Apolipoprotein E polymorphism and Alzheimer's disease

Judes Poirier, Jean Davignon, Daniel Bouthillier, Sandra Kogan, Philippe Bertrand, Serge Gauthier

Summary

Apolipoprotein E (apoE) is associated with Alzheimer's neurofibrillary tangles and β-amyloid protein in senile plaques. It also appears to play an important part in the redistribution of lipids that follows deafferentation and neurodegeneration in the brain. The gene for apoE is on chromosome 19, within the genomic region previously associated with late-onset familial Alzheimer's disease (AD).

We have studied apoE phenotype expression and the corresponding allele frequencies (ε2, ε3, ε4) in 91 patients with sporadic AD and 74 controls. There was a significant association between ε4 and sporadic AD (ε4 frequency 0 380 in AD and 0·122 in controls, $p < 0·01$). Analysis of ε4 allele frequency as a function of age revealed a bimodal distribution, with peaks at 65 and 75 years. In bearers of ε4 in whom AD develops this tended to happen earlier in life than in those with ε3 or ε2. The ε4/AD association was more pronounced in women. Octogenarians with AD had an ε4 allele frequency that was 3 times higher than one reported, in a different study, in healthy octogenarians.

ApoE may be an important susceptibility factor in the aetiopathology of sporadic AD.

Lancet 1993; **342:** 697–99

See Commentary page 695

Douglas Hospital Research Centre (J Poirier PhD, P Bertrand PhD), **McGill Centre for Studies in Aging** (J Poirier, S Kogan PhD, Prof S Gauthier MD), **and Department of Psychiatry** (J Poirier, S Gauthier), **McGill University, Quebec, Canada; and Clinical Research Institute of Montreal** (Prof J Davignon MD, D Bouthillier PhD) **and University of Montreal** (J Davignon)

Correspondence to: Dr Judes Poirier, McGill Centre for Studies in Aging, Douglas Hospital Research Centre, 6575 Lasalle Blvd, Verdun, Quebec, Canada H4H 1R3

Introduction

Apolipoprotein E (apoE) is a polymorphic protein associated with plasma lipoproteins. It interacts with the "remnant receptor" (apoE receptor) and the low-density-lipoprotein (LDL) receptor (apoE/B receptor) of the liver and other organs to modulate the catabolism of triglyceride-rich lipoprotein particles. ApoE is unique among apolipoproteins in that it has a special relevance to nervous tissue.[1] It is involved in the mobilisation and redistribution of cholesterol in repair, growth, and maintenance of myelin and neuronal membranes during development or after injury.[2-4]

The importance of apoE is underscored in Alzheimer's disease (AD) by its presence within the plaques and dystrophic neurites that characterise AD;[5,6] by the fact that apoE mRNA, which has a critical role during compensatory central-nervous-system sprouting and synaptogenesis,[1,7] is reduced in the hippocampus in AD,[8] and by the ability of apoE to bind tightly to the soluble and insoluble forms of β-amyloid.[6,9,10] Furthermore, linkage between apoE and apoCII gene loci on chromosome 19 has lately been reported in some cases of late-onset familial AD.[10,11] A preliminary report on apoE genotypes in some cases of familial AD showed an increase in the ε4 allele prevalence.[10]

ApoE is encoded by a gene on the long arm of chromosome 19, within a region previously associated with familial late-onset AD. Common apoE polymorphisms are determined by alleles designated ε4, ε3, and ε2.[4] The ancestral isoform of the protein is apoE3, and this has cysteine at residue 112 and arginine at residue 158, whereas arginine in apoE4 and cysteine in apoE2 are present at both sites. This polymorphism results in six apoE phenotypes—E2/2, E3/3, and E4/4 in homozygotes and E3/2, E4/2, and E4/3 in heterozygotes. ApoE2 has lower affinity for the LDL receptor than has either E3 or E4.[4] Lipoproteins associated with apoE4 are cleared more efficiently than the ones containing apoE3 and apoE2. The presence of apoE4 has some direct consequences for plasma cholesterol and lipoprotein levels and may alter brain reinnervation processes which rely heavily upon cholesterol and triglyceride transport by apoE.[1,7] The fact that sporadic AD patients have abnormally high plasma levels of lipoprotein-cholesterol[12,13] and poor reinnervation capacity[14-16] prompted us to study apoE polymorphism in a large cohort of sporadic cases of AD and controls living in eastern Canada. The apoE polymorphism profile of this population has been extensively characterised.[17] A detailed analysis of apoE genotype in healthy octogenarians from eastern Canada is also available.[18]

