom 134 patients were diagnosed as demented during follow-up.

Mortality

Information on the vital status of all 5290 participants was obtained at regular intervals from the municipal health authorities. Our studies on mortality are based on 21188 person-years of follow-up, in which 913 persons died.

Laboratory analysis

APOE genotyping was performed on coded samples without knowledge of the other measurements or diagnoses. Genotyping was performed using a polymerase chain reaction, as described in detail elsewhere.[9] Results were read by three persons independently. In case of any discrepancies, APOE genotyping was repeated. Serum total and high density lipoprotein (HDL) cholesterol were determined with an automated enzymatic procedure.[22]

Statistical analysis

Differences in baseline characteristics by the APOE genotypes were explored with the chi-square test for categorical data, and an analysis of variance (ANOVA) was used for continuous, normally distributed variables. Cox' proportional hazards models were used to assess the relative risks (RR) of myocardial infarction, stroke, dementia and death, which is presented with a 95% confidence interval (95% CI). The APOE genotypes were entered in the model as dummy variables, using the APOE3E3 group as the reference group. To adjust for confounding, age and sex were added to models on myocardial infarction, stroke and dementia. In addition, education level was added to models on dementia. For genotypes significantly related to a certain outcome, we used the population attributable risk to estimate the excess rate in population that is attributable to that specific APOE genotype. The population attributable risk was computed as the product of the difference in incidence rate with the APOE3E3 group, and the proportion of subjects with the particular APOE genotype. To estimate the survival curves for the various APOE genotypes, we used Kaplan-Meier analysis.

Results

The distribution of the APOE polymorphism in our study population was in Hardy-Weinberg equilibrium (X^2=0.5; df=3; P>0.2). Descriptive statistics are presented in *table 1*. As compared to the APOE3E3 group, persons with APOE2E3 had on average lower total cholesterol levels and higher levels of HDL cholesterol. By contrast, subjects with the APOE3E4 or APOE4E4 genotype had, a higher mean total cholesterol level than persons with APOE3E3, and a lower level of HDL cholesterol in serum. The APOE genotype was not related to a history of myocardial infarction or stroke at baseline. Dementia was more prevalent at baseline among APOE*4 carriers compared to persons without this allele.

Table 2 shows absolute and relative risks of myocardial infarction associated with the APOE polymorphism. Persons with the APOE4E4 genotype were at increased risk of myocardial infarction (RR 2.1; 95% CI 0.8 to 6.0), in particular men and individuals younger than 65 years, although numbers in subgroups were small. Overall, APOE3E4 carriers had a similar risk of myocardial infarction compared to persons with the APOE3E3 genotype. The APOE2E3 group had a slightly lower risk of myocardial infarction (RR 0.7; 95% CI 0.3 to 1.5), but this did not reach statistical significance. Similar findings were obtained when adjustments were made for both total and HDL cholesterol. For instance, the adjusted relative risk associated with APOE4E4 was 2.1 (95% CI 0.7 to 5.8). Also when we analysed the risk of fatal myo-

Table 1 – Baseline characteristics of the study population*

	APOE2E2 n=41	APOE2E3 n=698	APOE2E4 n=143	APOE3E3 n=3046	APOE3E4 n=1229	APOE4E4 n=133
Age	71.3 (9.3)	69.6 (9.2)	69.1 (8.9)	69.9 (9.1)	69.8 (9.0)	68.3 (8.1)
Women	61% (25)	61% (428)	57% (81)	55% (1688)	55% (681)	54% (72)
Primary education only	31% (12)	26% (177)	21% (28)	25% (722)	26% (303)	21% (27)
Cholesterol level (mmol/L)	6.59 (1.68)	6.32 (1.31)	6.47 (1.17)	6.59 (1.19)	6.75 (1.22)	6.81 (1.05)
HDL-cholesterol level (mmol/L)	1.30 (0.33)	1.40 (0.38)	1.35 (0.38)	1.33 (0.36)	1.31 (0.37)	1.29 (0.36)
Previous myocardial infarction	15% (6)	12% (81)	13% (18)	13% (393)	13% (151)	11% (14)
Previous stroke	3% (1)	3% (23)	8% (11)	4% (130)	4% (51)	4% (5)
Prevalent dementia	7% (3)	6% (40)	7% (10)	6% (177)	8% (98)	15% (20)

* Values are unadjusted means (SD) or percentages (numbers), based on all available information.

Table 2 – Risk of myocardial infarction associated with apolipoprotein E genotype*

	APOE2E2	APOE2E3	APOE2E4	APOE3E3	APOE3E4	APOE4E4
Overall						
Events/person-years	0/146	7/2176	3/452	51/9229	22/3659	4/395
RR (95% CI)	-	0.7 (0.3 to 1.5)	1.3 (0.4 to 4.1)	1 (reference)	1.0 (0.6 to 1.7)	2.1 (0.8 to 6.0)
Men						
Events/person-years	0/64	4/720	2/197	29/3832	12/1540	3/175
RR (95% CI)	-	0.8 (0.3 to 2.2)	1.4 (0.3 to 5.9)	1 (reference)	0.8 (0.4 to 1.7)	2.4 (0.7 to 8.0)
Women						
Events/person-years	0/82	3/1456	1/256	22/5397	10/2119	1/220
RR (95% CI)	-	0.6 (0.2 to 2.1)	1.2 (0.2 to 9.0)	1 (reference)	1.2 (0.6 to 2.7)	1.5 (0.2 to 11.2)
Age tertile 1 (< 65 yrs)						
Events/person-years	0/46	0/777	1/165	11/3470	5/1299	3/196
RR (95% CI)	-	-	2.2 (0.3 to 18.4)	1 (reference)	1.0 (0.3 to 3.0)	4.0 (1.1 to 14.3)
Age tertile 2 (65-73 yrs)						
Events/person-years	0/39	6/743	0/153	16/2919	9/1175	0/100
RR (95% CI)	-	1.7 (0.7 to 4.4)	-	1 (reference)	1.3 (0.6 to 3.2)	-
Age tertile 3 (> 73 yrs)						
Events/person-years	0/61	1/656	2/135	24/2840	8/1185	1/100
RR (95% CI)	-	0.2 (0.0 to 1.6)	2.1 (0.5 to 9.0)	1 (reference)	0.7 (0.3 to 1.8)	1.4 (0.2 to 10.7)

* Relative risk (RR) with 95% confidence interval (CI), adjusted for age and sex.

cardial infarction (n=23; excluding n=64 non-fatal myocardial infarctions), we obtained rather similar findings. When subjects with missing baseline data on a previous myocardial infarction were included (n=345), our findings did not change either.

As shown in *table 3*, the APOE genotype was overall not strongly related to the development of stroke during follow-up. Although persons with the APOE4E4 genotype had a 1.4-fold increased risk, this did not reach statistical significance (95% CI 0.5 to 3.9). Also subjects with APOE2E3 had a non-significantly increased risk of stroke (RR 1.3; 95% CI 0.9 to 2.1), but this was not consistently increased over the age strata. The APOE3E4 genotype was associated with a 40% lower risk of stroke in men, and with a 30% increased risk in women, but again, not statistically significantly. We detected an increased risk of stroke in APOE2E2 carriers younger than 65 years, but numbers were small. No consistent differences were observed across age strata. Furthermore, similar findings were obtained when restrictions were made to fatal stroke or ischaemic stroke.

Table 4 shows the absolute and relative risks of dementia for the various APOE genotypes. Persons with the APOE4E4 genotype had a more than five-fold higher risk of dementia than homozygotes for APOE*3, while heterozygotes for APOE*4 were at an almost two-fold increased risk. The APOE2E3 genotype was associated with a decreased risk of dementia, but not statistically significant (0.7; 95% CI 0.4 to 1.3). The relative risk of dementia associated with the APOE genotype was not consistently different across strata of sex. Among men, homozygotes for APOE*4 had an almost three-fold increased risk to become demented; women with the APOE4E4 genotype had an six-fold increased the risk of dementia. By contrast, one APOE*4 allele was associated with a higher relative risk of dementia among men as compared women. No major differences were observed between age strata. Although the relative risk of dementia was highest in the APOE4E4 group, the population attributable risk was found to be higher in persons with the APOE3E4 genotype (1.35 per 1000 person-years), than in homozygotes for APOE*4 (0.50 per 1000 person-years). Thus, each year, an estimated 185 per 100000 individuals aged 55 or over, may become demented as a consequence of carrying the APOE3E4 or APOE4E4 genotype instead of APOE3E3.

Table 5 shows that overall, mortality was similar between the APOE genotypes (logrank=0.69; df=5; *P*=0.98). However, in the youngest age tertile, mortality was almost two-fold increased in persons with the APOE2E4 or APOE4E4 genotype relative to homozygotes for APOE*3.

To further explore the role of APOE in longevity, the *figure* shows the APOE allele frequencies in 5-years age strata of the study population. The APOE*4 allele frequency decreased from 0.166 in 55 year-olds to 0.118 in subjects aged 90 or over. The change in APOE*4 frequency was estimated to be -0.005 (95% CI -0.009 to

Table 3 – Risk of stroke associated with apolipoprotein E genotype*

	APOE2E2	APOE2E3	APOE2E4	APOE3E3	APOE3E4	APOE4E4
Overall						
Events/person-years	1/168	26/2569	2/513	87/11279	34/4569	4/467
RR (95% CI)	0.7 (0.1 to 5.0)	1.3 (0.9 to 2.1)	0.6 (0.1 to 2.4)	1 (reference)	1.0 (0.7 to 1.5)	1.4 (0.5 to 3.9)
Men						
Events/person-years	1/72	9/938	2/206	36/4862	9/2027	2/205
RR (95% CI)	1.3 (0.2 to 9.7)	1.2 (0.6 to 2.5)	1.2 (0.3 to 5.1)	1 (reference)	0.6 (0.3 to 1.2)	1.5 (0.4 to 6.4)
Women						
Events/person-years	0/96	17/1631	0/307	51/6417	25/2542	2/262
RR (95% CI)	-	1.5 (0.8 to 2.5)	-	1 (reference)	1.3 (0.8 to 2.1)	1.3 (0.3 to 5.3)
Age tertile 1 (< 65 yrs)						
Events/person-years	1/49	3/887	0/183	7/3912	2/1472	0/194
RR (95% CI)	12.9 (1.6 to 108)	2.0 (0.5 to 7.7)	-	1 (reference)	0.8 (0.2 to 3.7)	-
Age tertile 2 (65-73 yrs)						
Events/person-years	0/46	2/839	2/173	20/3501	7/1490	2/128
RR (95% CI)	-	0.4 (0.1 to 1.9)	2.1 (0.5 to 8.8)	1 (reference)	0.8 (0.4 to 2.0)	2.7 (0.6 to 11.7)
Age tertile 3 (> 73 yrs)						
Events/person-years	0/72	21/843	0/157	60/3866	25/1606	2/145
RR (95% CI)	-	1.6 (1.0 to 2.7)	-	1 (reference)	1.1 (0.7 to 1.7)	1.1 (0.3 to 4.6)

* Relative risk (RR) with 95% confidence interval (CI), adjusted for age and sex.

Table 4 – Risk of dementia associated with apolipoprotein E genotype*

	APOE2E2	APOE2E3	APOE2E4	APOE3E3	APOE3E4	APOE4E4
Overall						
Events/person-years	1/91	10/1458	4/277	70/6180	42/2450	7/224
RR (95% CI)	1.0 (0.1 to 6.9)	0.7 (0.4 to 1.3)	1.7 (0.6 to 4.8)	1 (reference)	1.9 (1.3 to 2.8)	5.4 (2.4 to 11.8)
Men						
Events/person-years	0/36	0/545	2/117	17/2696	14/1098	1/96
RR (95% CI)	-	-	3.0 (0.7 to 12.9)	1 (reference)	2.3 (1.1 to 4.8)	2.9 (0.4 to 22.2)
Women						
Events/person-years	1/56	10/913	2/160	53/3484	28/1352	6/128
RR (95% CI)	1.6 (0.2 to 11.7)	1.0 (0.5 to 1.9)	1.4 (0.3 to 5.7)	1 (reference)	1.8 (1.1 to 2.8)	6.3 (2.7 to 14.8)
Age tertile 1 and 2 (55-73 yrs)†						
Events/person-years	0/54	0/1030	1/204	9/4177	7/1693	2/179
RR (95% CI)	-	-	2.2 (0.3 to 17.3)	1 (reference)	1.9 (0.7 to 5.0)	5.1 (1.1 to 23.8)
Age tertile 3 (> 73 yrs)						
Events/person-years	1/38	10/428	3/73	61/2003	35/2760	5/45
RR (95% CI)	1.0 (0.1 to 7.5)	0.8 (0.4 to 1.6)	1.6 (0.5 to 5.1)	1 (reference)	1.9 (1.2 to 2.9)	5.2 (2.1 to 13.0)

* Relative risk (RR) with 95% confidence interval (CI), adjusted for age, sex, and education;

† The coefficients did not converge when in first tertile because of limited number of dementia patients (n=5).

