Polymorphisms in the Apolipoprotein E Region and Severity of Multiple Sclerosis

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Abstract

Background: The apolipoprotein E (APOE) polymorphism is known to affect various neurologic disorders with different effects on the immune system and CNS repair. However, previous studies on possible modulation of the clinical course of multiple sclerosis (MS) by APOE polymorphism have been inconsistent.

Objective: To clarify the issue for MS patients' management and future research.

Methods: The present cross-sectional study investigated 81 patients with clinically proven MS and related their clinical and demographic findings to the allelic polymorphism of the APOE gene. The genotype distribution of patients with MS was compared with a comparison group of 93 asymptomatic elderly volunteers.

Results: Significant differences were found in the distribution of ε_4 allele between patients with MS and the comparison group (9.3% *vs.* 0.5%; p<0.001). An analysis of disease progression in 81 patients with MS indicated that APOE ε_4 carriers are more likely to be affected with severe disease.

Conclusion: The results obtained suggested that APOE genotype affected susceptibility to MS and indicated an association of the APOE ε_4 allele with a more severe course of the disease.
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Keywords • Multiple sclerosis • apolipoprotein E • polymorphism

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Introduction

The clinical course of multiple sclerosis (MS) is variable. Available data on the natural history of the disease suggest that approximately 50% of patients will have progressive MS within 10 years of their initial attacks and require some form of walking aid within 15 years after the onset of the disease. By contrast, 20% to 40% of patients experience a benign course of MS with minor disability after 10 years of diagnosis.[1]

This diversity may be, in part, due to different pathogenic mechanisms involved in the evolution of this disease. More recently, however, interest has also focused on genetic factors that may influence the prognosis of MS.[2] Apolipoprotein E (APOE) polymorphism has been a candidate for such investigations, is involved in lipid transport, and is shown to influence

Table 1: Demographic and clinical characteristics of the patients with MS.

Characteristic	Mean±SD (range)
Age at onset	23.9±8.5 (6-52)
Disease duration	7.5±5.8 (0.08-25)
Recurrences	4.7±4.9 (0-20)
EDSS	4.8±2.9 (0-9.5)
Progression Index	1.4±4.1 (0-36)
Annual relapse rate	1.1±1.9 (0-12)

Table 2: Distribution of EDSS in MS group.

EDSS	No. of patients (%)
<3	29 (35.8)
3 to<7	27 (33.3)
≥7	25 (30.9)

Apolipoprotein E (APOE) polymorphism has been a candidate for such investigations, is involved in lipid transport, and is shown to influence growth and repair of neurons.[3,4] The APOE gene is located on chromosome 19 and is polymorphic with ε_2, ε_3 and ε_4 being the most common alleles. The ε_4 allele has turned out to be a major risk factor for the early onset of Alzheimer's disease. Adverse recovery after head trauma or other injuries to the brain have also been linked to the ε_4 allele.[5-7] Because MS is characterized by repeated damage to the central nervous system (CNS) followed by attempts to repair, it is speculated that the APOE genotype could also affect the prognosis of the disease. Initially, support for these speculations came from the results of relatively small cross-sectional studies on MS that usually involved fewer than 100 patients and found a severe outcome in those with ε_4 allele.[8-10] Two subsequent investigations on larger number of patients yielded conflicting results. One study found no relationship between any of the APOE alleles or genotypes with disease progression, whereas in another study, a significantly faster progression of MS was observed in patients homozygous for the APOE ε_4 allele.[11,12]

In view of this discrepancy and the potential implications of an association between the APOE genotype and MS course, the present investigation was performed to clarify the issue for patients' management and future research.

Patients and Methods

Through collaboration between foregoing institutions and six hospitals in Tehran, 81 patients with clinically proven MS, who volunteered for a cross-sectional study, were identified according to Poser's criteria.[13] The clinical data collected were age at the onset of disease, duration of illness, disease severity according to the Expanded Disability Status Scale (EDSS), progression index and the number of relapses and medications.

