SHORT REPORT

The prevalence of apoE- ϵ 4 in Alzheimer's disease is age dependent

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Abstract

The ϵ 4 allele of apolipoprotein E is a risk factor for Alzheimer's disease. However, other yet unidentified factors might be involved. It has been suggested that the ϵ 4 allele might be relatively less relevant in Alzheimer's disease with onset before age 60 and after age 80. The aim was to evaluate the association of the ϵ 4 allele with Alzheimer's disease across a wide range of ages at onset. 156 patients with age at onset between 46 and 89 and 120 cognitively unimpaired subjects aged 53 to 89 as controls were studied. Age at onset in the cases and age in the controls were stratified into six groups (60 and younger, 60 to 64, 65 to 69, 70 to 74, 75 to 79, and 80 and older). Multivariable sex adjusted probit regression analysis was used to model ϵ 4 prevalences in cases and controls across age. The sex adjusted relation of ϵ 4 with age in controls was slightly negative with prevalence of 0.16 in the youngest and 0.09 in the oldest age groups. The sex adjusted relation in cases with Alzheimer's disease had a bell shaped curve with prevalence of 0.23 in the youngest age group, rising to 0.54 and 0.51 in the age groups 65 to 69 and 70 to 74, and decreasing to 0.12 in the oldest age group. It is concluded that the relation of the ϵ 4 allele with Alzheimer's disease is age dependent, indicating that other risk factors might be relevant in the younger and older ages.

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The prevalence of the ϵ 4 allele of apolipoprotein E (apoE) has been consistently shown to be higher in sporadic and familial Alzheimer's disease than cognitively normal elderly subjects. The reported prevalences range from about 0.30 to 0.50 in the different studies, as compared with prevalences of 0.10–0.18 in the controls.¹ However, some findings suggest that the reported prevalences of ϵ 4 in patients with Alzheimer's disease are differentially distributed across ages at the onset of disease.

The prevalence of ϵ 4 in patients developing presenile Alzheimer's disease (before 60–65

years of age) has been reported to be higher than in controls but lower than Alzheimer's disease developing after age 65.^{2–3} On the other hand, the ϵ 4 prevalence seems relatively lower in patients with Alzheimer's disease with very late onset—that is, after age 85–90.^{3–6} These findings imply that the risk of Alzheimer's disease due to the ϵ 4 allele might also be different across age groups and that the risk should be assessed on an age specific basis. To achieve a reliable estimate of the age associated risk, accurate estimates of the prevalence of the ϵ 4 allele across age in Alzheimer's disease cases and normal controls are needed.

Despite the many studies on apoE in dementia published in the past five years, data on the prevalence of the ϵ 4 allele in demented and normal controls throughout the range of ages typical of Alzheimer's disease (between 50–55 to 90–95) are scant. Most studies tend to truncate their samples to onset ages of 65–70 and younger or 75 and older,^{3–6} or to consider few (three or less) or very wide (for example, from 65 to 95) age strata, thus preventing accurate estimates of age specific prevalences.

The aim of the present study was to estimate the prevalence of the ϵ 4 allele across a wide range of ages at onset and to describe the relation between the ϵ 4 allele and age in Alzheimer's disease patients and elderly controls.

Methods

A total of 156 consecutive patients were recruited at the Alzheimer's Disease Unit, Brescia, Italy, from 1 June 1993 to 15 April 1994. All underwent a uniform clinical diagnostic investigation that has been described in detail elsewhere,^{7,8} and met NINCDS-ADRDA criteria for probable Alzheimer's disease. In the present study, data on age, sex, education, age at onset of dementia, Hachinski score, mini mental state examination (MMSE) at the time of first evaluation, and apoE ϵ 4 genotype were used.

All controls were cognitively unimpaired elderly subjects living in the community with a negative history of neurological disease. Seventy of them were recruited at the Alzheimer's Unit among patients' spouses, and 50 were hyperlipidaemic subjects of similar age (69.5 (SD 9.0) v 69.2 (SD 3.6) years) seen at an Ital-

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Demographic and clinical features of 156 patients with Alzheimer's disease and 120 controls

	Alzheimer's disease		Controls		p Value
	Mean (SD) or ratio (%)	Range	Mean (SD) or ratio (%)	Range	
Age*	69.2 (8.3)	46–89	69.4 (7.2)	53–89	NS
Sex (women)	119/156 (76.3%)	—	70/120 (58.3%)	—	0.001
Education (y)	6.0 (3.5)	1–24	8.5 (4.0)†	5–19	0.003
Disease duration (y)	4.6 (2.7)	0.5–12.8	—	—	—
Hachinski score	2.2 (2.0)	0–8	—	—	—
Mini mental state examination	12.4 (8.6)	0–27	28.8 (1.4)†	25–30	<0.0005
ε4 Allele‡	126/312 (40.4%)	—	29/240 (12.1%)	—	<0.0005

*Age at disease onset in cases and age at observation in controls.