Table 5 – Mortality as a function of the apolipoprotein E genotype*

	APOE2E2	APOE2E3	APOE2E4	APOE3E3	APOE3E4	APOE4E4
Overall						
Events/person-years	8/173	117/2819	27/566	522/12154	219/4964	20/512
RR (95% CI)	0.9 (0.3 to 2.4)	0.9 (0.7 to 1.2)	1.0 (0.6 to 1.7)	1 (reference)	1.1 (0.9 to 1.4)	0.9 (0.5 to 1.6)
Men						
Events/person-years	4/66	50/1046	14/242	237/5209	89/2190	10/223
RR (95% CI)	1.0 (0.4 to 2.8)	1.0 (0.8 to 1.4)	1.3 (0.7 to 2.2)	1 (reference)	0.9 (0.7 to 1.1)	1.1 (0.6 to 2.0)
Women						
Events/person-years	4/107	67/1773	13/323	285/6945	130/2774	10/290
RR (95% CI)	0.9 (0.3 to 2.4)	0.9 (0.7 to 1.2)	1.0 (0.6 to 1.7)	1 (reference)	1.1 (0.9 to 1.4)	0.9 (0.5 to 1.6)
Age tertile 1 (< 65 yrs)						
Events/person-years	0/50	12/1006	4/208	43/4266	16/1670	4/230
RR (95% CI)	-	1.2 (0.6 to 2.3)	2.0 (0.7 to 5.7)	1 (reference)	1.0 (0.5 to 1.7)	1.8 (0.6 to 5.0)
Age tertile 2 (65-73 yrs)						
Events/person-years	1/51	24/939	6/187	91/3762	38/1587	3/135
RR (95% CI)	0.8 (0.1 to 5.9)	1.0 (0.7 to 1.6)	1.3 (0.6 to 3.1)	1 (reference)	1.0 (0.7 to 1.4)	1.0 (0.3 to 3.2)
Age tertile 3 (> 73 yrs)						
Events/person-years	7/72	81/874	17/170	388/4126	165/1706	13/147
RR (95% CI)	0.9 (0.4 to 1.9)	1.0 (0.8 to 1.3)	1.0 (0.6 to 1.7)	1 (reference)	1.0 (0.8 to 1.2)	1.0 (0.5 to 1.7)

* Relative risk (RR) with 95% confidence interval (CI).

-0.001) for every five years. The decrease in the frequency of APOE*4 was to the benefit of the APOE*3 frequency (increase 0.013; 95% CI 0.003 to 0.022 per five years), as also the frequency of the APOE*2 allele slightly decreased with age (-0.007; 95% CI -0.015 to 0.000 per five years).

Discussion

Using data from a large prospective population-based study, we found that APOE*4 homozygotes had an increased risk of myocardial infarction, but that the incidence of stroke was not strongly related to the APOE polymorphism. Although APOE*4 carriers had an increased risk of dementia, the contribution of APOE*4 to the incidence of dementia was modest, reflecting the relatively low frequency of this allele. The APOE genotype appeared not to be a strong determinant of mortality up until 95 years of age.

In the interpretation of these findings, a major concern is that we could not determine the APOE genotype of the whole Rotterdam Study cohort. The group of subjects in whom APOE was measured, were on average younger (69.8 versus 72.2 years), included more males (44 versus 29%), and less persons who died during follow-up (17 versus 26%), than those with an undetermined APOE genotype. How-

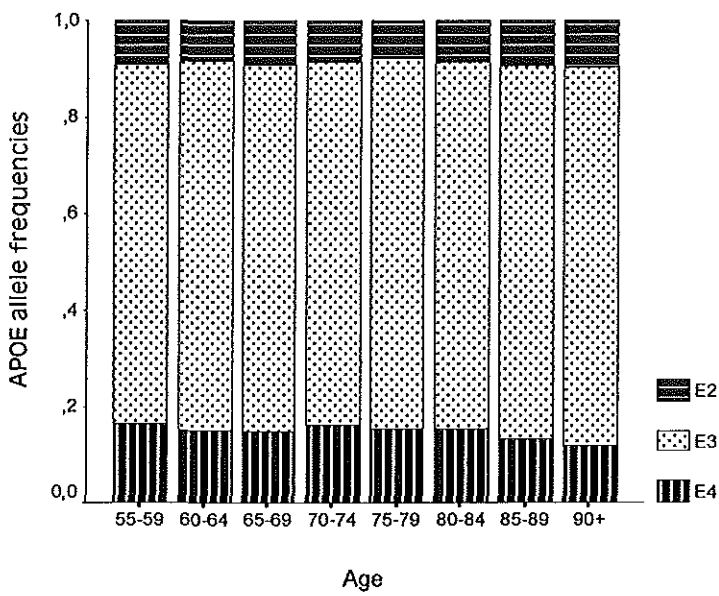

Figure – Apolipoprotein E allele frequencies as a function of age.

ever, the proportions of patients with a previous myocardial infarction, stroke or dementia were similar, and also the level of total and HDL cholesterol did not differ between those with and without a determined APOE genotype. Based on the above characteristics, persons with an unknown genotype do not specifically fit into one of the APOE genotype groups as observed in the present study. As the distribution of the APOE genotypes was in Hardy-Weinberg equilibrium, it seems further unlikely that selective APOE genotyping has occurred. Another issue is that there was complete follow-up on myocardial infarction or stroke in 91% of the participants in this study. However, the distribution of the APOE genotypes in persons with and without complete follow-up on vascular diseases was similar (X^2=3.9; df=5; P=0.57). Therefore, there seems not to be selective loss to follow-up. The determinant of this study, the APOE genotype, was accurately measured, without knowledge of the outcomes.[9] Based on these considerations, it seems unlikely that our study is subject to much measurement error or bias.

A possible limitation of the present study though, is that we could not explore the role of APOE in subtypes of myocardial infarction, stroke and dementia in detail. However, it should be noted that this study aimed to investigate the population impact of the APOE polymorphism. With regard to the implications for public health, associations with rarer subtypes of disease may be less relevant.

An advantage of this study is the population-based approach with a high response rate, which minimises the possibility of selection bias. There was complete follow-up with regard to survival. The occurrence of stroke and myocardial infarction during follow-up were ascertained using files of general practitioners, who cover the whole study population. As access to primary care is high in the Netherlands, also less severe events could be detected.

There is some controversy in the literature with regard to the association of the APOE genotype to myocardial infarction.[2-4] Although some studies reported an increased APOE*4 frequency in patients with coronary heart disease, it should be noted that the APOE*4 related summary odds ratio for coronary heart disease in a meta-analysis of cross-sectional studies did not exceed 1.3.[2] The two prospective population-based studies up until now are inconsistent.[3,4] One suggested that APOE*4 increases the risk of death from coronary heart disease,[3] while in the other survey, no association could be detected.[4] The number of patients in the present study exceeds the previous ones five times. We found that homozygotes for APOE*4 were at increased risk, in particular those younger than 65 years. This could imply that this genetic predisposition to myocardial infarction wanes with ageing. On the other hand, we cannot exclude that we missed some cases of 'silent' myocardial infarction, which becomes more and more frequent with aging.[16] Possible

underestimation of the number of myocardial infarctions during follow-up could further explain the relative low number of incident cases in our study (n=87).

The literature on APOE and stroke is rather inconsistent as well.[4-6] In cross-sectional studies, both an increased APOE*2 frequency,[24] as well as an increased APOE*4 frequency have been reported.[25] Of the three population-based cohort studies on APOE and stroke, two reported no association,[4,6] while a third suggested a decreased risk for APOE*2 carriers younger than 80 years.[5] It has been postulated that some of the inconsistencies in the literature on APOE and stroke may be explained by a modifying effect of age.[5] In our large study, with a wide age range, we did not find consistent evidence for this, although we cannot exclude that APOE may be associated with stroke before the age of 55 years. In our investigation, the APOE genotype was not strongly related to the development of stroke during follow-up.

This is the first study that quantified the absolute risk of dementia associated with the APOE polymorphism. Besides, we assessed its contribution to the incidence of dementia on a population level. Although carriers of the APOE*4 allele are obviously at increased risk of dementia, we found that the corresponding population attributable risk was modest, which results from the relative rarity of the allele. Although the APOE2E3 genotype was found to be inversely related, this effect was modest and did not reach statistical significance. Besides, any relative protective effect of APOE*2 on a population level would be meaningless, given the low APOE*2 allele frequency.

Previous studies on the APOE genotype and longevity suggested that APOE*2 increases, and that APOE*4 reduces life expectancy.[1,10,11] In these studies, the oldest elderly were compared to younger individuals. In the present study, we found till the age of 90, a weak decrease in the frequency of both APOE*2 and APOE*4. Although overall, APOE was found to be unrelated to survival, in the youngest age category, the APOE2E4 and APOE4E4 genotypes were associated with increased mortality. These observations are consistent with our observations on APOE and myocardial infarction and stroke, both major causes of death.

An ultimate goal for genetic studies is to provide information that will improve our ability to identify individuals at increased risk of disease. Although the APOE genotype is involved in the development of dementia, the population contribution of the risk allele, APOE*4, seems to be modest. Our study suggests that the APOE polymorphism does not play an important role in mortality or the risk of vascular diseases in the elderly.

References

1. Davignon J, Gregg RE, Sing F. Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis 1988;8:1-21.
2. Wilson PWF, Schaefer EJ, Larson MG, et al. Apolipoprotein E alleles and risk of coronary disease. A meta-analysis. Arterioscler Thromb Vasc Biol 1996;16:1250-1255.
3. Stengård JH, Zerba KE, Pekkanen J, et al. Apolipoprotein E polymorphism predicts death from coronary heart disease in a longitudinal study of elderly Finnish men. Circulation 1995; 91:265-269.
4. Kuusisto J, Mykkänen L, Kervinen K, et al. Apolipoprotein E4 phenotype is not an important risk factor for coronary heart disease or stroke in elderly subjects. Arterioscler Thromb Vasc Biol 1995;15:1280-1286.
5. Ferrucci L, Guralnik JM, Pahor M, et al. Apolipoprotein E ε2 allele and risk of stroke in the older population. Stroke 1997;28:2410-6.
6. Basun H, Corder EH, Guo Z, et al. Apolipoprotein E polymorphism and stroke ina population sample aged 75 years or more. Stroke 1996;27:1310-5.
7. Strittmatter WJ, Saunders AM, Schmechel D, et al. Apolipoprotein E: high-avidity binding to ß-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci USA 1993;90:1977-81.
8. Farrer LA, Cupples LA, Haines JL, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein R genotype and Alzheimer disease. A meta-analysis. JAMA 1997;278:1349-56.
9. Slooter AJC, Cruts M, Kalmijn S, et al. Risk estimates of dementia by apolipoprotein E genotypes from a population-based incidence study: the Rotterdam Study. Arch Neurol 1998;55:964-8.
10. Schächter F, Faure-Delanef L, Guénot F, et al. Genetic associations with human longevity at the APOE and ACE loci. Nature Genet 1994;6:29-32.
11. Galinsky D, Tysoe C, Brayne CE, et al. Analysis of the apo E/apo C-I, angiotensin converting enzyme and methylenetetrahydrofolate reductase genes as candidates affecting human longevity. Atherosclerosis 1997;129:177-83.
12. Hofman A, Grobbee DE, De Jong PTVM, et al. Determinants of disease and disability in the elderly: the Rotterdam elderly study. Eur J Epidemiol 1991;7:403-422.
13. Copeland JRM, Keleher MJ, Kellet JM, et al. A semi-structured clinical interview for the assessment of diagnosis and mental state in the elderly. The geriatric mental schedule. I. Development and reliability. Psychol Med 1976;6:439-49.
14. Folstein MS, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189-98.
15. Willems JL, Abreu LC, Arnaud P, et al. The diagnostic performance of computer programs for the interpretation of electrocardiograms. N Engl J Med 1991;325:1767-73.
16. De Bruyne MC, Mosterd A, Hoes AW, et al. Prevalence, determinants and misclassification of myocardial infarction in the elderly: the Rotterdam Study. Epidemiology 1997;8:495-500.
17. World Health Organization. International Statistical Classification of Diseases and Related Health Problems, 10th revision. Geneva, World Health Organization, 1992.
18. Ott A, Breteler MMB, Van Harskamp F, et al. Prevalence of Alzheimer's disease and vascular dementia: association with education. The Rotterdam Study. BMJ 1995;310:970-3.

19. Bots ML, Looman SJ, Koudstaal PJ, et al. Prevalence of stroke in the general population. The Rotterdam Study. Stroke 1996;27:1499-1501.
20. Ott A, Breteler MMB, Van Harskamp F, et al. The incidence and risk of dementia. The Rotterdam Study. Am J Epidemiol 1998;147:574-80.
21. American Psychiatric Association. Diagnostic and statistical manual of mental disorders, third edition, revised. Washington DC, American Psychiatric Association, 1987.
22. Van Gent CM, Vandervoort HA, De Bruyn AM, et al. Cholesterol determinations. A comparative study of methods with special reference to enzymatic procedures. Clin Chem Acta 1977;75:243-251.
23. Ott A. Van Rossum CTM, Van Harskamp F, et al. Education and the risk of dementia in a large population based study. The Rotterdam Study. Neurology. In press.
24. Couderc R, Mahieux F, Bailleul S, et al. Prevalence of apolipoprotein E phenotypes in ischemic cerebrovascular disease. A case-control study. Stroke 1993;24:661-4.
25. Pedro-Botet J, Sentí M, Nogués X, et al. Lipoprotein and apolipoprotein profile in men with ischemic stroke. Role of lipoprotein(a), triglyceride-rich lipoproteins, and apolipoprotein E polymorphism. Stroke 1992;23:1556-62.

8

GENERAL DISCUSSION

8

General discussion

In this general discussion, a brief overview will be given of the major findings together with observations from other studies. These findings will be discussed with special attention to the underlying mechanism. Finally, recommendations for future research will be given. The methodological aspects of the studies in this thesis are described in the discussion sections of the previous chapters, and will be briefly considered here together with our findings. As the primary topic of this thesis is dementia, this chapter will focus on the association between apolipoprotein E and dementia.

Background

It has been suggested that apolipoprotein E (APOE=gene; apoE=protein) is involved in atherosclerosis and dementia,[1,2] two conditions which severely disturb the quality of life of numerous elderly individuals. Atherosclerosis is an important cause of mortality,[3] while dementia is the major threat to the wellbeing of older people.[4] As the frequency of both atherosclerosis and dementia increases with advancing age, the two conditions are huge problems as the geriatric population is increasing at a considerable pace.[5,6] Atherosclerosis as well as dementia are heterogeneous disorders, with an incompletely understood aetiology.

