Apolipoprotein E4 allele in a population-based study of early-onset Alzheimer's disease

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Several studies have reported an association of the apolipoprotein E allele ε4 (APOE*4) to familial and sporadic late-onset Alzheimer's disease (LOAD). Here we report on the relationship between APOE*4 and early-onset Alzheimer's disease (EOAD) in a Dutch population-based study. The frequency of the APOE*4 allele was 2.3 times higher among EOAD cases compared to controls. Among patients, the allele frequency was 1.6 times higher in those with a positive family history than in those without. A significant increase in risk of EOAD was found for subjects homozygous for APOE*4 regardless of family history of dementia, but an increase in EOAD risk for APOE*4 heterozygotes could only be shown in subjects with a positive family history. Our study demonstrates a significant association between APOE*4 and EOAD which is modified by family history of dementia.

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by β amyloid deposits in the parenchymal senile plaques and cerebral blood vessel walls and by neurofibrillary tangles (NFT) within neurons of the cerebral cortex and hippocampus. Apolipoprotein E (apoE) is a polymorphic protein that plays a central part in the metabolism of cholesterol and triglycerides. ApoE has three major isoforms — E2, E3 and E4. Their biosynthesis is controlled by a single locus at chromosome 19q13.2 with three codominant alleles ε2, ε3 and ε4 (refs 1, 2).

Roses and colleagues have described a significant association between familial and sporadic late-onset Alzheimer disease (LOAD) and the ε4 allele of the apolipoprotein E gene (APOE*4)[3]. In familial LOAD, the onset age of AD decreased as the number of APOE*4 alleles increased[4]. The association of LOAD with the APOE*4 allele has been confirmed in a number of independent studies[5–10]. However, it remains to be determined if APOE*4 is a primary genetic cause, which by itself is sufficient to cause AD, or merely a genetic factor modulating the expression of the AD phenotype determined by another genetic or environmental cause. A biological role of apoE in the AD pathogenesis is suggested by immunohistochemical analysis showing that apoE is present in AD brain lesions[11,12]. Furthermore, *in vitro* experiments indicate that apoE in cerebral spinal fluid binds to β amyloid with high affinity[3,12].

Genetically AD is a heterogeneous disorder. LOAD has been linked to chromosome 19q13.2 in a linkage study of familial LOAD[13] and in association studies of familial and sporadic LOAD[3–10]. Early-onset familial AD (onset at or before 65 years: EOAD) has been linked to mutations of the β amyloid precursor protein (APP) gene on chromosome 21 (q21.2)[14–19], as well as to chromosome 14 (q24.3)[20–23]. Studies of familial EOAD have not yielded significant evidence for a relationship between APOE*4 and the risk of familial EOAD[7,10,24–26]. Family members with known APP717 and chromosome 14 mutations did not differ significantly in APOE*4 allele frequencies from population controls[7,24,26]. In one EOAD family in which there were seven living patients with the App670/671 double mutation, a yougner onset age was found in patients with a APOE*4 allele, with the youngest onset in the one patient homozygous for APOE*4 (ref. 25). The difference in onset age however, was not statistically significant.

We have examined the association between APOE*4 and EOAD in a population-based study of 175 EOAD patients, in whom the onset of disease was between 34 and 65 years. Our intent was to examine the modification of the association by family history of dementia. Our results show that APOE*4 homozygosity is sufficient to increase the risk of both familial and sporadic EOAD, and that an increase in risk of EOAD for APOE*4 heterozygotes could only be shown in subjects with a positive family history. We therefore postulate that in heterozygotes the APOE*4 allele by itself is not able to increase the risk of AD at an early age, but may modify the expression of the AD phenotype determined by other genetic and/or environmental factors underlying the familial aggregation of AD.

