SHORT REPORT

Association of the APOE ϵ 4 allele with disease activity in multiple sclerosis

Nikos Evangelou, Mandy Jackson, David Beeson, Jackie Palace

Abstract

Objectives—Allelic variants of the APOE gene are known to influence the course of many neurological diseases and there is increasing evidence that apolipoprotein E (APOE) is a pivotal component in reinnervation and dendritic remodelling after neuronal injury. Previous studies did not show significant differences in the APOE allele frequencies in multiple sclerosis compared with controls but did not examine for correlation with disease severity. This study explores the relation of APOE genotypes with the disease severity.

Methods—Ninety five patients with multiple sclerosis were studied. Age of onset, type, and activity of the disease were recorded prospectively and genotyping was performed according to standard protocols.

Results—APOE allele frequencies of the group as a whole, the relapsing group, or the primary progressive group were not significantly different from those reported from matched historical controls. The ϵ 4 allele was found to be more common in patients with a more aggressive type of multiple sclerosis (odds ratio=2.95, $p=0.03$).

Conclusions—Although APOE does not seem to be implicated in the early pathogenesis of the disease, patients possessing the ϵ 4 allele might have a reduced capacity for neuronal remodelling after relapses.

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Apolipoprotein E (APOE) is a protein that plays an important part in cholesterol transport, uptake, and redistribution.¹ It is the principal apolipoprotein expressed in the brain. It has been suggested that ApOE has a fundamental role in both normal neuronal metabolism and brain recovery after injury.²

The ϵ 4 allele is associated with an increased risk and earlier age of onset of both familial and sporadic Alzheimer's disease.³ In motor neuron disease the ϵ 4 allele is more common in the more severe bulbar onset compared with limb onset type.⁴ Patients with APOE ϵ 4 alleles who have haemorrhagic strokes have a poorer

prognosis⁵ and after head injury, ϵ 4 patients seem to be associated with poorer recovery than non- ϵ 4 patients.⁶ However, the association of APOE with a poorer prognosis in many neurological diseases has not been supported by all studies.⁷

In two studies, no APOE alleles have been found to be associated with an increased risk of developing multiple sclerosis^{8,9} although a large cohort has not been studied. The aim of this study was to examine whether there is an association of APOE alleles with the severity of the disease. Preliminary results of this work have been presented previously.¹⁰

Methods

The study was prospective. Between autumn 1996 and autumn 1997, 95 consecutive patients with clinically definite multiple sclerosis¹¹ were assessed by a single observer (JP). The type of multiple sclerosis (relapsing remitting, secondary progressive, primary progressive) and the duration of the disease since the onset of the first symptoms were noted. The number of relapses since onset was recorded and historically classified as completely or partially resolved. All documentation of relapses and recovery of function had been completed before the APOE genotyping. All patients were examined and the expanded disability status scale (EDSS) score¹² was recorded when the patient was not in a relapse.

DNA was extracted from peripheral blood by standard methods (Neucleon II kit, Scotlab, Strathclyde, UK). Genomic DNA was amplified by polymerase chain reaction (PCR) and the amplification products were digested with HhaI and subjected to electrophoresis on polyacrylamide gels.¹³

The study was approved by the Central Oxford research ethics committee.

STATISTICAL METHODS

The Pearson χ^2 test was used to compare APOE allele frequency between groups. Logistic regression was used to examine the relation of APOE alleles with disease activity (defined as the EDSS score divided by the disease duration since the onset of the first symptom). Tests were two sided with significance set at the $p=0.05$ level.

Department of Clinical Neurology, University of Oxford, UK

N Evangelou
M Jackson
D Beeson
J Palace

Correspondence to:
Dr Jackie Palace,
Department of Clinical Neurology, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE, UK.
Telephone 0044 1865 228551; fax 0044 1865 222717; email nikos@FMRIB.ox.ac.uk

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Figure 1 The APOE allele frequency distribution at the Oxford multiple sclerosis group is similar to previously published multiple sclerosis groups and multiple sclerosis controls.

Results

Ninety five patients (58 women and 37 men), all white, who fulfilled the clinical criteria of clinically definite multiple sclerosis were examined and genotyped at the APOE locus. Fifty two had relapsing-remitting, 32 secondary progressive, and 11 primary progressive types of the disease. The mean age of onset was 29.8 years. Their duration of disease had a median of 12 years and ranged from newly diagnosed to 43 years. Their EDSS ranged from 0 to 9 with a median of 4.5.

The APOE allele frequency distribution in our multiple sclerosis population was not significantly different from published controls for patients with multiple sclerosis from the south of Britain⁸ (χ^2 , $p=0.37$) or published neurological controls⁹ ($p=0.76$) (fig 1) or from the healthy European population.¹⁴ No significant difference was found between the APOE

allele frequency distribution of the primary progressive group of patients compared with the relapsing-remitting and secondary progressive group of patients ($p=0.20$).