Crucial in the development of atherosclerosis is the level of cholesterol in serum.[3,7] The APOE genotype is a determinant of serum cholesterol level, and has three common alleles, APOE*2, APOE*3 and APOE*4 which fully determine the respective apoE2, apoE3 and apoE4 isoforms.[1] Compared to homozygotes for APOE*3, the most common genotype, APOE*2 is associated with lower levels of

total and low density lipoprotein (LDL) cholesterol and with higher levels of high density lipoprotein (HDL) cholesterol, while APOE*4 has opposite effects.[1] Yet, findings on APOE and atherosclerosis have been inconsistent,[8,9] and it is not yet clear how APOE is related to mortality and vascular diseases, such as stroke and coronary heart disease.[10,11]

Besides its role in lipid metabolism, the APOE*4 allele is more prevalent in patients with Alzheimer's disease (AD) than in cognitively normal controls. Although this association is well established, the magnitude of the risk was not clear at the start of this study.[12-14] In addition, it remained to be established whether the APOE*2 allele exerts a protective effect, whether the APOE genotype is also involved in other dementing diseases than AD, and, related to the latter topic, whether APOE testing could be used in the differential diagnosis of dementia. Furthermore, it was unclear whether the level of apoE in serum contributes to the aetiology of AD, and whether APOE is related to AD progression and survival. Furthermore, the putative synergistic effects of APOE*4 and atherosclerosis deserved further investigation, as these are both common risk factors for dementia.[15] The aim of this thesis was to clarify the above issues.

Findings and methodological considerations

Apolipoprotein E in atherosclerosis and vascular disease

Some inconsistencies have emerged from studies on the APOE genotype and either atherosclerosis or vascular diseases.[8-11] It should be noted however that few population-based investigations have been performed on these topics. In the Rotterdam Study, APOE*4 was overall not related to atherosclerosis, although the APOE4E4 genotype was weakly associated with atherosclerosis in the abdominal aorta and peripheral arteries. The APOE*2 allele appeared to be inversely related to atherosclerosis (*chapter 5.2*). Adjusting for serum apoE levels strengthened the inverse association of APOE*2 and atherosclerosis, whereas adjusting for total and HDL cholesterol only slightly changed the estimates. These observations suggest firstly that serum apoE level is not an intermediate factor. Secondly, the main APOE*2 effects on atherosclerosis do not seem to result from its influence on lipid levels.

The association of APOE with myocardial infarction and stroke was studied in a follow-up setting. Persons with the APOE4E4 genotype had an increased risk of myocardial infarction, while subjects with APOE2E3 were at a slightly lower risk (*chapter 7*). Adjusting for total and HDL cholesterol did not change these findings, suggesting that the level of these lipids is not intermediate. The incidence of stroke was not strongly related to the APOE polymorphism. Although persons with the

APOE2E3 or APOE4E4 genotype had a slightly higher risk than homozygotes for APOE*3, this did not reach statistical significance. Mortality was overall not related to APOE, but increased in persons with either the APOE2E4 or the APOE4E4 genotype who were younger than 65 years.

Apolipoprotein E and dementia

Differential diagnosis As discussed in *chapter 3*, APOE typing for differential diagnostic purposes of dementia seems not to be valuable. However, both approaches used to explore this issue, i.e. a consideration based on the available literature (*chapter 3.1*), and an evaluation using data from a population-based study (*chapter 3.2*), have their limitations. The first method is subject to publication bias. It is questionable whether conclusions based on the second approach will apply to patients who have been referred to a clinic. Clinical patients differ in several characteristics, including the APOE*4 frequency, from cases derived from a population-based study.[16] The best way of evaluating the value of APOE testing in the differential diagnosis of dementia, is a study on patients who are referred because of a possible dementia syndrome, using autopsy confirmed diagnoses as a golden standard. An investigation like this has recently been published, and showed that APOE genotyping is indeed not informative when used as a single diagnostic test, but may improve specificity when used in conjunction with clinical characteristics.[17]

Risk of all dementia and Alzheimer's disease Estimates of the relative risk of AD associated with the APOE genotypes differ widely across studies.[18] The strongest effects of the APOE genotypes were reported in clinic and autopsy studies as compared to population-based studies (*table 1*).[19] In *chapter 2*, differences between clinic and population-based studies are discussed in more detail. *Table 1* compares findings from the Rotterdam Study with a meta-analysis of cross-sectional studies among Caucasians.[19] Most risk estimates from the Rotterdam Study were quite comparable with those derived from the meta-analysis. The exception is the APOE4E4 related relative risk of incident AD, which was much lower in the Rotterdam Study. Interestingly, the other two population-based incidence studies till so far, reported also a weak association between APOE*4 and dementia,[13,20] but confidence intervals were wide. Although the APOE4E4 related risk estimates of AD from population-based incidence studies are conceptually superior, precision is currently lacking.

Contradicting findings have been reported on the association between the APOE*2 allele and AD.[21-24] Long-term population-based follow-up studies will ultimately clarify whether, compared to the most common genotype APOE3E3, APOE*2 is protective or has similar effects. Taken into account the wide variety in observed effects, it is not likely that possible protective effects of APOE*2 will be impressive.

Table 1 – Odds ratio for Alzheimer's disease according to apolipoprotein E genotype in meta-analysis and Rotterdam Study*

	APOE2E2	APOE2E3	APOE2E4	APOE3E3	APOE3E4	APOE4E4
Meta-analysis[19]†						
Clinic/autopsy studies	0.6 (0.2 to 2.0)	0.6 (0.5 to 0.8)	2.6 (1.6 to 4.0)	1 (reference)	3.2 (2.8 to 3.8)	14.9 (10.8 to 20.6)
Population-based studies	0.9 (0.3 to 2.8)	0.6 (0.5 to 0.9)	1.2 (0.8 to 2.0)	1 (reference)	2.7 (2.2 to 3.2)	12.5 (8.8 to 17.7)
Rotterdam Study						
Prevalent cases	0.6 (0.1 to 6.3)	0.6 (0.3 to 1.2)	1.4 (0.3 to 6.6)	1 (reference)	1.5 (0.9 to 2.5)	13.2 (5.2 to 34.0)
Incident cases	-	0.5 (0.2 to 1.2)	1.1 (0.3 to 4.7)	1 (reference)	1.9 (1.2 to 3.0)	3.2 (1.0 to 10.3)

* Odds ratios with 95% confidence intervals;

† Based on cross-sectional studies among Caucasians (total sample size: n=5107 cases and n=6262 controls).

Non-Alzheimer's disease dementias While there is only one study that did not report an increased risk for APOE*4 carriers on sporadic AD,[25] much more controversy exists in the literature on APOE and the non-AD dementias, including vascular dementia, Lewy body disease, Parkinson's disease dementia, frontal lobe dementia and Creutzfeldt-Jakob disease.[18] We found that APOE*4 was also related to dementia with stroke, which included patients with either vascular dementia or AD with cerebrovascular disease (*chapter 4.1*). The lack of an observed association between the APOE*4 allele and the non-AD dementias in some autopsy studies, may be due to selection bias. As APOE*4 appears to be related to various neuropathological characteristics of AD,[26] diagnosing the non-AD dementias only in the absence of any evidence for AD, implies that APOE*4 carriers are selectively excluded.

Serum apoE level We found that serum apoE levels were lower in AD compared to non-demented controls (*chapter 4.2*). When we adjusted for the APOE genotype, the difference in serum apoE levels diminished. Therefore, it is unlikely that serum apoE level is related to AD, independently of the APOE genotype.

Prognosis of Alzheimer's disease Previous studies on APOE and the course of AD are based on patients who were included at different stages of disease. This hampers the comparison of the rate of decline, and could explain the inconsistencies in the literature. We included patients at a similar stage, i.e. before the onset of symptoms, and found that the APOE*4 allele was not a strong determinant of survival in AD (*chapter 4.3*). As change in cognitive function and severity of dementia were similar for AD patients with and without APOE*4, our study suggests that the progression of AD is not related to the APOE*4 allele.

Apolipoprotein E, atherosclerosis and dementia

When studying the association of APOE, atherosclerosis and cognitive function, APOE*4 carriers with atherosclerosis appeared to have the worst cognitive performance (*chapter 6.1*). Mild, non-significant effects on cognitive function were observed for atherosclerosis in the absence of APOE*4 and for APOE*4 in the absence of atherosclerosis.

In addition, the effects of both APOE*4 and atherosclerosis on dementia were found to be stronger than the sum of the effects of APOE*4 alone and atherosclerosis alone (*chapter 6.2*). This again suggests a synergistic interaction.[27]

As the APOE genotype was shown to be associated with atherosclerosis (*chapter 5.2*), and atherosclerosis was found to be related to dementia, we studied whether atherosclerosis could be intermediate in the association of APOE and dementia (*chapter 6.2*). The association between the APOE genotypes and dementia did not

essentially change when adjustments were made for atherosclerosis or other vascular factors. This suggests that atherosclerosis is not an intermediate factor.

For the majority of the population, namely those not carrying the APOE*4 allele, smoking increased the risk of dementia and AD (*chapter 6.3*). For subjects with the APOE*4 allele however, smoking was inversely associated with dementia, although this did not reach statistical significance.

Mechanisms

The reader is referred to *chapter 5.1* for a discussion of APOE in lipid metabolism and atherosclerosis. This section will focus on the involvement of APOE in the aetiology of the dementias. First, a review will be given on the role of APOE in AD, the most frequent type of dementia. This is followed by a discussion on the role of APOE in the response to damage to the brain, which is not necessarily the neurodegeneration as observed in AD.

Apolipoprotein E and Alzheimer's disease

This section starts with a discussion on the possible role of cerebral apoE level in the pathogenesis of AD. Afterwards, an overview is given of the role of the various apoE isoforms in the metabolism of the various components of the AD pathology.

ApoE level in the AD brain A small study reported that the APOE*4 allele was associated with lower levels of apoE in the hippocampus and cortex of AD patients, and the authors proposed that the harmful effects of APOE*4 are due to reduced levels of available apoE4 protein.[28] However, there is little support for this hypothesis. Several studies reported that apoE level in cerebrospinal fluid was unrelated to the APOE genotype.[29,30] By contrast, APOE*4 allele expression was found to be increased in the Alzheimer brain,[31] although it remains to be determined whether this is cause or consequence. Furthermore, a recently identified polymorphism may be more common in AD, and was suggested to increase APOE gene expression.[32] Although these observations could not be confirmed in a population-based study on early onset AD.[33]

This section will further discuss the role of the different apoE isoforms in the AD pathology. Important neuropathological characteristics of AD are extracellular neuritic plaques, intracellular neurofibrillary tangles and neuronal loss. The different apoE isoforms may be involved in all features, through various pathways.

Neuritic plaques The major component of the neuritic plaques is ß-amyloid. It has been shown that in the AD brain, the number of plaques and the burden of ß-amyloid is related to the APOE genotype.[26,34] APOE*4 carriers were found to have

an increased density of ß-amyloid,[26,34] while APOE*2 carriers had less ß-amyloid depositions.[26] It has been suggested that the differences in the deposition of ß-amyloid may result from isoform specific variation in the binding to ß-amyloid, but studies on this topic have not been consistent.[35-37] The apoE isoforms were further reported to have a specific effect on the formation, aggregability,[38] or clearance,[39] of ß-amyloid *in vitro*. Support for a role of apoE in the deposition of ß-amyloid came from a study on transgenic mice. Among mice that over-express human Amyloid Precursor Protein (APP), those with a deficiency for apoE had a sharp reduction in ß-amyloid deposition as compared to mice with native apoE.[40] These mice studies suggest thus that apoE is somehow involved in the deposition of ß-amyloid, but leave unanswered the specific consequences of the various APOE alleles. A weakness in the hypothesis that the APOE genotype is involved in AD because of its role in ß-amyloid metabolism, is that it is still controversial whether ß-amyloid deposition is directly involved in AD, or whether it is just a secondary consequence.[41] An argument in favour of this so called amyloid hypothesis is that several APP mutations are known that invariably lead to ß-amyloid deposition and to AD.[18] However, in the vast majority of AD cases, without abnormalities in the APP gene, the link between the presence of ß-amyloid in the brain and measures of dementia is tenuous, as several studies failed to find an association between the ß-amyloid burden and disease severity.[41,42]

Neurofibrillary tangles The number of neurofibrillary tangles correlates more closely with the severity of AD than the number of senile plaques.[42] The neurofibrillary tangles that occur in the cytoplasm of neurons in the AD brain contain hyperphosphorylated tau protein. Tau normally stabilises the skeleton of the cell, and this effect is though to be dependent on its phosphorylation.[43] The apoE protein was observed to be localised to neurofibrillary tangles. The binding of apoE to tau appears to differ between the isoforms. The apoE4 isoform may bind less strongly than apoE3, which was speculated to result in the development of neurofibrillary tangles.[44] In the absence of apoE3, tau binding sites are free and may pair with that of other tau proteins, thus forming neurofibrillary tangles. It should be noted that relatively high concentrations of both tau and apoE were used in these *in vitro* experiments.[44] However, support for the hypothesis that apoE is related to AD because of its role in tau metabolism was obtained by an *in vivo* study, which reported that tau was hyperphosphorylated in mice deficient for apoE.[45] Again, findings on mice carrying the various human APOE alleles remain to be reported.

Neuronal loss The number of neurons decreases with increasing severity of AD.[42] The cholinergic neurotransmitter system may be specifically impaired in AD, and attenuation of choline acetyltransferase (ChAT) activity is a consistent neurochemical

marker. The APOE genotype may have direct impact on cholinergic function, as knockout mice for apoE have shown synaptic damage and low ChAT activity.[46] Whether neurotransmitter impairments in apoE deficient mice are limited to the cholinergic system remains to be reported. By contrast, studies on APOE and ChAT activity in the AD brain have been inconsistent.[47-49] The loss of neurons in the AD brain was found to be unrelated to the APOE genotype.[42]

Apolipoprotein E in the response to cerebral injury

As described above, there are several possibilities for the involvement of the APOE genotype in the pathogenesis of AD. Besides, APOE may be related to AD because of its role in the response to cerebral injury,[50] for instance due to atherosclerosis. The latter option is compatible with several studies in this thesis.