†Based on the 70 controls seen at the Alzheimer's unit (see methods).

‡The denominator is twice the number of subjects.

ian lipid clinic. Cases and controls have been described previously.^{7,8}

ApoE genotype was derived by phenotype, as assessed with isoelectric focusing.⁹ Further details have been provided elsewhere.⁷

Written informed consent was obtained from patients or their primary care givers. Approval of the study was provided by the local ethics committee.

Statistical analysis was performed with the package SPSS release 5.0.¹⁰ Between group differences of continuous and dichotomous variables were assessed with a *t* test and a χ^2 test, respectively. The critical *p* value was set at 0.05. Cases and controls were stratified into six age groups: younger than 60, 60 to 64, 65 to 69, 70 to 74, 75 to 79, and 80 and older. As the aim of the study was to model the prevalence of the ε4 allele across age, multivariable probit regression was used in the analysis. Probit regression allows modelling prevalences by transforming them into probits, defined as the value of the standard normal curve below which the observed proportion of the area is found.¹⁰ Generalised linear models can be fitted to probits. The general formula of the probit regression model is:

$$\text{probit (prevalence)} = \text{intercept} + BX$$

where *B* is the regression coefficient and *X* is the predictor variable. As a preliminary analysis of the data suggested that the relation of the ε4 allele prevalence with age in Alzheimer's

disease cases was quadratic, a general quadratic regression model was used. Model fitting was assessed with Pearson's goodness of fit test. The significance of the regression coefficients was assessed with Wald statistics.

For some examples of the use of probit analysis in the biomedical literature the interested reader can refer to Hussain and Redmond,¹¹ Lyden and Lonzo,¹² and Gilsing *et al.*¹³

Results

The table shows that the age of the patients with Alzheimer's disease at onset of disease was similar to that of controls. Twenty four patients (15.4%) had onset of disease between ages 46 and 60, 26 (16.7%) between 60 and 64, 37 (23.7%) between 65 and 69, 26 (16.7%) between 70 and 74, 33 (21.2%) between 75 and 79, and 10 (6.4%) between 80 and 89. The number of the controls in the same age groups was nine (7.5%), 17 (14.2%), 55 (45.8%), 18 (15.0%), eight (6.7%), and 13 (10.8%). Forty one of the 156 cases (26%), seven of 24 (29%) of those with disease onset before 60, and two of 67 (3%) of the non-hyperlipidaemic controls had a family history of dementia in a first degree relative. None had an autosomal dominant inheritance pattern. Sex was not significantly different in non-hyperlipidaemic and hyperlipidaemic controls (women: 46/70 *v* 24/50; ($\chi^2=3.1$, *df*=1, *p*=0.08). The prevalence of the ε2 allele (data not shown) was six of 312 (2%) in the cases and 19/240 (8%) in the controls ($\chi^2=9.0$, *df*=1, *p*=0.003). No age effect on ε2 allele prevalence could be found in cases or controls.

A preliminary analysis was carried out to evaluate whether the association of the ε4 allele with age was different in the two control groups (patients' spouses and hyperlipaemic elderly subjects). The ε4 allele prevalence was 0.09 (12/140) and 0.16 (16/100, *p*=0.12 on χ^2 test), respectively. A general quadratic multivariable probit regression model showed that the shape of the association was not different from a straight line with similar intercept in both groups. Normolipaemic and hyperlipaemic controls were then merged and used as a unique group in analysis.

The figure shows that the observed ε4 allele prevalence was low in controls at all ages whereas in the Alzheimer's disease group it was maximal in the 65–74 age groups, and lower at the extreme ages. The most parsimonious model describing the ε4 allele prevalences in

Observed and expected prevalences of the ε4 allele by age group in patients with Alzheimer's disease and controls. Age is considered at observation in controls and at onset of disease in cases. Expected prevalences were computed from a multivariate probit regression model. Coefficients were as follows: age group (0–5 variable younger to older): $B=-0.07$ (SE 0.08), NS; case/control (0=control, 1=case) $\times$ age group: 0.84 (0.19), *p*<0.005; case/control $\times$ (age group)²: -0.17 (0.04), *p*<0.005; sex (0=female, 1=male): -0.10 (0.14), *p*<0.005; intercept of cases: -0.71 (0.18), *p*<0.005; intercept of controls: -0.97 (0.21), *p*<0.005.