In brief, "age at onset" was defined as the age when the first episode of neurological dysfunction suggestive of demyelinating disease was presented, and was obtained via patients

recall and verified through reviewing of medical records. Disabilities were assessed on admission by use of the EDSS[14] and were recorded in three categories; <3, 3 to <7, and ≥7. Relapses were defined as new or deteriorating neurologic signs or symptoms with duration of >24 hrs proceeded by a relatively stable or improving neurologic state for at least 30 days. Interval treatment was further subdivided into interferon therapy (n=32; 39.5%), no interval treatment (n=33; 40.7%) and other interval treatments such as IVIG, methotrexate, cyclophosphamide, dexamethasone and azathioprine (n=16; 19.8%). The course of MS was described as those with relapse and remission (n=41; 50.6%), secondary progressive (n=34; 41.9%) and primary progressive (n=6; 7.5%). All clinical data were obtained while blinded to the patients' genotype.

Ten (12.3%) patients had one or more affected family members and the remaining (n=71, 87.7%), were sporadic cases. All ethnically matched healthy people in comparison group (n=93) were asked about their age, ethnicity and their MS. The age for comparison group was selected as ≥50 yrs to minimize the probability of contracting MS in the future. Their mean age was 64.5±9.7 yrs. The patients included 20 men and 61 women.

APOE genotyping
APOE genotyping was done according to the standard procedures of extraction of high molecular weight DNA from peripheral whole blood, semi-nested PCR amplification, and *Hin6*I restriction enzyme digestion.

Disease severity
MS progression was defined by progression index (PI). It was calculated as a measure of accumulated disability over time for all patients as follows: *PI=EDSS/disease duration in years*. Analyses were also conducted to examine the relationship of APOE ε_4 carrier status with clinical variables.

Statistical analysis
After a comparison between distribution of APOE genotypes in patients with MS and controls, one-way ANOVA was used to find the overall differences in demographic and clinical variables between patients at p<0.05.
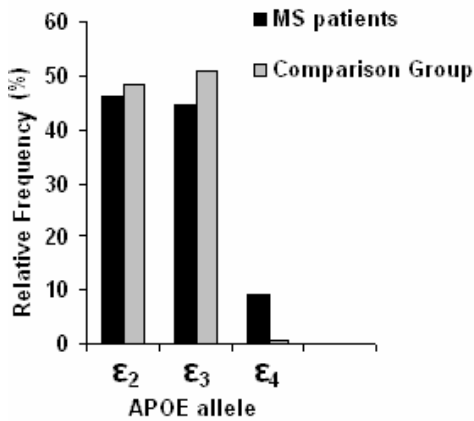
Fig 1: Distribution of APOE allele frequencies in MS patients and comparison groups.

Results

Eighty-one patients, all white, who fulfilled the criteria for clinically proven MS were examined and genotyped for APOE locus. As shown in Fig. 1, in patient group, ε_4 allele was significantly ($p<0.001$) more prevalent than in comparison group (9.3% *vs.* 0.5%). A summary of clinical variables among the patients with MS is shown in Table 1& 2. After classifying EDSS into three groups (<3, 3 to <7 and ≥7), significant differences were found between ε_4 positive and negative patients (Table 3) which indicated a significant association between ε_4 allele and the disease severity; the highest disease duration was observed in those homozygous for ε_4 allele ($p=0.082$). No significant differences were found with regard to the patient's mean age at the time of examination, age at onset of MS, and the distribution of gender. In addition, no significant difference was found between the APOE allele frequency distribution of the primary progressive group of patients as compared with those having relapse and remission and the secondary progressive group.

Three categories of disease severity included mild, moderate and severe. The severe form was characterized by EDSS≥7 ten years or less after disease onset. Mild MS with EDSS<3 that continued for more than 10 years after onset and the third group of patients were considered to have a moderate disease. The severe MS group included one patient with relapse and remission, 22 with secondary progressive and two with primary progressive MS. The mild MS group comprised 22 patients with relapse and remission and three with secondary progressive MS. The moderate MS group concluded 17 patients with relapse and remission and nine with secondary progressive MS.