Firstly, a synergistic effect was observed between APOE*4 and atherosclerosis on the risk of dementia and cognitive impairment (*chapters 6.1 and 6.2*). Secondly, the APOE polymorphism was found to be related to dementias of other aetiology than AD (*chapters 3.2, 4.1 and 4.2*). Dementia is a very heterogeneous condition which results from sufficient damage to the brain.[4] In persons with an impaired capability to recover from cerebral injury (supposed to be carriers of the APOE*4 allele), cerebral damage of whatever nature may lead to dementia, while others may have a higher chance to recover without loss of cognitive function. A schematic representation of the above findings is shown in *figure 1*.

These observations are thus compatible with the view that the consequence of atherosclerotic damage to the brain is more severe in presence of APOE*4. Besides, APOE*4 may interact with other types of damage including neurodegeneration, which would explain an association of APOE*4 with dementia in the absence of atherosclerosis. This section will further focus on the hypothesis of an impaired response to cerebral injury in APOE*4 carriers.

Other epidemiological studies A wide variety of studies have been performed on the outcome after cerebral damage as a function of the APOE genotype (*table 2*). Most studies suggest a synergistic effect between the APOE*4 allele and injury,

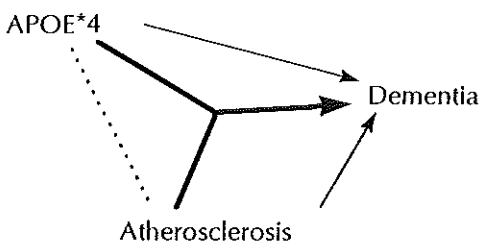

Figure 1 – Schematic representation of the interrelationship of APOE*4, atherosclerosis and dementia.
The thickness of the arrows indicates the strength of the association, a dotted line means no relationship.

which is compatible with an impaired recovery in APOE*4 carriers. However, two population-based studies could not confirm the observation that APOE*4 carriers are prone to dementia after head injury.[62,63] For the insulin resistance syndrome and smoking, only persons without the APOE*4 allele had an increased risk of AD.[64,65] There are at least two explanations for these inconsistencies. Firstly, it may suggest another, stronger interaction with the APOE genotype in the opposite direction, as discussed in *chapter 6.3* with regard to smoking. Secondly, these inconsistencies can be considered as arguments against the hypothesis that APOE*4 is related to the dementias because of a poor response to cerebral injury.

Neurobiological studies Cerebral injury was found to increase the production of apoE by astrocytes.[50] A brief episode of global ischaemia increased APOE gene transcription,[66] and led to an accumulation of apoE close to the microvessels, possibly due to alterations of the blood brain barrier.[67,68] Strong apoE immunoreactivity was also observed in neurons exhibiting signs of ischaemic cell damage.[68]

ApoE deficient mice exhibited impaired ability to recover as compared to wild-type mice after focal ischaemia or closed head injury.[69,70] Treating apoE knockout

Table 2 – Epidemiological studies on apolipoprotein E4 and outcome after brain injury

First author	Type of damage	Outcome
Synergistic effect		
Alberts[51]	intracerebral haemorrhage	mortality, functional outcome
Hofman[15]	atherosclerosis	dementia
Slooter[52]	atherosclerosis	cognitive decline
Kalmijn[53]	cerebrovascular disease	cognitive decline
Skoog[54]	white matter lesions	dementia
Mayeux[55]	head injury	Alzheimer's disease
Taesdale[56]	head injury	mortality, functional outcome
Jordan[57]	boxing	chronic neurologic deficits
Nicoll[58]	fatal head injury	deposition of ß-amyloid
Sorbi[59]	post-traumatic coma	recovery of consciousness
Tardiff[60]	cardiac bypass surgery	cognitive decline
Itzhaki[61]	herpes simplex-1 in brain	Alzheimer's disease
No synergistic effect		
Mehta[62]	head injury	dementia
O'Meara[63]	head injury	Alzheimer's disease
Ott[64]	smoking	dementia
Kuusisto[65]	insulin resistance syndrome	Alzheimer's disease

mice with intraventricular injections of either apoE3 or apoE4, improved learning capacity and restored neuronal degeneration.[71] The latter study suggest that the effects of apoE4 are not harmful in itself, but less beneficial than the effects of apoE3.

Transgenic mice expressing human apoE4 have larger infarct volumes following focal transient ischaemia compared to animals expressing human apoE3.[72] ApoE seems thus to be involved in neuronal repair following injury. There are various possibilities with regard to the underlying mechanism, which are not necessarily exclusive.

– *Mechanism: 1. neurotrophic effects*

Injured neurons and glia need large amounts of cholesterol and other lipids for plasma membrane biosynthesis. The supply of these hydrophobic molecules is made possible by apolipoproteins. ApoE has a special relevance for the central nervous system, as it is the predominant apolipoprotein in the brain, and other major plasma apolipoproteins such as apoA1 and apoB are absent.[73] Cells with excess lipids may release these compounds to apoE-lipid-complexes, which can be transported to cells requiring them for cell proliferation or repair. After injury, apoE expression by astrocytes increases, followed by an increase in LDL receptor activity in neurons which undergo dendritic proliferation and synaptogenesis.[50] After binding, the apoE-cholesterol-LDL receptor complex is believed to be internalised and degraded and the cholesterol is used for membrane and synapse formation.[50] The binding of apoE to the LDL receptor appeared to be isoform-specific.[74] Based on the poor recovery from brain injury as observed in APOE*4 carriers, it would be expected that binding of the apoE4 isoform to LDL receptors would be lowest. In contrast, binding of apoE2 containing lipoproteins appeared to be lowest in both rat astrocytes and neurons.[74]

In neuron culture experiments, apoE together with ß-VLDL could stimulate neurite extension and branching. These effects appeared to differ between the apoE isoforms. Addition of apoE3 led to extensive neurite outgrowth and elongation, while apoE4 was associated with short, stunted neurite extensions.[75] The effects of the apoE2 isoform remain to be reported.

Growth, adhesion and extension of neurites require interactions between the neuron and the extracellular substrate. ApoE binds to laminin, a component of the extracellular matrix, and this complex enhanced adhesion and branching of rat neurons, compared to laminin alone.[76] However, these effects were similar for apoE3 and apoE4.[76] Furthermore, there is evidence that apoE interacts with growth factors to support neuronal outgrowth and viability. The survival promoting effects of ciliary neurotrophic factor on hippocampal neurons was found to be potentiated by apoE, but apoE3 was as effective as the apoE4 isoform.[77]

– *Mechanism: 2. antioxidant properties*

Another way by which apoE may be involved in the repair after cerebral damage is by protecting against oxidative stress. Oxidative insults are believed to be involved in various neurodegenerative diseases.[78] The cytotoxic effects of ß-amyloid on neuronal cells could be blocked by physiological levels of apoE.[79] The antioxidant activity of apoE appeared to be isoform specific, with apoE2 being most effective, apoE3 moderately effective, and apoE4 least effective.[79] *In vivo* studies further showed a role for apoE in oxidative stress. Mice deficient for apoE express higher titres of antibodies against oxidised lipids as compared to control mice.[80] In addition, these knockouts have increased levels of 3-nitrotyrosine in their brain, a marker of oxidative stress.[81]

– *Mechanism: 3. role in inflammation*

A third possibility by which apoE may exert its beneficial effects is by modulating the glial response to inflammation. There is growing evidence that inflammatory processes are involved in the pathogenesis of the dementias. Various features of inflammation have been observed in the AD brain, including microglial activation, cytokine production, and complement activation.[82] Secondly, the use of non-steroidal anti-inflammatory agents appeared to be inversely associated with AD.[83] Furthermore, brain ischaemia, the principal cause of vascular dementia, is followed by an inflammatory response.[84]

ApoE is known to have immunomodulatory functions *in vitro*. These include the suppression of lymphocyte proliferation and synthesis of immunoglobulins,[73] as well as inhibition of TNF-α secretion.[85] Moreover, apoE3, but not apoE4, could block the activation of microglia, the phagocytes of the brain, due to treatment with secreted APP.[86] An *in vivo* study further suggested an immunomodulatory function of apoE, as mice deficient for apoE were found to have discrete abnormalities of cellular and humoral immune responses.[72] Therefore, apoE seems to be involved in the regulation of the inflammatory response, which may play a role in protecting neurons from secondary injury.

Discussion In conclusion, there are various possible mechanisms for a role of apoE in the response to cerebral injury, which are schematically represented in *figure 2*. Although these hypotheses are not necessarily exclusive, the hypothesis on neurotrophic effects is best supported. However, weaknesses in the view that apoE exerts its effects by transporting lipids to regenerating neurons, are the receptor binding experiments. *In vitro* studies on the antioxidant properties of the apoE isoforms are promising, but await experiments on mice carrying the various human APOE alleles. The role of apoE in inflammation has not yet been studied in detail for the various specific isoforms. With the exception of the *in vitro* experiments on apoE in oxidative

stress,[79] we are not aware of other reports that could explain the inverse association of APOE*2 with dementia.

Suggestions for future research

Apolipoprotein E in atherosclerosis and vascular disease

Given the varying results across studies, more research is needed to further explore whether the APOE genotype is related to atherosclerosis and vascular disease. Future investigations should ideally have a follow-up design in order to circumvent selective survival. Such studies, in which APOE genotyping is performed on material collected relatively early in life, could characterise participants who died before assessment of atherosclerosis.

If the APOE is truly related to atherosclerosis and vascular disease, independent of lipid levels, further studies should focus on other effects of the apoE isoforms than its role in lipid metabolism. These include possible anti-oxidative properties,[79,80] the involvement of apoE in inflammatory processes,[72,73] and the role of apoE in vessel wall homeostasis, such as the modulation of platelets aggregability and the proliferation and migration of smooth muscle cells and lymphocytes.[73,87]

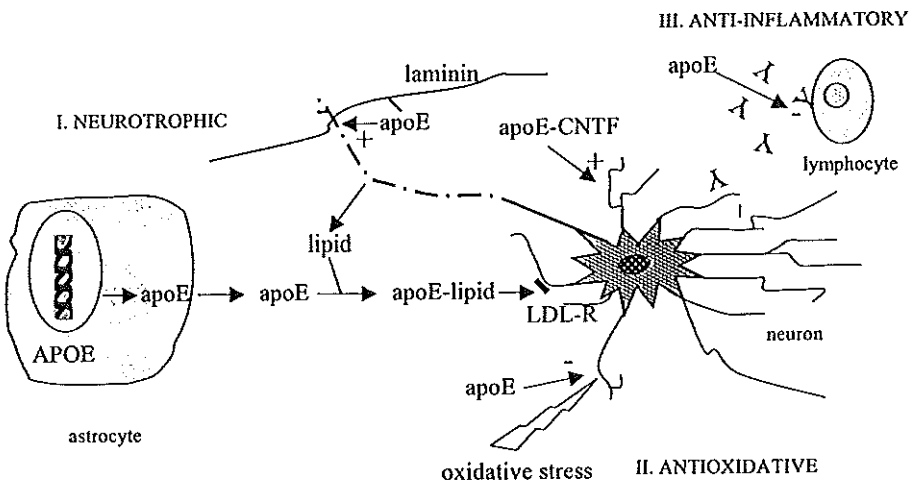

Figure 2 – Possible mechanisms for the role of apoE in the response to cerebral injury. APOE = apolipoprotein E gene; apoE = apolipoprotein E protein; CNTF = ciliary neurotrophic factor; LDL-R = low density lipoprotein receptor. A minus sign (–) indicates inhibition; a plus sign (+) indicates stimulation.

Apolipoprotein E genotype and Alzheimer's disease

The best way to further characterise the association of the APOE genotypes with AD is to use a population-based study, in order to avoid referral bias. Problems associated with selective survival can only be overcome in a follow-up setting. However, in these surveys it has been difficult to implement neuropathologic confirmation of the diagnosis. An estimated 10 percent of clinically diagnosed AD patients appear to have another dementing illness at autopsy.[88] This may result in non-differential misclassification, reducing statistical power. Nevertheless the problem of misclassification in population-based studies is outweighed by the opportunity to study gene-environment interactions, using prospectively collected data on exposure. More reliable risk estimates await longer follow-up. Pooling of various population-based cohort studies will increase the number of person-years observation. Although this obviously increases efficiency, pooling assumes that there are no important modifiers that differ between the pooled cohorts. Further, problems related to admixture could be encountered.[89] This means that the association under study may be confounded by differences in the frequency of both determinant and outcome between the pooled populations.

Apolipoprotein E genotype and non-Alzheimer's disease dementias

Studies on APOE and the non-AD dementias may be difficult as the distinction between different types of dementia can be a matter of interpretation, and inclusion of AD patients may happen. In particular the distinction between AD and vascular dementia may not be very clear.[90] Therefore, it is important to study all dementia as well as AD and vascular dementia separately. Besides, the frequency of some dementing illnesses is relatively low, and a more efficient approach than a population-based setting may be used. An alternative design, suitable for studies on Lewy body disease, frontal lobe dementia and Creutzfeldt-Jakob disease, is a case-referent study. The essence is that referent subjects are sampled from the same study base from which the cases emerge, and that sampling is independent of exposure status.[91] However, a case-referent design may hamper the study of characteristics before assignment as case or referent subject.

