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Apolipoprotein E ε4 allele is a risk factor for familial and sporadic presenile Alzheimer's disease in both homozygote and heterozygote carriers

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Abstract

While apoliprotein E (ApoE) ε4 allele is now a well established risk factor for familial and sporadic senile Alzheimer's disease (AD), its role in the development of the rarer presenile or early onset type is controversial. Early studies showed no association; later ones found enrichment for the ε4 allele in familial or sporadic types or both. We have ApoE genotyped a series of Scottish people (n = 85) with early onset AD. We find highly significant enrichment for both homozygote and heterozygote ApoE ε4 allele carriers in familial *and* sporadic early onset AD with a pattern closely resembling that in late onset AD.

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AD is a neurodegenerative disorder characterised by β amyloid peptide deposits in senile plaques and cerebral blood vessel walls, and neurofibrillary tangles (NFTs) within neurones of the cerebral cortex and hippocampus. ApoE has been found localised to senile plaques, vascular amyloid, and NFTs[1 2] and one allele, ε4, is significantly associated with familial and sporadic late onset AD.[2–8] ApoE is a 34 kDa protein that is involved in cholesterol and triglyceride transport, mediating their clearance from plasma by interaction with low density lipoprotein receptor.[9] Unlike other lipoproteins which are mainly synthesised in the liver, ApoE is also synthesised in astrocytes and oligodendrocytes in the central nervous system.[9] It has three isoforms corresponding to alleles ε2, ε3, and ε4. The isoform corresponding to ε4 shows high avidity binding to β amyloid peptide,[2] and it is suggested that this accelerates β amyloid deposition and so the development of AD.[1 10] Although numerous studies have now shown an association between the ApoE ε4 allele and both familial and sporadic late onset AD,[2–8] the relationship with presenile/early onset AD (<65 years) is still controversial. Initial studies failed to find a significant association between the ApoE ε4 allele and the risk of familial early onset AD.[11–13] However, most data in these early studies came from AD families with a mutation in the amyloid precursor protein (APP) gene or linked to a locus on chromosome 14. When, in later reports, patients were examined who did not have APP mutations and, based on age of onset, were less likely to be chromosome 14 linked cases,[14–16] positive associations with the ApoE ε4 allele were observed. This suggests that genetic heterogeneity among early onset AD must be taken into account when interpreting ApoE associations. Chartier Harlin *et al*[14] found the ε4 allele significantly raised in 34 sporadic early onset English AD cases, and Okuizumi *et al*[15] found similar findings in 44 sporadic early onset Japanese cases. The largest study of early onset AD to date, based on a population in the northern Dutch provinces and Rotterdam,[16] gave an overall frequency for the ε4 allele in early onset AD of 35%. When patients were subdivided by family history (positive n = 107 and negative n = 68) the ε4 allele was 1·6 times higher in the family history positive group (41%) compared to the family history negative group (25%). Further analysis showed that an increased risk of early onset AD exists for ε4 homozygote carriers regardless of family history of dementia, but increased risk of early onset AD for ApoE ε4 heterozygote carriers could only be shown in subjects with a positive family history. This suggested that while a single ε4 allele increases the risk of late onset AD, alone it is insufficient to increase the risk of onset of illness before the age of 65 years.[17] In order to address these questions further we have ApoE genotyped a cohort of serially collected Scottish cases with a definite or probable diagnosis of early onset AD and compared ApoE ε4 allele frequency in familial and sporadic cases with non-demented control groups.

Methods

PATIENT AND NORMAL DNA SAMPLES

Early onset AD (n = 85)

All met NINCDS criteria for definite (n = 19) or probable AD (n = 66).[17] Definite cases died in either psychiatric or geriatric wards and had moderate or numerous plaques and tangles in frontal and temporal cortex and hippocampus. Cases were collected by the MRC Brain Metabolism Unit between 1979 and 1988. Probable cases were identified in hospital wards or referred by clinical colleagues throughout the central belt of Scotland. Age of onset averaged 57 years (SD 6), (28 M, 57 F). Age at onset was defined as the age at which memory loss or change in behaviour was first noticed. All cases were screened for mutations of the amy-

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Table 1 Frequencies of ε2, ε3, and ε4 apolipoprotein E alleles in early onset AD, late onset AD, and non-demented control groups

	Allele frequencies		
	ε2	*ε3*	*ε4*
Early onset AD (n=85)	0·06	0·53	0·41
Late onset AD (n=68)	0·01	0·60	0·38
Non-demented controls (n=47)	0·03	0·84	0·13
Scottish population (n=400)	0·08	0·77	0·15

Highly significant ($p<0·001$) differences between ε4 frequency in early onset AD and both control groups. No significant differences between early onset and late onset AD groups.

loid precursor protein gene. None was found.[18] A systematic enquiry concerning a family history of dementia was made in all cases through spouses and first degree relatives and hospital case notes. Fourteen cases were defined as familial, where there was evidence of early onset AD in a first degree relative. Either clinical symptoms began before 65 or the patient required long term care before 70 years. Seventy-one cases were defined as sporadic early onset AD; either no AD or only late onset AD was identified in first degree relatives after systematic enquiry.

Late onset AD (n=68)

The mean age of death was 81 (range 69–91) (19 M, 49 F). All died in hospital and had clinical and neuropathological features to satisfy NINCDS criteria for diagnosis of definite AD.[18]

Aged, non-demented controls (n=47)

The mean age of death was 78 (range 67–91) (29 M, 18 F). All had a full neuropathological examination and showed no features of AD.