To quantify the aggressiveness of the disease, as other studies have done in the past, we divided the duration of the illness by the degree of disability to give a "severity score". The patients were then divided into two groups using the median "severity score" as the threshold and referred to as aggressive and mild. Hence for the purpose of this study, 50% of the patients were allocated into the "mild group" if they had a long duration of symptoms but had developed little disability and the remaining 50% were allocated to the "aggressive group" (fig 2A). As only four patients were homozygous for the APOE $\epsilon 4$ allele, all patients with at least one $\epsilon 4$ allele were treated as one group. Seventy per cent of the $\epsilon 4$ patients but only 44% of the non- $\epsilon 4$ patients had aggressive disease (see fig 2B; OR=2.95; 95% confidence interval (95% CI) 1.09–8.01, $p=0.03$). Similarly, only 8.3% of $\epsilon 4$ patients and 19.7% non- $\epsilon 4$ patients were classified as benign multiple sclerosis having an EDSS <3 with a duration of the disease for at least 10 years ($p=0.17$).

Because the progression of disability as measured by the EDSS is not linear throughout the duration of the disease we examined the duration of the disease in the two groups of patients, $\epsilon 4$ and non- $\epsilon 4$, and we found that mean duration was not significantly different ($\epsilon 4$: 5385 days, non- $\epsilon 4$: 5216 days, $p=0.87$).

In agreement with other studies,¹⁵ onset later in life was associated with a more aggressive disease ($p=0.0003$). None of the alleles was

Figure 2 Scatter plot of activity index (duration of disease in days/EDSS). (A) The median value of the severity score for all patients was calculated as shown by the horizontal line. (B) Patients were divided into $\epsilon 4$ and non- $\epsilon 4$ groups.

significantly associated with the age of onset (for $\epsilon 4$, linear regression $p=0.98$). The sex of the patients was not associated with the activity of the disease (χ^2 , $p=0.97$). Controlling for age of onset, sex, and the duration of the disease, the $\epsilon 4$ allele was still significantly associated with the disease activity (odds ratio=3.3; 95% CI 1.2–11.5, $p=0.02$).

In 36 patients we were able to obtain clinical information on individual relapses from the case notes including the extent of recovery. ApoE $\epsilon 4$ patients ($n=10$) had a higher percentage of partially recovered relapses (62%) as opposed to the non- $\epsilon 4$ patients ($n=26$) who more commonly had completely rather than partially (42%) resolved relapses. That difference was not significant when individual patients were compared (Mann-Whitney, $p>0.05$).

Discussion

Our findings suggest that the APOE $\epsilon 4$ allele is associated with more rapid progression of disability in multiple sclerosis.

There is an increasing amount of evidence implicating ApoE as an important molecule in neuronal homeostasis.^{16 17} Both animal models and pathological findings suggest that ApoE has an important role in the recovery after neuronal damage.

The association of APOE with Alzheimer's disease is now well established and postmortem data in such patients show that neurons from those with the APOE $\epsilon 4$ allele show both more severe degeneration but also significantly less plastic dendritic changes.¹⁸ We carried out a prospective study to examine the role of APOE in multiple sclerosis.

In agreement with previous studies^{8 9} we did not find the frequency of any APOE allele to be higher in patients with multiple sclerosis compared either with published neurological controls or from large European epidemiological studies. Although we did not examine normal controls, other groups have found that the relative frequencies of the APOE alleles did not show significant variation across different central European countries for healthy people.¹⁴ We did not find any significant difference between the primary progressive and the relapsing-remitting/secondary progressive group although the number of patients examined were small.

Perhaps the most important finding of our study was that the $\epsilon 4$ allele seems to be associated with a more aggressive course of the disease. This is supported by a study using a smaller group of patients.¹⁹ When our patients were divided up further to include a benign group, this trend persisted.

The mechanisms of recovery from relapses in multiple sclerosis are not well understood. Functional improvement could be attributed to reduction of oedema, remyelination, and neuronal plasticity, but conclusive studies dissect-

ing the relative importance of those mechanisms are still lacking. As most of the documented relapses in $\epsilon 4$ patients had a poor recovery, it could be speculated that neuronal remodelling is not as efficient as in $\epsilon 3$ patients.

In conclusion, we confirmed the results of other studies that the APOE genotype does not seem to be a risk factor for developing multiple sclerosis. However, it was seen to influence the rate of progression of the disease. Large scale studies are needed to confirm these findings and to examine whether the frequency of the APOE alleles is different in patients with primary progressive multiple sclerosis. Our clinical evidence seems to be in agreement with the proposed hypothesis that APOE has an important part to play in the recovery of the CNS after injury.

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