Subjects

Patients were derived from a population-based epidemiologic study of EOAD[27]. The study aimed at a

Table 1 Description of the study population

	Cases			Controls		
	Total	Men	Women	Total	Men	Women
Number (%)	175 (100%)	59 (34%)	116 (66%)	159 (100%)	64 (40%)	95 (60%)
Age at onset in years (s.d.)	57 (5.0)	56 (5.5)	57 (4.6)	–	–	–
Age at diagnosis in years (s.d.)	61 (4.2)	61 (4.3)	63 (4.2)	–	–	–
Attained age at study in years (s.d.)	63 (4.4)	63 (4.7)	63 (4.1)	63 (4.4)	63 (5.0)	63 (3.8)
Family history in first degree relatives (% of total)	107 (61%)	35 (59%)	72 (62%)	44 (28%)	18 (28%)	26 (27%)

complete ascertainment of all EOAD patients in four Northern provinces of the Netherlands and the area of metropolitan Rotterdam in whom onset was at or before the age of 65 years. Age at onset is defined as the age at which memory loss or change in behaviour is first noted[27]. For this study, the clinical diagnosis of AD was independently confirmed by two neurologists using a standardized protocol according to the NINCDS-ADRDA criteria for AD[27,28]. Of the 201 eligible patients, 198 (99%) participated in the study. For each patient a control subject was selected, matched for age (within 5 years) and place of residence. These controls were drawn randomly from the population register of the municipality of the patient at the time of diagnosis. Of all invited control subjects (n=325), 198 (61%) agreed to participate in the study. Cognitive status of all control subjects was tested and none of them showed symptoms of dementia at the time of the study.

For all patients and controls, detailed data on family history of dementia in first, second and third degree relatives were collected by interviewing a next of kin. To increase the validity, the family data were always verified by a second informant who was a sibling of the patient. The family history of patients and controls with no first degree relatives with dementia is classified as negative and of those with at least one first degree relative that suffered from dementia as positive. The pedigree is considered consistent with autosomal dominant inheritance of AD if there are at least three patients with dementia in two generations reported by relatives and if there are at least two patients with detailed medical records on the clinical diagnosis of AD. The pedigree structure of 19 out of the 198 patients was consistent with autosomal dominant inheritance. In 16 of these families, the proband was included in the APOE study presented here.

Blood samples for APOE typing were collected for a sample of 175 (89%) patients and 159 (80%) controls (Table 1). The mean age at onset of AD was 57 years (range:34–65 years), being lower than 60 years in 125 (71%) of the cases and lower than 50 years in 19 (11%) of the cases. In 62 (35%) of the cases, the diagnosis of AD was made at or before the age of 60 years. The attained age at the time of the study was similar for cases and controls.

Blood samples for DNA extraction were collected for a random sample of 100 patients. Their DNA was screened for mutations in exons 16 and 17 of *APP* by PCR sequencing (C.M.v.D. *et al.*, unpublished observations) and single-stranded conformational polymorphism analysis (SSCP)[29]. No mutations were found in any of the probands[29]. However, in the family of one proband (1302)[18], who did not carry a mutation in *APP* herself, a mutation in exon 17 of *APP* was found changing an amino acid at codon 692 (Ala to Gly)[18]. In this family, EOAD as well as cerebral haemorrhage due to amyloidosis was linked to the APP692 mutation[18]. The pedigrees of 10 of the 19 families with autosomal dominant inheritance of AD ascertained in our study population were informative for linkage studies. One family was conclusively linked to chromosome 14 (q24.3). Nine families could not be linked to chromosomes 14, 19 or 21. The probands of these 10 families were included in the present APOE analysis.

APOE*4 allele frequency

ApoE typing was performed in 175 patients and 159 age-matched controls (Table 1). In the case-series there were 107 patients with a positive family history of dementia, including the 16 patients with a pedigree structure consistent with autosomal dominant inheritance. Table 2 shows the APOE*4 allele distribution in patients and controls. The frequency of the APOE*4 allele in EOAD patients was 35% and 2.3 times higher than the APOE*4 allele frequency of 15% in controls. Stratification by family history of dementia showed that APOE*4 allele frequencies were similar for controls with a positive family history of dementia (18%) and controls with a negative family history (14%). Among patients, the APOE*4 allele frequency was 1.6 times higher in those with a positive family history (41%) than in those with a negative family history (25%). When the 16 autosomal dominant EOAD patients were excluded from the calculations, the frequency of the APOE*4 allele (37%) was still 1.5 times higher than in the patients with a negative family history. The APOE*4 allele frequencies were not significantly associated with sex or age in the patient or control series (Fig. 1). In the overall and the stratified analysis for sex, age at onset and family history of dementia, the frequency of the APOE*4 allele was significantly higher among EOAD cases (29–41%) as compared to controls (10–21%).