Patients and methods

Patients

The investigation was done at the McGill Centre for Studies in Aging in collaboration with the Clinical Research Institute of Montreal, with approval by institutional review boards. Informed consent was obtained from all patients. 91 consecutive ambulatory patients living near Montreal who were referred to the cognitive disorder clinic between May, 1990, and January, 1991, were screened. They all had a clinical diagnosis of probable AD[19] and most were in stage 3 or 4 of the Reisberg global deterioration scale. Controls consisted of healthy spouses of AD patients and elderly volunteers.

ApoE phenotype

Blood was obtained after a 12 h fast. Plasma was separated in the cold and frozen at −80°C until determination of phenotype with analytical isoelectric focusing followed by apoE goat antibodies and enzyme-conjugated rabbit anti-goat IgG detection.[20]

Results

The ages of controls were well matched with the patients' ages (table 1). As expected, cognitive scores (MMS) were lower in AD patients than in controls (table 1). The mean duration of AD was 3·98 (SD 0·36) years. The prevalence of Down's syndrome within the patient's family was similar for both groups. Additional AD or senility in the family (as assessed by questionnaire) was more common in the Alzheimer cohort than in the control cohort but dementia in the family of the Alzheimer's patients had no impact on the apoE phenotype distribution (not shown).

The apoE phenotype distribution and allele frequencies are given in table 2. We included the phenotype distribution profile of normolipidaemic individuals from eastern Canada as a reference.[18] The phenotypic profile shows enrichment of the E4/4 and E4/3 phenotypes in AD. The ε4 allele frequency is significantly higher (3-fold) in the Alzheimer population. The ε4 allele is thus much enriched in AD, and at the expense of both ε3 and ε2. Within the AD cohort, a marked enrichment of ε4 allele frequency is observed in women versus men.

Figure 1A shows the distribution of ε4 allele prevalence as a function of age, suggesting two peaks, at 56–66 and 75–85 years, in AD. When (figure 1B) the analysis was restricted to individuals who were ε4 homozygous or heterozygous the age distribution profile was similar for AD and controls, confirming that it is the overall prevalence of ε4 which is increased in AD.

An extended analysis for the 75–85-year-old AD patients was made possible by the large study of Davignon et al[18] on healthy octogenarians living in eastern Canada. Figure 2 illustrates the phenotype distribution of apoE in 236 healthy octogenarians and in the 37 octogenarians with AD in our study. The increase in apoE4/3 in the AD octogenarian population is accompanied by a reduction in E3/3 and E3/2.

	AD			Controls		
	All (n=91)	M (n=30)	F (n=61)	All (n=74)	M (n=29)	F (n=45)
Age (yr)	75 1 (10 3)	75 3 (11 1)	75 1 (9 8)	75 8 (9 6)	75 9 (10 4)	75 8 (9 0)
MMS	16 9 (6 6)	17 9 (7 6)	16 0 (5 9)	28 3 (1 9)	28 4 (1 9)	28 4 (1 9)
Cases in family	44 (48%)*	21 (23%)	18 (20%)	11 (15%)	3 (4%)	8 (11%)
Down's syndrome in family	2 (2%)	1 (1%)	1 (1%)	2 (3%)	1 (1%)	1 (1%)

Mean (SD) for age and MMS. *% of M + F.

Table 1: **Demographic and clinical characteristics**

	Normo-lipidaemic (n=102)	AD			Controls		
		All (n=91)	F (n=61)	M (n=30)	All (n=71)	F (n=45)	M (n=29)
Phenotype (%)							
E4/4	3 9	13†	13	13	3	2	3
E3/3	61 7	33†	25	50	57	62	48
E2/2	2 0	0	0	0	0	0	0
E4/3	20 6	48†	56	33	19	18	21
E3/2	9 8	3†	3	3	22	18	28
E4/2	2 0	2†	3	0	0	0	0
Allele (frequency)							
ε4	0 152	0 38*	0 426‡	0 300	0 122	0 113	0 137
ε3	0 770	0 59*	0 542‡	0 684	0 770	0 799	0 725
ε2	0 078	0 03	0 032‡	0 016	0 088	0 088	0 138

*p < 0 01 and †p < 0 001 versus controls; ‡p < 0 01 versus men.