Population data were also available for ApoE allele frequencies in Scotland[19] (n=400). Mean age was 53 (SD 0·22). ApoE allele frequencies in the last three groups have already been described.[19 20]

ApoE ε4 allele status was determined on genomic DNA samples from the first three groups by polymerase chain reaction (PCR) amplification of ApoE gene sequences followed by restriction isotyping. The method used was essentially that described by Wenham *et al*[21] with the exception that 6% metaphor agarose gels were used for product visualisation.

Results

Table 1 gives the ApoE allele frequencies for each of the four groups. Highly significant ($p<0·001$) differences were observed in ApoE ε4 allele frequency in both early and late onset AD when compared to non-demented control groups. By contrast, no significant differences were detectable between the dementia groups themselves or between the two control groups. There was also no correlation within either group of ApoE ε4 status with age of onset of AD.

Table 2 gives ApoE ε4 allele numbers and percentages in early onset AD and control groups, as well as separate figures for family history positive and negative cases. Highly significant ($p<0·001$) differences in ε4 allele numbers were found when the early onset AD group as a whole was compared to the non-demented controls or the Scottish population ($\chi^2=21·6$ and 72·3 respectively, 2 df). These differences remained highly significant when we analysed separately the family history positive ($\chi^2=13·1$, and 39·4, 2 df) and family history negative cases subgroups ($\chi^2=19·1$ and 63·1, 2 df).

Table 3 gives the ODDs ratios for early onset AD associated with the ApoE ε4 allele. The genotype frequencies of controls are estimated from population allele frequencies under Hardy-Weinberg assumptions. The risk of early onset AD is 4·6 times higher for carriers of at least one ApoE ε4 allele compared to subjects without an ApoE ε4 allele. The greatest increase is in those with a family history, but does not differ significantly from those without a family history. It is also increased more than four-fold in homozygous ε4 carriers compared to heterozygous carriers.

Table 2 Apolipoprotein E ε4 alleles in early onset AD

ApoE ε4 allele	*All early onset AD (n=85)*	*Family history*		*Controls*	
		+ve (n=14)	*−ve (n=71)*	*Non-demented (n=47)*	*Population (n=400)*
Frequency	0·41	0·46	0·40	0·13	0·15
Copy number					
2	15 (17·6%)	3 (21%)	12 (17%)	1 (2%)	4 (1%)
1	40 (47%)	7 (50%)	33 (47%)	10 (22%)	110 (27%)
0	30 (35·2%)	4 (28%)	26 (37%)	36 (76%)	286 (71%)

Table 3 Odd ratios for early onset associated with the ApoE ε4 allele

Number of ApoE ε4 alleles (0 is reference)	*All*	*Positive family history*	*Negative family history*
1 or 2	4·88 (2·96; 8·02)	6·65 (2·00; 22·1)	4·60 (2·70; 7·85)
1	3·85 (2·29; 6·48)	5·06 (1·42; 18·0)	3·67 (2·10; 6·41)
2	16·7 (7·99; 35·0)	25·1 (5·19; 120·9)	15·4 (6·97; 34·1)

Reference is genotype frequencies estimated from population controls under Hardy-Weinberg assumption.
( )=95% confidence limits.

$$\text{Odds ratio} = \frac{\text{Prob (affected/carrier [family history])}}{\text{Prob (affected/non-carrier [family history])}}$$

Figures in columns 3 and 4 require the assumption that genotype frequencies in the general population do not depend on the family history.

Discussion

Our study shows an increased risk of early onset AD in ApoE ε4 allele carriers. The ε4 allele frequency was at least 2·5 times greater in the early onset AD group than in both non-demented control samples irrespective of family history, and the increase was highly statistically significant. The increased risk of early onset AD was greatest in ε4 homozygotes (16 times) but was also substantial in heterozygotes (3·7 times) compared to non-ε4 carriers. The differences between family positive and family negative cases were not statistically significant. Our results agree, therefore, with Van Duijn *et al*[16] in showing raised ε4 allele frequency in familial early onset AD and with Chartier Harlin *et al*[14] and Okuizumi *et al*[15] in showing a raised ε4 frequency in sporadic cases. We differ from Van Duijn *et al*[16] in finding a significant ($p<0·001$) increase in sporadic cases of ε4 heterozygotes as well as homozygotes.

The reasons why earlier studies failed to show ApoE ε4 allele increase in early onset AD have already been discussed. They were often based on small numbers and may have lacked statistical power, but more important was selection bias. Cases examined were mostly derived from rare families with mutations of the APP gene or linked to chromosome 14. They might be expected to differ in terms of genetic risk factors from the commoner early onset cases present in the general population. Indeed when such latter cases are examined, as in the Dutch and our series, the results are remarkably similar. The only differences in the ApoE ε4 allele frequencies are in the sporadic cases where, in contrast to the Dutch series, ε4 heterozygote carriers were significantly increased. This discrepancy may be because of population differences. Also the inclusion criteria for familiality differ between the two studies. The Dutch group included as familial all cases where a first degree relative had AD, irrespective of age of onset. In our series we classified as familial cases only those with a first degree relative with early onset AD (see above). The two sets of findings are therefore not directly comparable.

The main implication of our data is that the ApoE ε4 allele is a risk factor for early onset AD. While the risk is greatest in homozygotes it is also raised in ε4 heterozygotes. It is a risk factor in familial and sporadic cases. The findings are similar to those observed in late onset AD. This means that the relative risk does not change, that is, if AD occurs in ε4 carriers it is as likely to be the commoner late onset AD as in non-ε4 carriers. However, our findings again raise the issue as to whether from a clinicoepidemiological point of view the two should not be considered as a single population. Finally, the study highlights the risk in extending to early onset AD as a whole observations made on rare subgroups of autosomal dominant families where findings may be locus specific.

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