Alzheimer's disease cases and controls was one modelling the ε4 allele prevalences as a straight line in controls and as a parabola in cases (Pearson's goodness of fit test: $\chi^2=43.2$, $df=17$, $p<0.0005$).

Discussion

We have shown that the prevalence of the ε4 allele in Alzheimer's disease is dependent on age at onset. The relation is quadratic (roughly bell shaped), with low prevalences in patients with onset at ages 60 and earlier and 80 and older, and the greatest prevalence between ages 65 and 75. These data are preliminary to the estimate of the risk of Alzheimer's disease due to the ε4 allele and indicate that the risk for prevalent and incident Alzheimer's disease must be estimated across age.

Some previous studies have suggested the age at onset dependency of the ε4 allele in sporadic and familial Alzheimer's disease.^{4 14-16} Although most studies have either truncated ages below 65-70 or above 75, or have few age strata, they indicate overall higher prevalences between the ages of 60 and 70, with lower prevalences in younger and older ages.³

The findings of the present study are relevant in various ways. Firstly, they underline the need to consider age specific risks of the ε4 allele in prospective studies. Cross sectional studies that disregard the effect of age have shown a very close association of the ε4 allele carrier status with prevalent Alzheimer's disease (risk of 6.2, 95% confidence interval 4.9 to 7.8 in a large series of combined studies).¹ When age at onset is taken into account, the risk of ε4 allele homozygous subjects rises from about five at age 40 to 15 at age 60 then decreases to about two at age 90. The risk of ε4 allele heterozygous subjects rises from about two, goes up to four, and decreases to two at the same ages.¹⁷ The risk of ε4 allele carriers for incident Alzheimer's disease computed in prospective studies across all ages is lower (2.2 to 3.7 in different studies),^{18 19} and the possible rise and fall in risk across ages has not yet been considered.

Secondly, the sensitivity and specificity of the ε4 allele as an adjunct test for the diagnosis of Alzheimer's disease might also be reconsidered.^{20 21} The detection of an ε4 allele in a patient with probable Alzheimer's disease has been claimed to be predictive of definite Alzheimer's disease with 100% specificity.²¹ However, our results suggest that this might not be true in Alzheimer's disease with very old and very young age of onset and that age specific sensitivities and specificities should be computed.

Thirdly, these data confirm the presence of factors for Alzheimer's disease other than the ε4 allele, either genetic or environmental, that might be of pathogenic relevance mainly in the younger and older ages.⁵ The ε2 allele, which has been shown to be protective for Alzheimer's disease,²² might be one such factor. As previously reported,⁸ its prevalence in our controls is higher than in cases. However, no effect of age on the ε4 allele could be found in our subjects. The relevance of non-ε4 factors in Alzheimer's

disease is in agreement with recent findings suggesting that the proportion of all cases of Alzheimer's disease attributable to the ε4 allele is only about 14%.¹⁸ Although the computation of attributable proportions is doubtless a simplification of the complex phenomenon linking risk factors to diseases, it nevertheless gives a rough estimate of the epidemiological impact of a risk factor.

Some limitations of this study should be acknowledged. Firstly, we were not able to estimate age, sex, and education specific risks of the ε4 allele for Alzheimer's disease but only age specific ones. Reports in the literature suggest that the ε4 allele associated risk for Alzheimer's disease might be different in men and women.^{17 23} Furthermore, recent data suggest an interaction between the ε4 allele and education.²⁴ We assessed the effect of sex by including it in the multivariate model, but it did not prove significant. The effect of education could not be assessed for the low number of subjects of the strata when the sample was broken down also by education. This also prevented the evaluation of interaction effects of age with sex and education. Studies with a larger subject base will be needed to reliably consider this issue. Secondly, a proportion of the controls were hyperlipidaemic subjects. The association of the ε4 allele with a hyperlipidaemic state is well known,^{8 25} and the prevalence of the ε4 allele in hyperlipidaemic patients was accordingly higher, albeit non-significantly, than in normolipidaemic controls (0.09 *v* 0.16). However, the shape of the association was not different from a flat line in both groups, thus supporting their being treated as a single group for the purpose of this study. Lastly, it must be acknowledged that alternative explanations, such as differential accuracy of the clinical diagnosis of Alzheimer's disease with age, might also explain our findings.

In conclusion, we propose that the enrichment of the ε4 allele in the Alzheimer's disease population is concentrated in the age range of 65 to 75 years. This prompts revision of the current beliefs on the use of apoE genotyping for diagnostic purposes, indicates the need to assess age specific risks in prospective studies, and suggests the presence of factors other than apoE in the pathogenesis of Alzheimer's disease in younger and older ages.

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