Apolipoprotein E gene expression and protein level

Investigations on cerebral apoE levels should be extended, as existing studies are limited, and included only few brains.[28] To detect genetic variability in APOE expression, sequencing of the possible regulatory elements of the APOE gene, should be followed by studies on transcription activity, and *in vitro* binding experiments to nuclear extracts. Mutations which seem to be functionally relevant, can be further in-

vestigated in association studies. The contribution of newly identified genes to the occurrence of disease should be disclosed in prospective, population-based studies with, ideally, autopsy confirmation of the diagnoses.

Apolipoprotein E genotype, atherosclerosis and dementia

Epidemiological studies The observed interaction between the APOE*4 allele and atherosclerosis on cognitive function (*chapter 6.1*) and dementia (*chapter 6.2*) should be confirmed in other population-based studies with long duration of follow-up. A matter of concern in these studies is the assessment of atherosclerosis. The presence of atherosclerosis in the brain is, during life, difficult to investigate directly. Related to cerebral arteriosclerosis, hypoperfusion, and ischaemia are cerebral white matter lesions,[92,93] which can be observed with magnetic resonance imaging (MRI). It should be further examined whether the cognitive consequences of these white matter lesions are indeed more pronounced in carriers of the APOE*4 allele, compared to persons with other APOE genotypes, as has been observed recently.[54] Neuro-imaging studies, ideally implemented in a population-based setting,[54,94] will make an important contribution to this field. In addition, the same type of study may reveal whether damaging of the white matter by atherosclerosis is more pronounced in carriers of the APOE*4 allele, which would further support the hypothesis of an impaired recovery from cerebral injury in APOE*4 carriers. Other indicators of atherosclerosis, vascular pathology or hypoperfusion may be used to unravel the exact nature of the association between the APOE genotype, atherosclerosis and dementia. These include cerebral hypoperfusion, as estimated by single photon emission computed tomography (SPECT), blood pressure, haemostatic factors and lipid levels.

Follow-up studies are needed to determine whether the putative synergistic effects of APOE*4 and atherosclerosis can be extended to other functions than cognition. In particular, it remains to be disclosed whether the functional outcome after stroke, usually a reflection of atherosclerosis, is dependent upon the APOE genotype. The possible role of APOE in the response to injury should further be explored in relation to the outcome after other types of cerebral damage, including trauma, radiation, and encephalitis.

Neuropathological studies In addition, the association between APOE, atherosclerosis and dementia should be further explored in neuropathological studies. Autopsies are usually performed on selected patients. Restriction to cases who died of external causes may overcome possible selection bias, but numbers will probably be small.

The observation that the AD pathology is more often related to clinical dementia in the presence of cerebral infarcts,[90] is very interesting and should be confirmed. Such an autopsy study may further reveal whether the degree of atherosclerotic

changes of the cerebral arteries is associated with dementia and whether there is any modification by the APOE genotype.

Experiments on genetically modified animals The introduction of the various human APOE genotypes into mice as well as the generation of APOE-knockout mice, has already proven to be useful in the study of the consequences of ischaemia and other types of cerebral injury. As this is a rapidly evolving area, it is to be expected that multiple genes will be incorporated in order to resemble closely the disease process (*figure 3*). In the future, transgenic animals may provide a substrate with which to test pharmaceutical compounds that may mimic apoE2 or apoE3 protection or protect against the relatively harmful effect of apoE4.

Apolipoprotein E genotype, smoking and dementia

Further population-based cohort studies are needed to reveal whether the consequences of smoking on the risk of dementia, truly differ between carriers and non-carriers of the APOE*4 allele. It should be further investigated whether smoking might have neuroprotective properties in some individuals,[95-97] and whether selective impairment of cholinergic neurotransmission occurs in association with APOE*4.[47,48] Positron emission tomography may be used for these purposes when more specific radioligands have been generated.

Apolipoprotein E in therapeutic trials on dementia

In future trials of drugs generated to alter the development or progression of AD, the APOE genotypes should be considered in the evaluation of the observed effects, as APOE seems to be involved in many neurochemical processes. In randomised clinical trials, randomisation may be done after APOE genotyping in order to yield symmetrical distributions of the APOE genotypes across the different arms of the trial. Further, in the analysis of a randomised clinical trial, analyses in strata of the APOE genotypes, may reveal whether any observed effect is modified by the APOE polymorphism.

Furthermore, the observation that intraventricular infusion of apoE improved learning capacity and neuronal structure in apoE deficient mice,[71] suggests that apoE may have therapeutic potential. Further studies are necessary to develop agents with similar effects as apoE, which can pass the blood-brain-barrier. Given the developments in genetic testing, eventually, neuroprotective treatment may be administered to brain injured persons with a genetic predisposition for poor outcome.

Figure 3 Reproduced with permission of Kamagurka.

References

1. Davignon J, Gregg RE, Sing F. Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis 1988;8:1-21.
2. Strittmatter WJ, Saunders AM, Schmechel D, et al. Apolipoprotein E: high-avidity binding to ß-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci USA 1993;90:1977-81.
3. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993;362: 801-9.
4. Cummings JL. Dementia: the failing brain. Lancet 1995;345:1481-9.
5. Van Duijn CM. Epidemiology of the dementias: recent developments and new approaches. J Neurol Neurosurg Psychiatry 1996;60:478-488.
6. Hart- en vaatziekten in Nederland. Cijfers over ziekte en sterfte. Nederlandse Hartstichting. Den Haag, 1997.
7. Pooling Project Research Group. Relationship of blood pressure, serum cholesterol, smoking habit, relative weight and ECG abnormalities to incidence of major coronary events: final report of the pooling project. J Chron Dis 1978;31:201-306.
8. Hixson JE and the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. Apolipoprotein E polymorphism affect atherosclerosis in young males. Arterioscler Thromb 1991;11:1237-44.
9. De Andrade M, Thandi I, Brown S, et al. Relationship of the apolipoprotein E polymorphism with carotid artery atherosclerosis. Am J Hum Genet 1995;56:1379-90.
10. Stengård JH, Zerba KE, Pekkanen J, et al. Apolipoprotein E polymorphism predicts death from coronary heart disease in a longitudinal study of elderly Finnish men. Circulation 1995; 91:265-9.
11. Kuusisto J, Mykkänen L, Kervinen K, et al. Apolipoprotein E4 phenotype is not an important risk factor for coronary heart disease or stroke in elderly subjects. Arterioscler Thromb Vasc Biol 1995;15:1280-6.
12. Corder EH, Saunders AM, Strittmatter WJ, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 1993;261:921-3.
13. Henderson AS, Easteal S, Jorm AF, et al. Apolipoprotein E allele ε4, dementia, and cognitive decline in a population sample. Lancet 1995;346:1387-90.
14. Myers RH, Schaefer EJ, Wilson PWF, et al. Apolipoprotein E ε4 association with dementia in a population-based study: the Framingham study. Neurology 1996;46:673-7.
15. Hofman A, Ott A, Breteler MMB, et al. Atherosclerosis, apolipoprotein E, and the prevalence of dementia and Alzheimer's disease in the Rotterdam Study. Lancet 1997;349:151-4.
16. Tsuang D, Kukull W, Sheppard L, et al. Impact of sample selection on APOE ε4 allele frequency: a comparison of two Alzheimer's disease samples. J Am Geriatr Soc 1996;44:704-7.
17. Mayeux R, Saunders AM, Shea S, et al. Utility of the apolipoprotein E genotype in the diagnosis of Alzheimer's disease. N Engl J Med 1998;338:506-11.
18. Slooter AJC, Van Duijn CM. Genetic epidemiology of Alzheimer's disease. Epidemiol Rev 1997;19:107-19.
19. Farrer LA, Cupples LA, Haines JL, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein R genotype and Alzheimer disease. A meta-analysis. JAMA 1997; 278:1349-56.

20. Evans DA, Beckett LA, Field TS, et al. Apolipoprotein E ε4 and incidence of Alzheimer disease in a community population of older persons. JAMA 1997;277:822-4.
21. Slooter AJC, Cruts M, Kalmijn S, et al. Risk estimates of dementia by apolipoprotein E genotypes from a population-based incidence study: the Rotterdam Study. Arch Neurol 1998;55:964-8.
22. Corder EH, Saunders AM, Risch NJ, et al. Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. Nat Genet 1994;7:180-4.
23. Van Duijn CM, De Knijff P, Wehnert A, et al. The apolipoprotein E ε2 allele is associated with an increased risk of early-onset Alzheimer's disease and a reduced survival. Ann Neurol 1995; 37:605-10.
24. Sorbi S, Nacmias B, Forleo P, et al. ApoE allele frequencies in Italian sporadic and familial Alzheimer's disease. Neurosci Lett 1994;177:100-2.
25. Lannfelt L, Lilius L, Nastase M, et al. Lack of association between apolipoprotein E allele ε4 and sporadic Alzheimer's disease. Neurosci Lett 1994;169:175-8.
26. Polvikoski T, Sulkava R, Haltia M, et al. Apolipoprotein E, dementia, and cortical deposition of ß-amyloid protein. N Engl J Med 1995;333:1242-7.
27. Rothman KJ. Modern epidemiology. Boston, Litle, Brown, 1986.
28. Bertrand P, Poirier J, Oda T, et al. Association of apolipoprotein E genotype with brain levels of apolipoprotein E and apolipoprotein J (clusterin) in Alzheimer disease. Mol Brain Res 1995;33:174-8.
29. Landén M, Hesse C, Fredman P, et al. Apolipoprotein E in cerebrospinal fluid from patients with Alzheimer's disease and other forms of dementia is reduced but without any correlation to the apoE4 isoform. Dementia 1996;7:273-8.
30. Lehtimäki T, Pirttilä, Mehta PD, et al. Apolipoprotein E (apoE) polymorphism and its influence on apoE concentrations in the cerebrospinal fluid in Finnish patients with Alzheimer's disease. Hum Genet 1995;95:39-42.
31. Lambert J-C, Pérez-Tur J, Dupire M-J, et al. Distortion of allelic expression of apolipoprotein E in Alzheimer's disease. Hum Mol Genet 1997;6:2151-4.
32. Bullido MJ, Artiga MJ, Recuero M, et al. A polymorphism in the regulatory region of APOE associated with risk for Alzheimer's dementia. Nature Genet 1998;18:69-71.
33. Roks GW, Cruts M, Bullido MJ, et al. The 491-A/T polymorphism in the regulatory region of APOE and early-onset Alzheimer's disease. Submitted.
34. Gomez-Isla T, West HL, Rebeck GW, et al. Clinical and pathological correlates of apolipoprotein E ε4 in Alzheimer's disease. Ann Neurol 1996;39:62-70.
35. Strittmatter WJ, Weisgraber KH, Huang DY, et al. Binding of human apolipoprotein E to synthetic amyloid ß peptide: isoform-specific effects and implications for late-onset Alzheimer disease. Proc Natl Acad Sci USA 1993;90:8098-8102.
36. LaDu MJ, Falduto MT, Manelli AM, et al. Isoform-specific binding of apolipoprotein E to ß-amyloid. J Biol Chem 1994;269:23403-6.
37. Zhou Z, Smith JD, Greengard P, et al. Alzheimer amyloid-ß peptide forms denaturant-resistent complex with type ε3 but not type ε4 isoform of native apolipoprotein E. Mol Med 1996;2:175-80.
38. Ma J, Yee A, Brewer HB Jr, et al. Amyloid-associated proteins α_1-antichymotrypsin and apolipoprotein E promote assembly of Alzheimer ß-protein into filaments. Nature 1994;372:

92-4.
39. Rebeck GW, Harr SD, Strickland DK, et al. Multiple, diverse senile plaque-associated proteins are ligands of an apolipoprotein E receptor, the α_2-macroglobulin receptor/low-density-lipoprotein receptor-related protein. Ann Neurol 1995;37:211-7.
40. Bales KR, Verina T, Dodel RC, et al. Lack of apolipoprotein E dramatically reduces amyloid ß-peptide deposition. Nat Genet 1997;17:263-4.
41. Terry RD. The pathogenesis of Alzheimer's disease: an alternative to the amyloid hypothesis. J Neuropathol Exp Neurol 1996;55:1023-5.
42. Gomez-Isla T, Hollister R, West H, et al. Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. Ann Neurol 1997;41:17-24.
43. Crowther RA. Tau protein and paired helical filaments of Alzheimer's disease. Curr Opin Struct Biol 1993;3:202-6.
44. Strittmatter WJ, Saunders AM, Goedert M, et al. Isoform-specific interactions of apolipoprotein E with microtuble-associated protein tau: implications for Alzheimer's disease. Proc Natl Acad Sci USA 1994;91:11183-6.
45. Genis I, Gordon I, Sehayek E, et al. Phosphorylation of tau in apolipoprotein E-deficient mice. Neurosci Lett 1995;199:5-8.
46. Gordon I, Grauer E, Genis I, et al. Memory deficits and cholinergic impairments in apolipoprotein E-deficient mice. Neurosci Lett 1995;199:1-4.
47. Beffert U, Poirier J. Apolipoprotein E, plaques, tangles and cholinergic dysfunction in Alzheimer's disease. Ann N Y Acad Sci 1996;777:166-74.
48. Soininen H, Kosunen O, Helisalmi S, et al. A severe loss of choline acetyltransferase in the frontal cortex of Alzheimer patients carrying apolipoprotein ε4 allele. Neurosci Lett 1995; 187:79-82.
49. Svensson A-L, Warpman U, Hellström-Lindahl E, et al. Nicotinic receptors, muscarinic receptors and choline acetyltransferase activity in the temporal cortex of Alzheimer patients with differing apolipoprotein E genotypes. Neurosci Lett 1997;232:37-40.
50. Poirier J. Apolipoprotein E in animal models of CNS injury and in Alzheimer's disease. Trends Neurosci 1994;17:525-30.
51. Alberts MJ, Graffagnino C, McClenny C, et al. ApoE genotype and survival from intracerebral haemorrhage. Lancet 1995;346:575.
52. Slooter AJC, Van Duijn CM, Bots ML, et al. Apolipoprotein E genotype, atherosclerosis, and cognitive decline: the Rotterdam Study. J Neural Transm 1998;553:17-29.
53. Kalmijn S, Feskens EJM, Launer LJ, et al. Cerebrovascular disease, the apolipoprotein e4 allele, and cognitive decline in a community-based study of elderly men. Stroke 1996;27: 2230-5.
54. Skoog I, Hesse C, Aevarsson Ó, et al. A population study of apoE genotype at the age of 85: relation to dementia, cerebrovascular disease, and mortality. J Neurol Neurosurg Psychiatry 1998;64:37-43.
55. Mayeux R, Ottman R, Maestre G, et al. Synergistic effects of traumatic head injury and apolipoprotein-ε4 in patients with Alzheimer's disease. Neurology 1995;45:555-7.
56. Teasdale GM, Nicoll JAR, Murray G, et al. Association of apolipoprotein E polymorphism with outcome after head injury. Lancet 1997;350:1069-71.
57. Jordan BD, Relkin NR, Ravdin LD, et al. Apolipoprotein E ε4 associated with chronic