Table 2: **Apo E phenotype and allele frequencies**

Analysis of the gene dosage effect revealed a correlation between the age of onset and the ε4 allele copy number ($p = 0{\cdot}027$) in AD. As age of onset increased, ε4 allele copy number decreases (ie, average ages of onset for ε4/4, ε4/3, and ε3/3 are 70·4, 74·5, and 77·6, respectively).

Discussion

Plasma lipoprotein concentrations are modulated by apoE genotype and differences in aminoacid sequence of apoE are major determinants of plasma cholesterol within a population. ApoE has a key role in the clearance of cholesterol from plasma and reverse cholesterol transport and the avidity of apoE-containing lipoproteins for lipoprotein receptors increases from apoE2 to apoE3 to apoE4. Plasma cholesterol, LDL-cholesterol, and apoB rise and apoE falls with increasing allele number (ε2 to ε3 to ε4). Our results indicate that the ε4 allele is increased in frequency in sporadic AD, consistent with the report of Strittmatter et al[10] on ε4 frequency in late-onset familial AD. Those workers reported an ε4 prevalence of 0·52

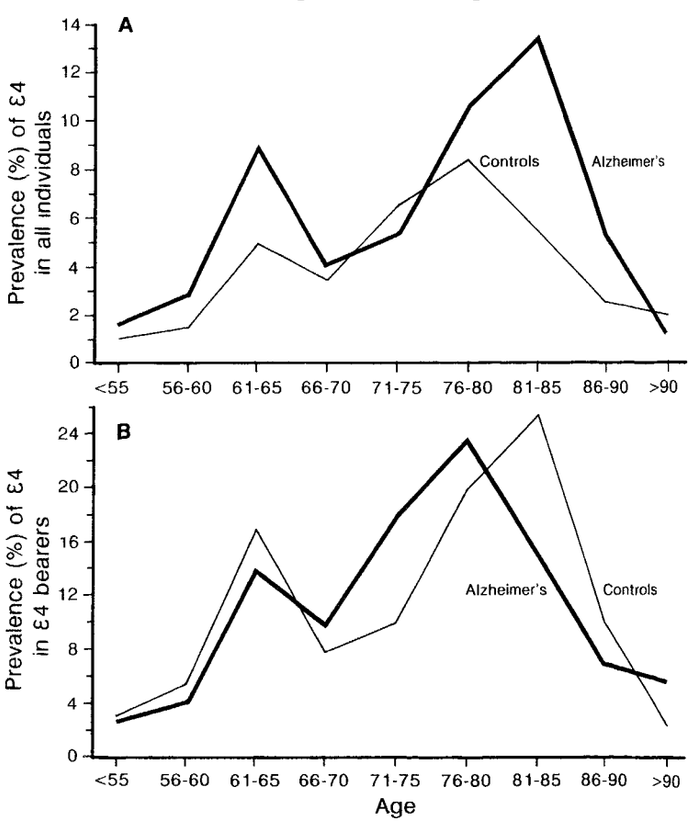

Figure 1: **Distribution of ε4 allele prevalence by age**

(A) All individuals in AD and control groups. (B) Individuals homozygous or heterozygous for ε4.

