

SHORT REPORT

No effect of the $\alpha 1$ -antichymotrypsin A allele in Alzheimer's disease

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Abstract

The apolipoprotein E (ApoE)- $\epsilon 4$ allele is associated in a dose dependent manner to an increased risk for Alzheimer's disease. However, the ApoE- $\epsilon 4$ allele effect does not account for all patients with Alzheimer's disease, and the existence of other genetic risk factors has been postulated. Kamboh *et al* reported an association between Alzheimer's disease and the A allele of $\alpha 1$ -antichymotrypsin (Aact) gene, which was not confirmed in a larger series more recently analysed. The ApoE and Aact genotypes were analysed in 314 patients with Alzheimer's disease and 173 healthy controls, confirming the dose dependent effect of the ApoE- $\epsilon 4$ allele. Nevertheless, even using odds ratios adjusted for age and sex, there was no significant effect of the Aact genotype on Alzheimer's disease or on the ApoE- $\epsilon 4$ allele associated risk for Alzheimer's disease.

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Keywords: $\alpha 1$ -antichymotrypsin (Aact); apolipoprotein E (ApoE); Alzheimer's disease

Alzheimer's disease represents the major cause of dementia in elderly people. In a small subset of patients, mostly those with early onset,¹ there is autosomal dominant inheritance. In these cases, mutations have already been found in three genes: amyloid precursor protein on chromosome 21, presenilin 1 on chromosome 14, and presenilin 2 on chromosome 1.² These mutations account for a small percentage of the patients. An association between late onset Alzheimer's disease and the apolipoprotein E (ApoE)- $\epsilon 4$ allele has been reported.³ This finding has been confirmed by numerous studies and extended to early onset Alzheimer's disease.⁴⁻⁵ However, the ApoE genotype does not influence the age at onset in patients with presenilin 1 mutations.⁶⁻⁷ The effect of ApoE- $\epsilon 4$ allele is dose dependent, the risk for Alzheimer's disease increasing with the number of ApoE- $\epsilon 4$

alleles.⁸ Because ApoE- $\epsilon 4$ allele is found in no more than 50% of the patients and many ApoE- $\epsilon 4$ allele carriers do not develop Alzheimer's disease, other genetic risk factors, acting alone or in conjunction with the ApoE- $\epsilon 4$ allele, may exist.

The gene coding for $\alpha 1$ -antichymotrypsin (Aact) is an obvious candidate, as its product binds to β -amyloid peptide in brains of patients with Alzheimer's disease and promotes the polymerisation of β -amyloid peptide into amyloid filaments.⁹⁻¹⁰ Recently, Kamboh *et al* reported the modification of the ApoE- $\epsilon 4$ allele associated risk for Alzheimer's disease by an A/T polymorphism within the signal peptide of Aact.¹¹ In carriers of the ApoE- $\epsilon 4$ allele, the Aact-AA genotype conferred an increased risk for Alzheimer's disease compared with the Aact-AT or Aact-TT genotypes. We have analysed the ApoE and Aact genotypes in a larger series of patients with Alzheimer's disease and controls.

Materials and methods**PATIENTS**

The patients and control subjects were all white people living in France. A total of 314 unrelated patients (221 women and 93 men), fulfilling the clinical criteria for probable Alzheimer's disease,¹² were included after exclusion of those belonging to families with autosomal dominant inheritance and with age at onset under 60 in all patients. The mean age at onset was 66.7 (SD 11.8) years; 173 healthy white subjects (91 women and 82 men) matched for age and sex were used as controls. The mean age at examination of the controls was 61.7 (SD 10.1) years.

GENOTYPING

Blood samples were obtained, after informed consent, from all subjects, and genomic DNA was extracted directly from blood using standard techniques. The ApoE and Aact genotypes were determined by the polymerase chain reaction restriction technique as described.¹¹⁻¹³

STATISTICAL ANALYSIS

Allele frequencies were estimated by allele counting. Allele and genotype frequencies were

Table 1 Distribution of ApoE and Aact genotypes

ApoE genotypes	Aact genotypes													
	AA				AT				TT				Total	
	AD		Control		AD		Control		AD		Control		AD	Control
	n	%	n	%	n	%	n	%	n	%	n	%	n	n
2/2	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0
2/3	1	0.10	8	0.32	5	0.50	13	0.52	4	0.40	4	0.16	10	25
2/4	2	0.40	2	0.40	3	0.60	2	0.40	0	0.00	1	0.20	5	5
3/3	38	0.30	20	0.20	60	0.48	50	0.50	28	0.22	30	0.30	126	100
3/4	35	0.30	9	0.22	58	0.49	25	0.61	25	0.21	7	0.17	118	41
4/4	13	0.24	0	0.00	30	0.54	2	1.00	12	0.22	0	0.00	55	2
Total	89		39		156		92		69		42		314	173

compared by χ^2 analysis and the exact Fisher's test when appropriate. Standard logistic regression analysis was used to estimate odds ratios (ORs), adjusted for age and sex. The discrete variables were treated as 0/1 dummy with four variables for the ApoE genotypes (genotype $\epsilon 2/\epsilon 2$ was not found) and two variables for the Aact genotypes. Interactions between ApoE and Aact loci were also tested. The differences between models were tested using the likelihood ratio criterion.