Table 2 APOE*4 allele distribution in EOAD cases and controls

No. of APOE*4 alleles	All		Positive family history		Negative family history	
	Patients n=175	Controls n=159	Patients n=107	Controls n=44	Patients n=68	Controls n=115
2	29 (17%)	5 (3%)	22 (21%)	2 (5%)	7 (10%)	3 (3%)
1	63 (36%)	38 (24%)	43 (40%)	12 (27%)	20 (30%)	26 (23%)
0	83 (47%)	116 (73%)	42 (39%)	30 (68%)	41 (60%)	86 (75%)

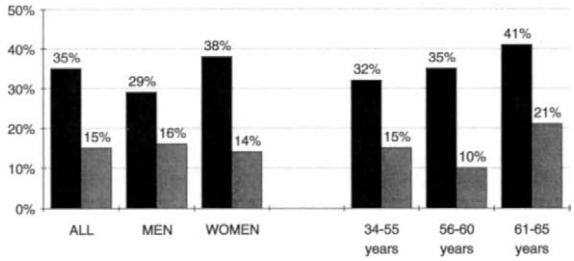

Fig. 1 APOE*E allele frequencies by sex and age. Black, cases; grey, controls.

Risk estimation

Risk of EOAD was 3.0 times highers for carriers of at least one APOE*4 allele as compared to subjects without an APOE*4 allele (Table 3). The association with EOAD was strongest in those with a positive family history. For subjects with a positive family history, the risk of EOAD was 3.3 times increased for APOE*4 carriers as compared to a 1.9 times increase in risk in those with a negative family history. The association between EOAD and the APOE*4 allele was not statistically significant for subjects with a negative family history (p=0.06).

When we take the number of APOE*4 alleles into consideration, the percentage of homozygotes (21%) for the APOE*4 allele in patients with a positive family history was significantly higher when compared to patients with a negative family history (10%) (Table 2). Comparing cases and controls, the risk of EOAD for the APOE*4 homozygote was signficantly higher than the risk for the heterozygote. There was an increase in risk of EOAD for the APOE*4 heterozygote for those with a positive family history and in the APOE*4 homozygote for those with a positive family as well as those with a negative history of dementia (Table 3). For APOE*4 heterozygotes with a negative family history, a non-significant odds ratio was found. Exclusion of the 16 autosomal dominant EOAD patients resulted in only minor changes in the risk estimates.

Family history and onset age

Among subjects that did not carry the APOE*4 allele, the risk of EOAD was 2.9 (95% CI 1.6–5.6) times higher for those with a positive family history of dementia as compared to those with a negative family history. For carriers of the APOE*4 allele, the risk was 5.0 (95% CI 2.1–11.8) times higher for those with a positive family history. These findings indicate that the APOE*4 allele cannot fully explain familial aggregation of EOAD.

Table 3 Odds ratios for EOAD associated with the APOE*4 allele

No. of APOE*4 alleles (0 is reference)	All	Positive family history	Negative family history
1 or 2	3.0 [1.9;4.7][a]	3.3 [1.6;7.0]	1.9 [1.0;3.7]
1	2.3 [1.4;3.8]	2.6 [1.2;5.7]	1.6 [0.8;3.2]
2	8.1 [3.0;21.8]	7.9 [1.7;36.0]	4.9 [1.2;19.9]

[a]95% confidence interval, in square brackets.

In our population-based series of EOAD patients, the APOE*4 allele was not associated with onset age. The mean onset age was 57.1 (s.d. 4.7) years in carriers of one or more APOE*4 allele and 56.5 (s.d. 5.2) in non-carriers. Among patients with a positive family history, the mean onset age was 57.4 (s.d. 4.8) years in APOE*4 carriers and 56.7 (s.d. 5.1) years in non-carriers. Among patients with a negative family history, the mean onset age was 56.5 (s.d. 4.4) years in APOE*4 carriers and 56.3 (s.d. 5.5) years in non-carriers.