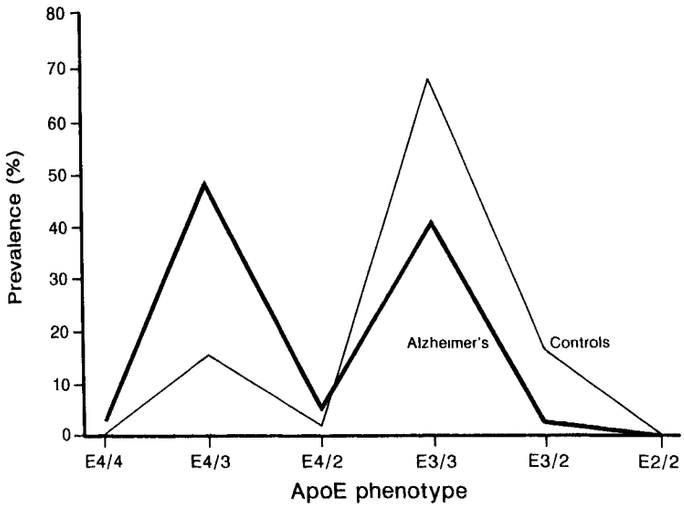

Figure 2: **ApoE phenotype distribution in AD and control octogenarians**

whereas this frequency was 0·38 in our sporadic cases. In our sporadic AD patients with age at onset greater than 70 the ε4 frequency was 0·36, indicating that factors other than age account for the differences between the familial and the sporadic series. The familial study measured allele frequency by polymerase chain amplification on post-mortem genomic DNA whereas the phenotype was examined in a living population of AD patients; furthermore families of several ethnic origins were used in the familial study while the controls were from France. Our AD patients and controls are both from eastern Canada, and the vast majority of them were born in the Province of Quebec. Since apoE allele frequencies vary in different populations[21] it is crucial to select AD and control groups, with a uniform genetic background.

In AD we found two peaks in ε4 prevalence, at 55–65 and 75–85 years. This is consistent with the concept of an early and a late onset form of AD. Those two entities are also found in familial AD, for which distinct chromosomal loci have been reported.[11,22] Interestingly, when all the elderly individuals from our study and those healthy octogenarians from Davignon et al[18] are pooled (n = 418), 83% of all the homozygote ε4/4 individuals were diagnosed with AD. This ratio is similar to the 91% ratio reported for the familial cases.

Analysis of the octogenarians revealed an increase in E4/3 phenotype in AD but not in E4/4, which might have been expected. However, ε4 homozygotes are at high risk of atherosclerosis[23] and may therefore have been selected against by age.

ApoE can interact directly with βA4 amyloid to form a stable adduct[6,10] and it is also found in senile plaques and neurofibrillary tangles.[5,6] This co-localisation of apoE with the major neuropathological features of AD plus the enrichment of the ε4 allele suggest some relationship to the cause of AD. One possibility is that the age-related decline in cell number and lipid content that happens normally in the human brain is exacerbated by the presence of the ε4 allele(s) in susceptible individuals. Furthermore, if apoE disrupts lipase activity[24] in the brain and alters the transport of cholesterol and phospholipids in brain areas vulnerable to ageing, a direct consequence would be aberrant and/or defective reinnervation and poor synaptic plasticity. For example, reports on cerebral cortex integrity in AD have revealed a 45% decrease in presynaptic terminal density in the neocortex[25] and significant synaptic losses in the frontal and temporal cortices.[26]

We thank Mrs Nicole Aumont and Nathalie Buisson for their technical support. Supported by grants from the American Alzheimer Association (JP), National Institute on Ageing (AG-10003; JP), Medical Research Council of Canada (JP), and LCDC (SG).