traumatic brain injury in boxing. JAMA 1997;278:136-40.
58. Nicoll JAR, Roberts GW, Graham DI. Apolipoprotein E ε4 allele is associated with deposition of amyloid ß-protein following head injury. Nature Med 1995;1:135-7.
59. Sorbi S, Nacmias B, Piacentini S, et al. ApoE as a prognostic factor for post-traumatic coma. Nature Med 1995;1:852.
60. Tardiff BE, Newman MF, Saunders AM, et al. Preliminary report of a genetic basis for cognitive decline after cardiac operations. Ann Thorac Surg 1997;64:715-20.
61. Itzhaki RF, Lin W-R, Shang D, et al. Herpes simplex virus type 1 in brain and risk of Alzheimer's disease. Lancet 1997;349:241-4.
62. Mehta KM, Ott A, Slooter AJC, et al. Head trauma with loss of consciousness and risk of dementia and Alzheimer's disease. The Rotterdam Study. Submitted.
63. O'Meara ES, Kukull WA, Sheppard L, et al. Head injury and risk of Alzheimer's disease by apolipoprotein E genotype. Am J Epidemiol 1997;146:373-84.
64. Ott A, Slooter AJC, Hofman A, et al. Smoking and the risk of dementia and Alzheimer's disease in a population-based cohort study: the Rotterdam Study. Lancet 1998;351:1840-3.
65. Kuusisto J, Koivisto K, Mykkänen L, et al. Association between features of the insulin resistance syndrome and Alzheimer's disease independently of apolipoprotein E4 phenotype: cross sectional population based study. BMJ 1997;315:1045-9.
66. Ali SM, Dunn E, Oostveen JA, et al. Induction of apolipoprotein E mRNA in the hippocampus of the gerbil after transient global ischemia. Mol Brain Res 1996;38:37-44.
67. Hall ED, Oostveen JA, Dunn E, et al. Co-expression of amyloid precursor protein and apolipoprotein E in the selectively vulnerable hippocampus following transient forebrain ischemia in gerbils. Exp Neurol 1995;135:17-27.
68. Kida E, Pluta R, Lossinsky AS, et al. Complete cerebral ischemia with short-term survival in rat induced by cardiac arrest. II. Extracellular and intracellular accumulation of apolipoproteins E and J in the brain. Brain Res 1995;674:341-6.
69. Laskowitz DT, Sheng H, Bart RD, et al. Apolipoprotein E-deficient mice have increased susceptibility to focal cerebral ischemia. J Cereb Blood Flow Metab 1997;17:753-8.
70. Chen Y, Lomnitski L, Michaelson DM, et al. Motor and cognitive deficits in apolipoprotein E-deficient mice after closed head injury. Neuroscience 1997;80:1255-62.
71. Masliah E, Samuel W, Veinbergs I, et al. Neurodegeneration and cognitive impairment in apoE-deficient mice is ameliorated by infusion of recombinant apoE. Brain Res 1997;751: 307-14.
72. Laskowitz DT, Roses AD. Apolipoprotein E: an expanding role in the neurobiology of disease. Alz Rep 1998;1:5-12.
73. Mahley RW. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science 1988;240:622-30.
74. Guillaume D, Bertrand Ph, Dea D, et al. Apolipoprotein E and low-density lipoprotein binding and internalization in primary cultures of rat astrocytes: isoform-specific alterations. J Neurochem 1996;66:2410-8.
75. Nathan BP, Bellosta S, Sanan DA, et al. Differential effects of apolipoproteins E3 and E4 on neuronal growth in vitro. Science 1994;264:850-2.
76. Huang DY, Weisgraber KH, Strittmatter WJ, et al. Interaction of apolipoprotein E with laminin increases neuronal adhesion and alters neurite morphology. Exp Neurol 1995;136:

251-7.
77. Gutman CR, Strittmatter WJ, Weisgraber KH, et al. Apolipoprotein E binds to and potentiates the biological activity of ciliary neurotrophic factor. J Neurosci 1997;17:6114-21.
78. Beal MF. Aging, energy, and oxidative stress in neurodegenerative diseases. Ann Neurol 1995;38:357-66.
79. Miyata M, Smith J. Apolipoprotein E allele-specific antioxidant activity and effects on cytotoxicity by oxidative insults and ß-amyloid peptides. Nat Genet 1996;14:55-61.
80. Palinski W, Ord VA, Plump AS, et al. ApoE-deficient mice are a model of lipoprotein oxidation in atherogenesis. Demonstration of oxidation-specific epitopes in lesions and high titers of autoantibodies to malondialdehyde-lysine in serum. Arterioscler Thromb 1994;14: 605-16.
81. Matthews RT, Beal MF. Increased 3-nitrotyrosine in brains of apoE-deficient mice. Brain Res 1996;718:181-4.
82. Eikelenboom P, Zhan S-S, Van Gool WA, et al. Inflammatory mechanisms in Alzheimer's disease. TIPS 1994;15:447-50.
83. Andersen K, Launer LJ, Ott A, et al. Do nonsteroidal anti-inflammatory drugs decrease the risk for Alzheimer's disease? The Rotterdam Study. Neurology 1995;45:1441-5.
84. Garcia JH, Liu K, Yoshida Y, et al. Influx of leucocytes and platelets in an evolving brain infarct (Wistar rat). Am J Pathol 1994;144:188-99.
85. Laskowitz DT, Goel S, Bennet ER, et al. Apolipoprotein E suppresses glial secretion of TNF-α. J Neuroimmunol 1997;76:70-4.
86. Barger SW, Harmon AD. Microglial activation by Alzheimer amyloid precursor protein and modulation by apolipoprotein E. Nature 1997;388:878-81.
87. Mazzone T. Apolipoprotein E secretion by macrophages: its potential physiological functions. Curr Opinion Lipidol 1996;7:303-7.
88. Galasko D, Hansen LA, Katzman R, et al. Clinical-neuropathological correlations in Alzheimer's disease and related dementias. Arch Neurol 1994;51:888-95.
89. Khoury MJ, Beaty TH, Cohen BH. Fundamentals of genetic epidemiology. Oxford University Press. New York, 1993.
90. Snowdon DA, Greiner LH, Mortimer JA, et al. Brain infarction and the clinical expression of Alzheimer disease. The nun study. JAMA 1997;277:813-7.
91. Miettinen OS. Estimability and estimation in case-referent studies. Am J Epidemiol 1976;103:226-35.
92. Van Swieten JC, Van den Hout JH, Van Ketel BA, et al. Periventricular lesions in the white matter on magnetic resonance imaging in the elderly: a morphometric correlation with arteriolosclerosis and dilated perivascular spaces. Brain 1991;114:761-74.
93. Meyer JS, Kawamura J, Terayama Y. White matter lesions in the elderly. J Neurol Sci 1992;110:1-7.
94. Breteler MMB, De Leeuw FE, De Groot JC, et al. Frequency, causes and consequences of cerebral white matter changes: the Rotterdam scan study. Neuroepidemiology 1998. In press.
95. Benwell MEM, Balfour DJK, Anderson JM. Evidence that tobacco smoking increases the density of (-)-[^{3}H]Nicotine binding sites in human brain. J Neurochem 1988;50:1243-7.
96. Marchi M, Raiteri M. Nicotinic autoreceptors mediating enhancement of acetylcholine re-

lease become operative in conditions of 'impaired' cholinergic presynaptic function. J Neurochem 1996;67:1974-81.

97. Sjak-Shie NN, Meyer EM. Effects of chronic nicotine and pilocarpine administration on neocortical neuronal density and [³H]GABA uptake in nucleus basalis lesioned rats. Brain Res 1993;624:295-8.

9

Summary

9.1

Summary

This thesis describes the role of the apolipoprotein E genotype (APOE; apoE=protein) in atherosclerosis and dementia. These are two major disorders with increasing impact in our ageing society. The consequence of atherosclerosis, vascular disease, is the leading cause of mortality and disability. Dementia severely disturbs the quality of life, and is feared by numerous older persons.

High levels of total and LDL cholesterol and low levels of HDL cholesterol predispose to the development of atherosclerosis. The level of these lipids in serum is partly determined by the APOE polymorphism. Besides, the APOE genotype was found to be involved in Alzheimer's disease, the most common type of dementia.

For the results presented in this thesis, data were used from the Rotterdam Study, a population-based cohort study among inhabitants of the suburb Ommoord in Rotterdam. In total, 7983 persons (response 78%) participated, who were aged 55 years or over at baseline. Atherosclerosis was non-invasively assessed by the presence of plaques in the common carotid arteries, calcifications of the abdominal aorta, the common carotid artery intima-media wall thickness, and the ankle to arm blood pressure index. The mini mental state examination was used to assess cognitive function. Dementia was diagnosed using neuropsychological testing, neurological examination, and neuro-imaging. Events of interest, including death, myocardial infarction and stroke, were recorded after baseline examinations. After on average 2.1 follow-up years, 6315 (88%) of the 7215 subjects who were still alive participated in the second wave of the Rotterdam Study.

Chapter 2 provides a review on the genetic epidemiology of Alzheimer's disease. Up until now, various autosomal dominant mutations have been identified in three genes. These encode for the amyloid precursor protein (APP; chromosome 21), pre-

senilin 1 (PS-1; chromosome 14) and presenilin 2 (PS-2; chromosome 1). However, these mutations are all extremely rare. More common is the APOE*4 allele, and its effects are probably modified by various genetic and environmental factors. Findings on other susceptibility genes have been difficult to reproduce. It is to be expected that other, yet unknown genes are involved in the aetiology of Alzheimer's disease.

Our studies suggest that APOE testing is not informative in the differential diagnosis of dementia. This issue was first considered using the available literature on APOE and the non-Alzheimer's disease dementias ***(chapter 3.1).*** As APOE*4 seems to be associated with the most important alternative diagnosis for Alzheimer's disease, vascular dementia, and possibly also with other dementing diseases, it is not to be expected that APOE testing can detect Alzheimer cases in a population of dementia patients.

We investigated this topic further among 249 mildly to moderately dementia patients who participated in the Rotterdam Study ***(chapter 3.2).*** APOE genotyping did not change diagnostic certainty (likelihood ratio was 0.75 based on the presence of one APOE*4 allele; likelihood ratio was 1.38 based on homozygosity testing), and seems therefore of little value in the differential diagnosis of dementia.

Chapter 4.1 describes the association between the APOE genotype and dementia with stroke, which included either vascular dementia or Alzheimer's disease with cerebrovascular disease. This issue was studied using data from the Rotterdam Study and the Washington Heights Study, a population-based follow-up study performed in Northern Manhattan, New York. As no major differences were observed between the two sites, we pooled the data from both studies. Relative to APOE3E3, persons with the APOE4E4 genotype had a nearly 7-fold increased risk of dementia with stroke (OR=6.9; 95% CI 1.6 to 29.4) while the OR associated with the APOE3E4 genotype was nearly 2-fold increased (OR=1.8; 95% CI 1.2 to 2.7).

In ***chapter 4.2*** we evaluated the role of serum apoE level in Alzheimer's disease. Serum apoE level was lower in Alzheimer patients than in non-demented controls (0.75 µmol/l (SD 0.35), versus 0.83 µmol/l (SD 0.40)). This finding is in accordance with lower serum apoE levels as observed in carriers of the APOE*4 allele, who are over-represented among Alzheimer patients. After adjustments were made for the APOE genotype, the difference in serum apoE levels largely disappeared. This observation suggests that the level of apoE in serum does not contribute to the pathogenesis of Alzheimer's disease, independent of the APOE genotype.

We further studied whether the APOE genotype is related to the course of Alzheimer's disease ***(chapter 4.3).*** For this purpose, 97 Alzheimer patients were included at a similar stage i.e. before the onset of symptoms, and followed for up to 5 years. The change in cognitive function and the severity of dementia were similar for Alzheimer patients with and without the APOE*4 allele. The median survival after

the onset of symptoms did not differ between the two groups either. However, mortality rate was higher in APOE*4 carriers after adjusting for age (1.5; 95% CI 0.8 to 2.8), as carriers of the APOE*4 allele were younger at dementia onset.

Chapter 5.1 briefly summarises the role of apoE in atherosclerosis. Besides its function in the metabolism of lipoproteins, apoE is involved in several processes in the arterial wall. ApoE may inhibit the aggregability of platelets and modulate lymphocyte function. In addition, apoE may interact with the extracellular matrix, thereby influencing the retention of lipoproteins.