Results

Table 1 shows the distribution of the ApoE and Aact genotypes in controls and patients. Controls were in Hardy-Weinberg equilibrium for ApoE and Aact genotypes. The ApoE allele distributions were significantly different in patients and controls ($P < 10^{-10}$); ApoE- $\epsilon 4$ frequency was significantly higher in patients (0.37 *v* 0.14, $P < 10^{-10}$), and corresponded to a decrease in the frequency of the ApoE- $\epsilon 2$, more than the $\epsilon 3$ allele. Thus the ApoE- $\epsilon 2$ allele frequency was significantly lower in patients than in controls (0.02 *v* 0.09, $P = 0.0001$). No significant differences in Aact genotype ($P = 0.38$) or allele ($P = 0.23$) distributions were found between patients and controls. Stratification of the data according to the presence or absence of the ApoE- $\epsilon 4$ allele disclosed no difference in the Aact-A frequency between controls and patients with Alzheimer's disease. Among those with $\epsilon 4/\epsilon 4$ and $\epsilon 3/\epsilon 4$ genotypes, the frequency of the Aact-A allele was 0.53 in patients and 0.52 in controls, ($P = 0.89$). Among those with $\epsilon 3/\epsilon 3$ genotype, the values were 0.53 and 0.48 respectively ($P = 0.26$).

The logistic regression analysis (table 2) showed that age ($P = 10^{-6}$), sex ($P = 0.02$), and ApoE- $\epsilon 4/\epsilon 4$ ($P = 10^{-6}$), $\epsilon 3/\epsilon 4$ ($P = 0.001$), and $\epsilon 2/\epsilon 3$ genotypes ($P = 0.0045$) were significant predictors of Alzheimer's disease. The following genotypes had no significant effect: ApoE- $\epsilon 2/\epsilon 4$ ($P = 0.34$), Aact-A/A ($P = 0.44$), and A/T ($P = 0.64$). The dose dependent effect of the ApoE- $\epsilon 4$ allele was confirmed: $\epsilon 4/\epsilon 4$ homozygotes had a higher risk (OR=20.9, 95% confidence interval (95% CI) 4.8–90.4) than $\epsilon 4/\epsilon 3$ heterozygotes (OR=2.3, 95% CI 1.4–3.8). Those with ApoE- $\epsilon 2/\epsilon 3$ had a lower risk (OR=0.30, 95% CI 0.1–0.7), whereas the adjusted OR for ApoE- $\epsilon 2/\epsilon 4$ genotype was not significantly different from 1 (OR=0.45, 95 CI

Table 2 Odds ratio (OR) for developing Alzheimer's disease for both the ApoE and the Aact genotypes

Genotype	OR	95% CI
ApoE:		
3/3	1.0	Referent group
3/4	2.3	1.4–3.8
4/4	20.9	4.8–90.4
2/4	0.45	0.1–2.3
2/3	0.30	0.1–0.7
Aact:		
T/T	1.0	Referent group
A/T	0.88	0.5–1.5
A/A	1.29	0.7–2.5

Odds ratios are adjusted for age and sex.

0.09–2.3). None of the 12 two way interactions between ApoE and Aact genotypes were significant ($P > 0.10$).

Discussion

Our data, using patients with Alzheimer's disease and controls from the same population, confirmed the dose dependent effect of the ApoE- $\epsilon 4$ allele with ORs similar to those calculated in previous studies.⁴ In the initial association study of Aact polymorphism in Alzheimer's disease,¹¹ the Aact-A allele increased the risk associated with the ApoE- $\epsilon 4$ allele in a dose dependent manner. In our series, including a larger number of patients with Alzheimer's disease, we found no significant difference between the Aact allele and genotype distributions in patients compared with controls, and no modification of the risk associated with the ApoE- $\epsilon 4$ allele by Aact genotypes. These results are very similar to those recently reported by Haines *et al*,¹⁴ but do not confirm the findings of Kamboh *et al*.¹¹ This difference is not likely to be due to sample size, as the number of patients was larger in the study of Haines *et al*¹⁴ ($n = 576$) and in ours ($n = 314$) than in that of Kamboh *et al*¹¹ ($n = 222$). In addition, age and sex were taken into account as covariables in two studies, but not in the study of Kamboh *et al*.¹¹ The results of Kamboh *et al*¹¹ might be explained by random fluctuations in the control group between ApoE- $\epsilon 4$ allele and non ApoE- $\epsilon 4$ allele carriers.

In conclusion, our study shows that the risk conferred by the ApoE- $\epsilon 4$ allele is not modified by the A/T polymorphism in the signal sequence of the Aact gene. Additional analyses in more varied populations will permit more definite conclusions to be drawn as to the influence of the Aact-A allele on Alzheimer's disease.

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