Families with autosomal dominant inheritance

In 16 out of the 19 families with autosomal inheritance of AD, DNA from an EOAD patient was available for APOE typing. Among probands, the frequency of the APOE*4 allele was 59%, which was significantly higher than the allele frequency among the other patients with a positive family history (37%). Among probands 88% carried at least one APOE*4 allele and 31% were homozygous. APOE was typed in all affected relatives in the 19 families. In two families (1104 and 1083), none of the patients carried an APOE*4 allele. With the exception of two families (1270 and 1302)[18], tested affected relatives within a family were fully concordant for the APOE genotype. In one (1302) of the two discordant families, EOAD and cerebral haemorrhage due to amyloidosis were linked to a mutation at codon 692 of the APP gene[18]. Therefore, the APOE data from this family were excluded from the present analysis. In agreement with the increased risk of AD associated with the APOE*4 allele, the age at onset in the other family (1270) decreased with increasing number of APOE*4 alleles. The onset in subjects with the E3E3 genotype was 78 years (n=2), the E3E4 genotype 71 years (n=1) and the E4E4 genotype 47 years (n=1).

Discussion

Our study shows that the risk for EOAD increases for subjects that carry the APOE*4 allele. A significant increase in risk of EOAD exists for E4 homozygous subjects regardless of family history of dementia. An increase in risk of EOAD for APOE*4 heterozygotes could only be shown in subjects with a positive family history. APOE has been associated with lipid metabolism and the risk of cardiovascular disease[30]. As patients with cardiovascular disease are more likely to be excluded from the case series according to the NINCDS-ADRDA protocol for the clinical diagnosis of AD, but may occur in the control population[28], there is a potential bias in studies of the role of the APOE gene in AD. However, this does not explain our findings because having excluded all patients and controls with cardiovascular disease, the risk estimate for the APOE*4 allele remained significant (OR APOE*4 3.1; 95% CI 1.8-5.3).

Although there is ample evidence for an association between familial and sporadic LOAD and APOE*4 (refs 3–10) studies of familial EOAD have not confirmed an association with APOE*4 (refs 7,10,24–26). Our population-based study including 175 EOAD patients provides empirical evidence that the risk for familial as well sporadic EOAD is increased for subjects that carry the APOE*4 allele, being highest for the familial form of EOAD. The lack of association in earlier studies[7,10,24–26] may be explained by the limited statistical power[10] and the occurrence of selection bias when studying families selected for linkage analysis[7,24–26].

In our population, 20% of the familial AD patients and 31% of the patients with autosomal dominant EOAD were homozygous for APOE*4. This higher percentage compared to previous studies of LOAD (13–17%)[4,6,10] corroborates the finding of Corder *et al.*[4] suggesting that in familial LOAD, the onset age decreased as the number of APOE*4 alleles increased. In that study[4], the average age at onset in familial LOAD was estimated to be 68 years in subjects with two APOE*4 alleles. Our data show that the onset of AD in APO-E4E4 homozygotes may be considerably earlier, as the mean age at onset of AD in our study was 57 years (ranging from 34 to 65 years). In our population-based case-series of EOAD patients, we could not show an association between the APOE*4 allele and onset age in the patients, regardless of family history. We cannot exclude the possibility, however, that the truncation of the age of onset distribution of our study population at age 65 years may have limited the statistical power to show an effect of the APOE*4 allele.

We have found a significant increase in risk for EOAD for APO-E4E4 homozygotes, being highest in those with a positive family history of dementia. However, a significant association was also observed for subjects without family history. Assuming that family history of dementia is a valid proxy measurement for other genetic factors, this finding suggests that APOE*4 homozygosity is sufficient to increase the risk of EOAD in the absence of other genetic factors. An important question to be resolved in future epidemiologic studies is to determine what percentage of the APOE*4 homozygotes, with or without a family history of AD, develop either EOAD or LOAD.