In ***chapter 5.2*** the association between the APOE genotype and atherosclerosis was evaluated, using data from the Rotterdam Study. We found overall no relationship between the APOE*4 allele and atherosclerosis, although the APOE4E4 genotype was weakly associated with atherosclerosis in the abdominal aorta and peripheral arteries. By contrast, the APOE*2 allele appeared to be inversely associated with atherosclerosis. Adjusting for serum apoE levels strengthened the association between APOE*2 and atherosclerosis, whereas adjusting for total and HDL cholesterol only slightly changed the estimates. These findings suggest that serum apoE level is not an intermediate factor. Secondly, the main APOE*2 effects on atherosclerosis seem not to result from its influence on lipid levels.

Furthermore, the effects of both APOE and atherosclerosis were studied in relation to cognitive function ***(chapter 6.1),*** and dementia. Carriers of the APOE*4 allele performed worse on the mini mental state examination than persons without this allele. Furthermore, cognitive function in persons with atherosclerosis was worse as compared to subjects without evidence of atherosclerosis. When the study population was further stratified, we found that APOE*4 carriers with atherosclerosis had the worst cognitive performance. Mild, non-significant effects on cognitive function observed for atherosclerosis in the absence of APOE*4, and for APOE*4 in the absence of atherosclerosis. Our findings suggest a synergistic effect of APOE*4 and atherosclerosis with regard to cognitive performance.

In ***chapter 6.2*** we studied the combined effects of APOE and atherosclerosis on dementia. The consequences of both APOE*4 and atherosclerosis were stronger than the sum of the effects of APOE*4 alone and atherosclerosis alone. This again suggests a synergistic interaction. Besides, as the APOE genotype was shown to be associated with atherosclerosis, and atherosclerosis was found to be related to dementia, we studied whether atherosclerosis could be intermediate in the association of APOE and dementia. The association between the APOE genotypes and dementia, did not essentially alter when adjustments were made for atherosclerosis or other vascular factors. This suggests that atherosclerosis is not an intermediate factor.

Chapter 6.3 evaluates the association of APOE, smoking and dementia. Although smoking is a well known risk factor for atherosclerosis, the combined effects

of smoking and APOE*4 on the occurrence of dementia, were opposite from the combined effects of atherosclerosis and APOE*4. Among APOE*4 carriers, current smoking was associated with a lower risk of Alzheimer's disease (OR=0.6; 95% CI 0.1 to 4.8), as compared to non-smokers. Among non-APOE*4 carriers, current smokers had a higher risk of Alzheimer's disease than never smokers (OR=4.6; 95% CI 1.5 to 14.2). These observations suggest an interaction between APOE*4 and smoking in the aetiology of Alzheimer's disease. In this chapter it is discussed that smoking could affect the cholinergic system, counterbalancing the putative impairment associated with APOE*4.

Chapter 7 describes the population impact of the APOE genotype with regard to vascular diseases, dementia and mortality. Persons with APOE4E4 younger than 65 years were at increased risk of myocardial infarction. However, the incidence of stroke was not strongly related to the APOE polymorphism. Although carriers of the APOE*4 allele were at increased risk of dementia, the contribution of APOE*4 to the incidence of dementia on a population level, was modest, which reflects the relatively low frequency of the APOE*4 allele. An estimated 185 per 100000 individuals aged 55 or over, become demented each year as a consequence of carrying APOE3E4 or APOE4E4 instead of the APOE3E3 genotype. Mortality was overall not related to APOE, but increased in persons with either the APOE2E4 or the APOE4E4 genotype who were younger than 65 years.

Chapter 8 summarises our findings together with observations from other studies and methodological considerations. The discussion of the mechanism underlying our observations focuses on the involvement of APOE in the neuropathology of Alzheimer's disease. APOE may be related to Alzheimer's disease because of its role in the response to cerebral injury, for instance due to atherosclerosis. As this hypothesis is compatible with several studies in this thesis, it was discussed in more detail. There are various mechanisms possible how apoE may play a role in the response to cerebral injury. ApoE is involved in the transport of components for plasma membranes to injured neurons, and may interact with the extracellular matrix, or with growth factors. Furthermore, apoE could protect against oxidative stress, and may modulate the glial response to inflammation. Finally, recommendations for future research are given.

9.2

Samenvatting

Dit proefschrift beschrijft de rol van het apolipoproteïne E genotype (APOE; apoE=eiwit) in atherosclerose en dementie. Deze twee stoornissen spelen in onze vergrijzende samenleving een steeds belangrijker rol. De consequentie van atherosclerose, hart- en vaatziekten, is de belangrijkste oorzaak van morbiditeit en mortaliteit. Dementie is een zeer ingrijpende aandoening voor de patiënt en zijn omgeving, en wordt gevreesd door vele ouderen.

Hypercholesterolemie en lage spiegels HDL cholesterol verhogen het risico op atherosclerose. De concentratie van deze lipiden in serum wordt gedeeltelijk bepaald door het APOE polymorfisme. Daarnaast bleek het APOE genotype een rol te spelen bij de ziekte van Alzheimer, de meest voorkomende vorm van dementie.

Voor de resultaten die gepresenteerd worden in dit proefschrift, werd gebruik gemaakt van gegevens van het Erasmus Rotterdam Gezondheid en Ouderen (ERGO) onderzoek, een bevolkingsonderzoek onder inwoners van de Rotterdamse wijk Ommoord. In totaal deden 7983 personen hieraan mee (respons 78%), die allen 55 jaar of ouder waren bij aanvang van het onderzoek. De aanwezigheid van atherosclerose werd niet-invasief gemeten middels het voorkomen van plaques in de arteriae carotides communes, calcificaties in de aorta abdominalis, de intima-media wanddikte van de arteriae carotides communes, en de enkel-arm index. De 'mini mental state examination' werd gebruikt om het cognitief functioneren te beoordelen. De diagnose dementie werd gebaseerd op neuropsychologisch onderzoek, neurologisch onderzoek en beeldvormend onderzoek. Sterfte, evenals het optreden van een myocardinfarct of een cerebrovasculair accident werd geregistreerd na inclusie. Na gemiddeld 2.1 jaar werden 6315 (88%) van alle nog in leven zijnde 7215 deelnemers voor de tweede keer onderzocht.

Hoofdstuk 2 geeft een overzicht van de genetische epidemiologie van de ziekte van Alzheimer. Tot nu toe zijn er verscheidene autosomaal dominante mutaties gevonden in drie genen. Deze coderen voor het amyloïd precursor proteïne (APP; chromosoom 21), preseniline 1 (PS-1; chromosoom 14) en preseniline 2 (PS-2; chromosoom 1). Echter, deze mutaties zijn alle buitengewoon zeldzaam. Veel frequenter is het APOE*4 allel, de effecten hiervan worden waarschijnlijk gemodificeerd door verschillende genetische en omgevingsfactoren. Bevindingen naar andere 'kwetsbaarheidsgenen' konden echter niet altijd gereproduceerd worden. Het ligt in de lijn der verwachting dat er meer genen een rol spelen in de etiologie van de ziekte van Alzheimer.

APOE genotypering lijkt niet informatief te zijn in de differentiaal diagnostiek van dementie. In ***hoofdstuk 3.1*** wordt dit onderwerp beschouwd gebruikmakend van de literatuur over APOE en de niet-Alzheimer dementieën. APOE*4 is waarschijnlijk ook geassocieerd met de meest frequente type dementie na de ziekte van Alzheimer, vasculaire dementie, en mogelijk eveneens met andere vormen van dementie. Het is daarom niet waarschijnlijk dat APOE genotypering gebruikt zal kunnen worden om Alzheimer patiënten te onderscheiden van mensen met een andere vorm van dementie.

De diagnostische waarde van APOE genotypering werd verder geëvalueerd onder 249 mild tot matig ernstig demente patiënten die deelnamen aan het ERGO onderzoek ***(hoofdstuk 3.2).*** APOE genotypering verhoogde de diagnostische zekerheid niet (waarschijnlijkheidsverhouding was 0.75 gebaseerd op de aanwezigheid van één APOE*4 allel; waarschijnlijkheidsverhouding was 1.38 gebaseerd op testen voor twee APOE*4 allelen), en lijkt daarom van weinig waarde in de differentiaal diagnostiek van dementie.

Hoofdstuk 4.1 beschrijft de relatie tussen het APOE genotype en 'dementie met beroerte', een verzamelterm voor vasculaire dementie en de ziekte van Alzheimer met cerebrovasculaire pathologie. Dit onderwerp werd onderzocht met gegevens van de ERGO studie en the Washington Heights Study, een cohort onderzoek onder de algemene bevolking van het noordelijk deel van Manhattan in New York. Aangezien er geen grote verschillen waren tussen de twee populaties, werden de gegevens gecombineerd. Vergeleken met het APOE3E3 genotype, hadden dragers van twee APOE*4 allelen een bijna 7-keer hoger risico op dementie met beroerte (OR=6.9; 95% BI 1.6 tot 29.4) terwijl dragers van het APOE3E4 genotype een bijna twee keer verhoogd risico hadden (OR=1.8; 95% BI 1.2 tot 2.7).

Hoofdstuk 4.2 behandelt de rol van serum apoE bij de ziekte van Alzheimer. De concentratie apoE in serum was lager bij Alzheimer patiënten dan bij niet-demente controles (0.75 µmol/l (SD 0.35), versus 0.83 µmol/l (SD 0.40)). Deze bevinding past bij de lagere serum apoE concentraties die gemeten worden bij

dragers van het APOE*4 allel, die over-vertegenwoordigd zijn onder Alzheimer patiënten. Na correctie voor het APOE genotype, verdween het verschil in serum apoE spiegels grotendeels. Dit wijst erop dat de concentratie apoE in serum geen determinant lijkt te zijn van de ziekte van Alzheimer, onafhankelijk van het APOE genotype.

We onderzochten verder of het APOE genotype gerelateerd is aan het beloop van de ziekte van Alzheimer ***(hoofdstuk 4.3).*** Hiervoor werden 97 Alzheimer patiënten geïncludeerd op het moment dat zij nog geen symptomen vertoonden, en tot 5 jaar gevolgd. De verandering in het cognitief functioneren en de ernst van de dementie waren gelijk voor Alzheimer patiënten met en zonder het APOE*4 allel. De mediane overleving na het begin van de symptomen was eveneens gelijk voor de twee groepen. Echter, de mortaliteit onder APOE*4 dragers was hoger na correctie voor leeftijd (1.5; 95% BI 0.8 tot 2.8), aangezien patiënten met het APOE*4 allel jonger waren bij aanvang van de dementie.

Hoofdstuk 5.1 vat kort de rol samen van apoE bij atherosclerose. Naast de functie in het metabolisme van lipoproteïnen, is apoE betrokken bij verschillende processen in de arteriële vaatwand. ApoE kan de aggregatie van trombocyten remmen en de functie van lymfocyten beïnvloeden. Daarnaast kan apoE een interactie aangaan met de extracellulaire matrix, en daardoor de ophoping van lipoproteïnen beïnvloeden.

In ***hoofdstuk 5.2*** wordt de associatie tussen het APOE genotype en atherosclerose bestudeerd, gebruikmakend van gegevens van de ERGO studie. Wij vonden overall geen relatie tussen het APOE*4 allel en atherosclerose, hoewel er een zwak verband bestond tussen het APOE4E4 genotype en atherosclerose in de aorta abdominalis en de perifere arteriën. Het APOE*2 allel daarentegen bleek omgekeerd geassocieerd te zijn met atherosclerose. Correctie voor serum apoE spiegels versterkte de inverse associatie tussen APOE*2 en atherosclerose, terwijl correctie voor totaal en HDL cholesterol slechts weinig invloed had. Deze bevindingen suggereren ten eerste dat de spiegel van apoE in serum geen intermediaire factor is. Ten tweede, de belangrijkste effecten van APOE*2 op atherosclerose lijken niet het resultaat te zijn van het APOE*2 effect op lipiden spiegels.

Verder werden de effecten van zowel APOE als atherosclerose bestudeerd in relatie tot het cognitief functioneren ***(hoofdstuk 6.1),*** en dementie. Dragers van het APOE*4 allel presteerden slechter op de 'mini mental state examination' dan personen zonder dit allel. Daarnaast was het cognitief functioneren van mensen met atherosclerose slechter vergeleken met personen zonder aanwijzingen voor atherosclerose. Wanneer we de onderzoekspopulatie verder stratificeerden, vonden we dat APOE*4 dragers met atherosclerose het slechtst presteerden. Geringe, niet-significante effecten wat betreft het cognitief functioneren was gerelateerd aan athero-

sclerose zonder APOE*4, en voor APOE*4 zonder atherosclerose. Deze bevindingen doen een synergistisch effect van APOE*4 en atherosclerose vermoeden ten aanzien van het cognitief functioneren.

In ***hoofdstuk 6.2*** bestudeerden we de gecombineerde effecten van APOE en atherosclerose met betrekking tot dementie. De consequentie van zowel APOE*4 als atherosclerose was sterker dan de som van de afzonderlijke factoren. Dit suggereert wederom een synergistische interactie. Daarnaast bestudeerden we in hoeverre atherosclerose een intermediare factor zou kunnen zijn in de associatie van APOE met dementie, aangezien het APOE genotype geassocieerd bleek te zijn met atherosclerose, en atherosclerose gerelateerd was aan dementie. De associatie tussen het APOE genotype en dementie veranderde nauwelijks na statistische correctie voor atherosclerose of andere vasculaire factoren. Dit suggereert dat atherosclerose geen intermediare factor is.

Hoofdstuk 6.3 bespreekt de relatie tussen APOE, roken en dementie. Hoewel roken een bekende risico factor is voor atherosclerose, verschillen de gecombineerde effecten van roken en APOE*4 ten aanzien van het voorkomen van dementie, van de gecombineerde effecten van atherosclerose en APOE*4. Onder dragers van het APOE*4 allel, hadden rokers een lager risico op de ziekte van Alzheimer (OR=0.6; 95% BI 0.1 tot 4.8), dan mensen die nooit hadden gerookt. Onder niet-APOE*4 dragers hadden rokers een hoger risico om de ziekte van Alzheimer te krijgen dan mensen die nooit hadden gerookt (OR=4.6; 95% BI 1.5 tot 14.2). Deze bevindingen suggereren een interactie tussen APOE*4 en roken in de etiologie van de ziekte van Alzheimer. Roken zou invloed uit kunnen oefenen op het cholinerge systeem, en daarmee de nadelige effecten van APOE*4 kunnen compenseren.