Discussion

Our finding suggests that the APOE ε_4 allele is associated with a more severe disability in MS patients. There is an increasing amount of evidence implicating APOE as an important molecule in neuronal homeostasis.[15,16] Both animal models and pathological findings suggest that APOE plays an important role in the recovery after neuronal damage.[17,18] 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 ε_4 allele show a more severe degeneration but also significantly less plastic dendritic changes.[19]

Our results support the previously suggested hypothesis that APOE may be involved in modulation of clinical expression. Specifically, we observed significant associations between APOE ε_4 and a more severe form of MS ($p=0.019$).

Several studies have reported an association of the APOE ε_4 allele and a more severe disease, as assessed by older age at onset, by disease activity/progression index[20-23] or by faster rate of disease progression.[24] Fewer studies have reported APOE effects on MS susceptibility, although one case-control study reported a higher risk of developing MS for APOE $\varepsilon_4/\varepsilon_4$ homozygotes.[25] However, the results of this study provide strong support for MS susceptibility by the ε_4 allele of the APOE gene. Limited studies have examined the association of the APOE ε_2 allele with MS phenotypes. One study[26] suggested that remyelination might be impaired in individuals with the APOE ε_2 allele, since the APOE ε_2 isoform was shown to have reduced affinity for receptors in glial cells. Another study[27] reported some evidence of a protective effect of the APOE ε_2 allele and showed that the time required to reach the secondary progressive state for patients, whose initial disease was of relapse and remission type, was significantly

ε_4 Allele	n	EDSS group		
		<3	3 to<7	≥7
Negative	148	25(36.8)	26(38.2)	17(25.0)
Positive	14	4(30.8)	1(7.7)	8(61.5)
Total	162	29(35.8)	27(33.3)	25(30.9)

Table 3: Distribution of ε_4 allele in EDSS groups

longer for those with APOE $\varepsilon_2/\varepsilon_3$ genotypes than for patients with $\varepsilon_3/\varepsilon_3$ and $\varepsilon_3/\varepsilon_4$ APOE genotypes. In our data, the presence of the ε_2 allele seemed to exert a protective role against the onset of the progressive form of the disease.

In conclusion, the APOE genotype seems to be a risk factor for developing MS. 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 MS. Our clinical evidence seemed to be in agreement with the proposed hypothesis that APOE has an important part to play in the recovery of the CNS after injury.

References

1 Weinshenker BG: The natural history of multiple sclerosis. *Neurol Clin* 1995; **13**: 119-46.

2 Weinshenker BG, Kantarci OH: Seeking genes for MS: big risks for big gains. *Neurology* 2000*;* **54**:542-4.

3 Mahley RW: Apolipoprotein E: Cholesterol transport protein with expanding role in cell biology. *Science* 1988**; 240**: 622-30.

4 Ignatius MJ, Gebicke-Haerter PJ, Skene JH, et al. Expression of apolipoprotein E during nerve degeneration and regeneration. *Proc Natl Acad Sci USA* 1986*;* **83**:1125-9.

5 Poirier J: Apolipoprotein E in the brain and its role in Alzheimer's disease. *J Psychiatr Neurosci* 1996; **21**: 128-34.

6 Lichtman SW, Seliger G, Tycko B, Marder K: Apolipoprotein E and functional recovery from brain injury following postacute rehabilitation. *Neurology* 2000*;* **55**: 1536-9.

7 Laskowitz, DT.Horsburgh K., Roses, AD. Apolipoprotein E and the CNS response to injury. *J Cereb Blood Flow Metab* 1998; **18**, 465-71.

8 Sylantiev C, Chapman J, Chilkevich O: The ApoE 4 allele and progression of disability in multiple sclerosis. *Neurology* 1998*;* **50**(Suppl): A 150.