A significant association between EOAD and APOE*4 heterozygosity could only be shown in subjects with a positive family history. The fact that the odds ratio was not statistically significant increased among subjects with a negative family history suggests that carrying one APOE*4 allele may not be sufficient to increase the risk of AD before the age of 65. As a number of studies have reported an association of LOAD with the APOE*4 allele[3–10] we consider it likely that an age-related factor may be necessary to increase the risk of AD among carriers of one APOE*4 allele.

The lack of association between the APOE*4 heterozygous genotype and EOAD among subjects with a negative family history as well as the higher risk of EOAD associated with the APO-E4E4 genotype for patients with a positive family history indicates that the APOE*4 allele may modify the expression of other genetic factors underlying the familial aggregation in those with a positive family history. This is supported by the finding that among APOE*4 carriers, the risk of EOAD increased for those with a positive family history of dementia. Also the extremely high percentage (88%) of carriers of the APOE*4 allele in families with evidence for autosomal inheritance of AD, while none of the families showed statistically significant evidence for linkage to chromosome 19, is compatible with this hypothesis.

To our knowledge, this is the first study that shows there is a significant association between APOE*4 and EOAD, which is modified by family history of dementia. Our study indicates that APOE*4 homozygosity may be sufficient to increase the risk of EOAD in the absence of other apparent genetic factors, and suggests that in APOE*4 heterozygosity, interaction with other genetic or age related, factors may be necessary to increase the risk of EOAD. Finally, the APOE*4 allele cannot fully explain familial aggregation of EOAD as among APOE*4 carriers as well as non-carriers the risk of EOAD increased significantly for those with a positive family history of dementia.

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Methodology

APP mutation analysis. All patient DNA's were analysed for the APP717(Val to Ile)[14] mutation by restriction enzyme digestion with *BclI* of the PCR product obtained by amplification of exon 17 of the APP gene using the primers and amplification conditions as described[31]. In addition we used SSCP analysis to screen for the APP670/671 mutation[19] in exon 16 and the APP717(Val to Gly)[15], APP692(Ala to Gly)[18] and APP693(Glu to Gln)[32] mutations in exon 17 of the *APP* gene[33]. In the SSCP analysis exons 16 and 17 were amplified in the presence of α-^{32}P dCTP using published primer and amplification conditions[31,34]. After denaturation the PCR products were loaded on a 1× Hydrolink gel for the APP717 mutations and on a 1× Hydrolink gel containing 10% glycerol for the APP670/671, APP692 and APP693 mutations and electrophoresed during 22 h at 10 W. Under these conditions the different mutations are visible as bands with altered mobility are apparent in positive control DNA's containing the respective mutation. In nine patients belonging to families with an autosomal dominant inheritance of AD, the sequence of exons 16 and 17 was determined.

APOE analysis. For 100 randomly selected patients, genomic DNA was used for genotyping. The APOE gene was amplified in the presence of α-^{32}P dCTP using primers and amplification conditions as described[35]. The PCR product was digested with *HhaI* and fragments were separated by electrophoresis on a 6% non-denaturing polyacrylamide gel[24]. For the remaining 75 patients and all controls, ApoE typing was performed in stored serum samples frozen at –80 °C. A rapid micromethod was used based on isoelectric focusing of delipidated serum samples followed by immunoblotting[36] using a polyclonal anti-apoE antiserum.

Statistical analysis. Allele frequencies for patients and controls were assessed by counting alleles and calculating sample proportions. The Z statistic was used to compare allele frequencies between groups[37]. When comparing allele frequencies between groups, p values lower than 0.05 are regarded statistically significant. The strength of association between a genotype and EOAD was estimated as the odds ratio, which is an estimate of the relative risk (that is, the risk of EOAD for genotype carriers divided by the risk of EOAD for non-carriers)[38]. Odds ratios are presented with 95% confidence intervals based on Woolf's method[38]. If there is no association between the genotype and EOAD, the odds ratio will equal 1. If there an increased risk of EOAD associated with the genotype, the odds ratio will be higher than 1, while an odds ratio lower than 1 is anticipated if the genotype is associated with a decreased risk of EOAD. The risk of EOAD is considered statistically significant ($p<0.05$) increased or decreased if the 95% confidence interval does not include unity.

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