References

1 Poirier J, Hess M, May PC, Finch CE. Apolipoprotein E- and GFAP-RNA in hippocampus during reactive synaptogenesis and terminal proliferation. *Mol Brain Res* 1991; **11:** 97–106.
2 Boyle JK, Zoellner CD, Anderson LJ, et al. A role for apolipoprotein E, apolipoprotein A-1, and low density lipoprotein receptors in cholesterol transport during regeneration and remyelination of rat sciatic nerve. *J Clin Invest* 1989; **83:** 1015–31
3 Leblanc AC, Poduslo JF. Regulation of apolipoprotein E gene expression after injury of the rat sciatic nerve. *J Neurosci Res* 1990; **25:** 162–71.
4 Mahley RW. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science* 1988; **240:** 622–30.
5 Namba Y, Tomonaga M, Kawasaki H, et al. Apolipoprotein E immunoreactivity in cerebral amyloid deposits and neurofibrillary tangles in Alzheimer's disease. *Brain Res* 1991; **541:** 163–66.
6 Wisniewski T, Frangione B. Apolipoprotein E: a pathological chaperone protein in patients with cerebral and systemic amyloid. *Neurosci Lett* 1991; **135:** 235–38.
7 Poirier J, Baccichet A, Dea D, Gauthier S. Role of hippocampal cholesterol synthesis and uptake during reactive synaptogenesis in adult rats. *Neuroscience* 1993; **55:** 81–90.
8 Poirer J, Hess M, May PC, Pasinetti G, Finch CE. Astroglial gene expression during reactive synaptogenesis. In: Abraham EH, Tanaka K, eds. Basic and therapeutic strategies in Alzheimer's and Parkinson's disease. New York: Plenum Press, 1991:191–94.
9 Wisniewski T, Golabek A, Matsubara E, et al. Apolipoprotein E: binding to soluble Alzheimer's B-amyloid. *Biochem Biophys Res Commun* 1993; **192:** 359–365.
10 Strittmatter WJ, Saunders AM, Smechel D, et al. Apolipoprotein E: high-avidity binding to the B-amyloid and increased frequency of type 4 allele in late onset familial Alzheimer's disease. *Proc Natl Acad Sci USA* 1993; **90:** 1977–81.
11 Schellenberg GD, Boehnke M, Wijsman EM, et al. Genetic association and linkage analysis of the apolipoprotein CII locus and familial Alzheimer's disease. *Ann Neurol* 1992; **31:** 223–27.
12 Mucjkle TJ, Roy JR. High-density lipoprotein cholesterol in differential diagnosis of senile dementia. *Lancet* 1985; i: 1191–92.
13 Guibilei F, D'Antona R, Antonini R, et al. Serum lipoprotein pattern variation in dementia and ischemic stroke. *Acta Neurol Scand* 1989; **81:** 84–86.
14 Ransmayr G, Cervera P, Hirsch E, et al. Choline acetyl transferase-like immunoreactivity in the hippocampal formation of controls subjects and patients with Alzheimer's disease. *Neuroscience* 1989; **32:** 701–14.
15 Represa A, Duyckerts C, Tremblay E, et al. Is senile dementia of the Alzheimer type associated with hippocampal plasticity? *Brain Res* 1988; **457:** 355–59.
16 Honer WG, Dickson DW, Gleeson J, et al. Regional synaptic pathology in Alzheimer's disease. *Neurobiol Aging* 1992; **13:** 375–82.
17 Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. *Atherosclerosis* 1988; **8:** 1–20.
18 Davignon J, Bouthillier D, Nestruck AC, et al. Apolipoprotein E polymorphism and atherosclerosis: insights from a study in octogenarians. *Trans Am Clin Climatol Assoc* 1987; **99:** 100–10.
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 the department of Health and Human services task force on Alzheimer's disease. *Neurology* 1984; **34:** 939–44.
20 Bouthillier D, Sing CF, Davignon J: Apolipoprotein E phenotyping with a single method: application to the study of informative mating. *J Lipid Res* 1983; **24:** 1060–69.
21 Hallman DM, Boerwinckle E, Saha N, et al. Apolipoprotein E polymorphism: a comparison of allele frequencies and effects in nine populations. *Am J Hum Genet* 1991; **49:** 338–49.
22 Schellenberg GD, Bird TD, Wijsman EM, et al. Genetic linkage evidence for a familial Alzheimer's disease locus on chromosome14. *Science* 1992; **58:** 668–71.
23 Cumming AM, Robertson F. Polymorphism at the apo E locus in relation to risk of coronary disease. *Clin Genet* 1984; **25:** 310–18.
24 Yamada N, Murase T. Modulation, by apolipoprotein E, of lipoprotein lipase activity. *Biochem Biophys Res Commun* 1980; **94:** 710–15.
25 Masliah E, Terry RD, DeTeresa RM, et al. Immunohistochemical quantification of synapse-related protein synaptophysin in Alzheimer's disease. *Neurosci Lett* 1989; **103:** 234–39.
26 Davies CA, Mann DMA, Sumpter PQ, et al. Quantitative morphometric analysis of the neuronal and synaptic content of the frontal and temporal cortex in patients with Alzheimer's disease. *J Neurol Sci* 1987; **78:** 151–64.