9 Dousset V,Gayou A, Brochet B, Iron A: ApoE polymorphism in multiple sclerosis. *Mult Scler* 1998; **4**: 357.

10 Evangelou N, Jackson M, Beeson D, Palace J: Association of the ApoE epsilon4 allele with disease activity in multiple sclerosis. *J Neurol Neurosurg Psychiatry* 1999*;* **67**: 203-5.

11 Weatherby SJ, Mann CL, Davies MB, et al. Polymorphisms of apolipoprotein E: outcome and susceptibility in multiple sclerosis. *Mult Scler* 2000*;* **6**: 32-36.

12 Hogh P, Oturai A, Schreiber K, et al. Apolipoprotein E and multiple sclerosis: impact of the epsilon-4 allele on susceptibility, clinical type and progression rate. *Mult Scler* 2000; **6**: 226-30.

13 Poser CM, Paty DW, Scheinberg L, et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Ann Neurol* 1983*;***13**: 227-31.

14 Kurtzke JF: Rating neurologic impairment in multiple sclerosis: an Expanded Disability Status Scale (EDSS). *Neurology* 1983**; 33**:1444-52.

15 Nathan BP, Chang KC, Bellosta S, et al. The inhibitory effect of apolipoprotein E4 on neurite outgrowth is associated with microtubule depolymerization. *J Biol Chem* 1995*;* **270**:19791-9.

16 Masliah E, Mallory M, Veinbergs I, et al. Alterations in apolipoprotein E expression during aging and neurodegeneration. *Prog Neurobiol* 1996; **50**: 493-503.

17 Boyles J, Zoellner C, Anderson L, et al. A role of apolipoprotein E, apolipoprotein A-I, and low-density lipoprotein receptors in cholesterol transport during regeneration and remyelination of the rat sciatic nerve. *J Clin Invest* 1989; **83**:1015-31.

18 Smith K, Kapoor R, Felts P: Demyelination; the role of reactive oxygen and nitrogen species. *Brain Pathol* 1999; **9**: 69-92.

19 Arendt T, Schindler C, Bruckner MK, et al. Plastic neuronal remodeling is impaired in patients with Alzheimer's disease carrying apolipoprotein 4 allele. *J Neurosci* 1997; **17**:516-29.

20 Dousset V, Gayou A, Brochet B, Iron A: ApoE polymorphism in multiple sclerosis. *Mult Scler*1998; **4**: 357.

21 Evangelou N, Jackson M, Beeson D, Palace J: Association of the APOE ε4 allele with disease activity in multiple sclerosis. *J Neurol Neurosurg. Psychiatry*1999*;* **67**: 203-5.

22 Lucotte G, Mercier G, Clavel C: Apolipoprotein E-ε4 allele, the major risk factor for Alzheimer's disease, is also involved in the severity of multiple sclerosis. *Alzheimer's Rep* 2000*;* **3**:151-4.

23 Fazekas F, Strasser-Fuchs S, Kollegger H, Berger T, et al. Apolipoprotein E epsilon 4 is associated with rapid progression of multiple sclerosis. *Neurology* 2001*;* **57**: 853-7.

24 Chapman J, Vinokurov S, Achiron A, Karussis DM, et al. APOE genotype is a major predictor of long-term progression of disability in MS. *Neurology* 2001*;* **56**: 312-6.

25 Høgh P, Oturai A, Schreiber K, Blinkenberg M, et al. Apolipoprotein E and multiple sclerosis: impact of the -4 allele on susceptibility, clinical type and progression rate. *Mult Scler* 2000; **6**: 226-30.

26 Carlin C, Murray L, Graham D, Doyle D, Nicoll J: Involvement of apolipoprotein E in multiple sclerosis: absence of remyelination associated with possession of the APOE epsilon2allele. *J Neuropathol Exp Neurol* 2000*;* **59**: 361-7.

27 Ballerini C, Campani D, Rombola G, Gran B, et al. Association of apolipoprotein E polymorphism to clinical heterogeneity of multiple sclerosis. *Neurosci Let* 2000; **296**: 174-6.