Hoofdstuk 7 beschrijft de invloed van het APOE genotype op populatie niveau met betrekking tot hart- en vaatziekten, dementie en sterfte. Personen met het APOE4E4 genotype die jonger waren dan 65 jaar, hadden een verhoogd risico om een myocardinfarct te krijgen. Daarentegen was de incidentie van het cerebrovasculair accident niet duidelijk gerelateerd aan het APOE polymorfisme. Hoewel dragers van het APOE*4 allel een verhoogd risico hadden op dementie, was de bijdrage van APOE*4 aan de incidentie van dementie op populatie niveau gering. Dit weerspiegelt de relatief lage frequentie van het APOE*4 allel. Naar schatting 185 per 100000 mensen van 55 of ouder, worden jaarlijks dement omdat zij tenminste drager zijn van APOE3E4 of APOE4E4, in plaats van het APOE3E3 genotype. De mortaliteit was overall niet gerelateerd aan APOE, maar verhoogd onder personen met het APOE2E4 of het APOE4E4 genotype, jonger dan 65 jaar.

Hoofdstuk 8 vat onze bevindingen samen met die van andere studies en besteedt aandacht aan enkele methodologische aspecten. De bespreking van het mechanisme dat ten grondslag ligt aan onze epidemiologische bevindingen concentreert

zich op de rol van APOE in de neuropathologie van de ziekte van Alzheimer. APOE zou een rol kunnen spelen in de pathogenese vanwege zijn rol in het herstel na cerebrale schade, bijvoorbeeld vanwege atherosclerose. Deze hypothese werd gedetailleerder besproken aangezien deze compatibel is met verschillende studies van dit proefschrift. Er zijn verschillende mechanismen mogelijk wat betreft de rol van apoE in het herstel na cerebrale schade. ApoE is betrokken bij het transport van componenten voor plasma membranen naar beschadigde neuronen, en zou een interactie aangaan met de extracellulaire matrix, of met groeifactoren. Ook zou apoE kunnen beschermen tegen oxidatieve stress, en zou de inflammatoire reactie van glia cellen kunnen moduleren. Ten slotte werden suggesties gedaan voor toekomstig onderzoek.

Acknowledgements
Dankwoord

Dit proefschrift kwam tot stand dank zij samenwerking met verschillende instituten en personen die ik hier graag met name zou willen noemen.

In de eerste plaats dank ik mijn promotor, prof. dr A. Hofman. Beste Bert, ik heb de afgelopen jaren enorm veel van je geleerd. De door jou opgezette epidemiologie opleiding was ontzettend waardevol. Ik heb meer oog gekregen voor 'major diseases and major determinants' in plaats van 'zeldzame postzegels'. Jouw systematische manier van denken en vertrouwen in de goede afloop hebben me zeer gestimuleerd.

In de tweede plaats dank ik natuurlijk dr C.M. van Duijn. Beste Cock, ik ben je in de loop van de jaren erg gaan waarderen vanwege je humor en je onconventionele stijl. Wat het onderzoek betreft heb je op beslissende momenten een essentiële rol gespeeld. Je enthousiasme en ambitie bleken erg besmettelijk te zijn. Ik hoop dat we in de toekomst samen nog meer onderzoek kunnen doen.

Onder leiding van prof. C. Van Broeckhoven zijn ontelbare APOE typeringen uitgevoerd. Christine, bedankt voor je hulp bij het vinden van 'onderdak'. De accuratesse van Marc Cruts heb ik erg gewaardeerd. Ik hoop dat Hubert Backhovens, Marleen van den Broeck, Sally Serneels, Joke De Voecht en Anita Wehnert nooit meer nachtmerries zullen hebben van alle ERGO samples.

Jeannette Vergeer dank ik voor de gigantische hoeveelheid werk die ze verzet heeft. Daarnaast hebben Juliana Bronzova, Bianca de Graaf, Angela Jacobs, Hilda Kornman, Toos Stehman een belangrijke bijdrage geleverd aan het isoleren van DNA.

Monique Breteler gaf leiding aan de dementie diagnostiek binnen ERGO; Jules Claus, F. van Harskamp, Sandra Kalmijn, Inge de Koning en Maarten de Rijk dank ik voor hun bijdrage hieraan. Met name wil ik Alewijn Ott danken voor zijn 'monnikenwerk' en zijn geduld toen ik ingewerkt moest worden.

Hoewel dr M.L. Bots en prof. dr D.E. Grobbee naar Utrecht verhuisden, bleef er altijd gelegenheid voor overleg over de atherosclerose metingen. Een belangrijke bijdrage werd verder geleverd door dr J.C.M. Witteman.

Ik dank prof. dr L.M. Havekes en dr P. de Knijff voor hun bijdragen, waaronder het (laten) bepalen van honderden serum apoE spiegels.

It was a real privilege to work in the group of prof. R. Mayeux, one of the leading neuro-epidemiological groups in the world. Dear Richard, I really enjoyed working at your department, because of the friendly atmosphere and stimulating comments. I also liked working with Ming-Xin Tang, and thank Jo Biggs for company.

Dr W.A. van Gool en Carl Moons dank ik voor hun bijdrage aan het hoofdstuk over diagnostiek.

Ik realiseer me heel goed dat het onmogelijk is om binnen vier jaar te promoveren zonder dat er al veel werk gedaan is. Alle medewerkers van ERGO dank ik hiervoor, evenals de 7983 deelnemers. Verder dank ik hierbij alle huisartsen en verpleeghuisartsen die ERGO deelnemers in hun praktijk hebben. Zonder hun bijdrage is zo'n groot populatie-onderzoek onmogelijk.

Bij de statistici van onze afdeling kon ik altijd even binnen lopen. Van met name Bettina Hansen, Maria de Ridder en Theo Stijnen heb ik de afgelopen jaren erg veel geleerd.

Anna Bosselaar en Hilda Kornman wil ik bedanken voor het maken van afspraken met de deelnemers van het onderzoek beschreven in paragraaf 4.3. Ik kan iedereen aanraden de layout van het proefschrift door Anna te laten verzorgen.

Kamagurka dank ik voor zijn toestemming om een van zijn cartoons op te mogen nemen.

Genoeglijk zat ik met de andere promovendi van onze afdeling in hetzelfde schuitje. Het siert de Willies (Norbert Vaessen en Gerwin Roks) dat zij hun naam als Geuzen droegen. Mijn maten wil ik met name noemen: drieëneenhalf jaar lang hebben Casper Bijkerk en ik met de ruggen naar elkaar gezeten. Vanwege zijn enorme relativeringsvermogen en humor kijk ik er met veel plezier op terug. Jan-Cees de Groot was altijd te vinden voor iets 'maatschappelijks'; ik hoop dat het daar nog vaak van zal komen. Frank-Erik de Leeuw wist het van co-assistent tot maître de cérémonie te schoppen; ik vind het erg leuk om samen opgeleid te worden tot neuroloog.

Mijn ouders dank ik ervoor dat ze alles voor hun kinderen over hebben; overigens zonder ooit iets terug te vragen.

Barbara dank ik voor ALLES.

Curriculum vitae

Arjen Slooter was born on July 3, 1968 in remote Roermond. He graduated in 1986 at the 'Fioretti College' in Lisse, and started studying Medicine at the Vrije Universiteit Amsterdam in the same year. During his studies, he did clinical electives at the Isles of Barra (Scotland) and Man (Man), worked as a student-practitioner in family medicine, and was involved in experimental and clinical research in neurology. In 1994, he obtained his medical degree, and worked for 9 months as a resident in neurology at the Sint Lucas Ziekenhuis, Amsterdam (head: dr J.A.L. Vanneste). In 1995, he began the studies described in this thesis at the Department of Epidemiology & Biostatistics, Erasmus University Medical School, Rotterdam (head: prof. dr A. Hofman), and was trained as an epidemiologist. Part of this work was done at the Sergievsky Center, College of Physicians and Surgeons, Columbia University, New York (head: prof. R. Mayeux). From February 1999 he will continue doing research, and will start his training as a neurologist at the University Hospital Utrecht (head: prof. dr J. van Gijn). First, he is going to make a world tour with his wife, Barbara Benard.

Correspondence after January 15, 1999 to: Hugo de Grootstraat 20b, 3581 XT Utrecht

List of publications

Slooter AJC. Perinatale asfyxie en de kans op infantiele encefalopathie, huisarts-geneeskundige aspecten. Huisarts Wet 1993;36:416-9.

Taphoorn MJB, *Slooter AJC,* Lanting P, Deijen JB, Faes TJC. Pupillary and cardiovascular autonomic function in the narcolepsy-cataplexy syndrome. Sleep-wake research in the Netherlands 1993;4:193-4.

Slooter AJC, Breteler MMB, Ott A, Van Broeckhoven C, Van Duijn CM. APOE genotyping in differential diagnosis of Alzheimer's disease. Lancet 1996;348:334.

Slooter AJC, Van Gool WA, Van Duijn CM. De plaats van apolipoproteïne-E-genotypering in de differentiaaldiagnostiek van dementie. Ned Tijdschr Geneeskd 1996; 140:2073-6.

Hofman A, Ott A, Breteler MMB, Bots ML, *Slooter AJC,* Van Harskamp F, Van Duijn CM, Van Broeckhoven C, Grobbee DE. Atherosclerosis, apolipoprotein E, and the prevalence of dementia and Alzheimer's disease in the Rotterdam Study. Lancet 1997;349:151-4.

Slooter AJC, Tang M-X, Van Duijn CM, Stern Y, Ott A, Bell K, Breteler MMB, Van Broeckhoven C, Tatemichi TK, Tycko B, Hofman A, Mayeux R. Apolipoprotein ε4 increases the risk of dementia with stroke. A population-based investigation. JAMA 1997;277:818-21.

Slooter AJC, Van Duijn CM. Genetic epidemiology of Alzheimer's disease. Epidemiol Rev 1997;19:107-19.

Ott A, *Slooter AJC,* Hofman A, Van Harskamp F, Witteman JCM, Van Broeckhoven C, Van Duijn CM, Breteler MMB. Smoking and the risk of dementia and Alzheimer's disease in a population-based cohort study: the Rotterdam Study. Lancet 1998;351:1840-3.

Slooter AJC, Cruts M, Kalmijn S, Hofman A, Breteler MMB, Van Broeckhoven C, Van Duijn CM. Risk estimates of dementia by apolipoprotein E genotypes from a population-based incidence study: the Rotterdam Study. Arch Neurol 1998;55:964-8.

Slooter AJC, De Knijff P, Hofman A, Cruts M, Breteler MMB, Van Broeckhoven C, Havekes LM, Van Duijn CM. Serum apolipoprotein E level is not increased in Alzheimer's disease: the Rotterdam Study. Neurosci Lett 1998;248:21-4.

Slooter AJC, Van Duijn CM, Bots ML, Ott A, Breteler MMB, De Voecht J, Wehnert A, De Knijff P, Havekes LM, Grobbee DE, Van Broeckhoven C, Hofman A. Apolipoprotein E genotype, atherosclerosis, and cognitive decline: the Rotterdam Study. J Neural Transm 1998;S53:17-29.

Van der Cammen TJM, Verschoor CJ, Van Loon CPM, Van Harskamp F, De Koning I, Schudel WJ, *Slooter AJC,* Van Broeckhoven C, Van Duijn C. Risk of left ventricular dysfunction in patients with probable Alzheimer's disease with APOE*4 allele. J Am Geriatr Soc. In press.

De Rijk MC, Breteler MMB, Ott A, *Slooter AJC,* Van Duijn CM, Van Broeckhoven C, Van der Meché FGA, Hofman A. Apolipoprotein E genotype, Parkinson's disease, and dementia in Parkinson's disease: the Rotterdam Study. Submitted.

Mehta KM, Ott A, *Slooter AJC,* Van Duijn CM, Hofman A, Breteler MMB. Head trauma with loss of consciousness and risk of dementia and Alzheimer's disease. The Rotterdam Study. Submitted.

Slooter AJC, Havekes LM, Bots ML, De Knijff P, Cruts M, Grobbee DE, Hofman A, Van Broeckhoven C, Witteman JCM, Van Duijn CM. Apolipoprotein E and atherosclerosis: the Rotterdam Study. Submitted.

Slooter AJC, Bronzova J, Witteman JCM, Van Broeckhoven C, Hofman A, Van Duijn CM. Oestrogen use and early onset Alzheimer's disease. A population-based study. Submitted.

Slooter AJC, Houwing-Duistermaat JJ, Van Harskamp F, Cruts M, Van Broeckhoven C, Breteler MMB, Hofman A, Stijnen Th, Van Duijn CM. Apolipoprotein E and progression of Alzheimer's disease. The Rotterdam Study. Submitted.

Slooter AJC, Cruts M, Ott A, Bots ML, Witteman JCM, Hofman A, Van Broeckhoven C, Breteler MMB, Van Duijn CM. Atherosclerosis is not intermediate in the association of apolipoprotein E and dementia: the Rotterdam Study. Submitted.

Dermaut B, Cruts M, *Slooter AJC,* Van Gestel S, De Jonghe C, Backhovens H, Vanderstichele H, Vanmechelen E, Breteler MMB, Hofman A, Hendriks L, Van Duijn CM, Van Broeckhoven C. The Glu318Gly mutation in presenilin-1 is not causally related to dementia: the Rotterdam Study. Submitted.

Tol J, *Slooter AJC,* Van Duijn CM. Genetic factors in early- and late-onset Alzheimer's disease. Submitted.