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AN INVESTIGATION OF HUMAN APOLIPOPROTEIN E POLYMORPHISMS IN MULTIPLE SCLEROSIS PATIENTS OF IRAN

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ABSTRACT

Multiple sclerosis (MS) is a chronic inflammatory disorder of the central nervous system, with a complex etiology that includes a strong genetic component. The chromosome 19q13 region surrounding the apolipoprotein E (APOE) gene has shown consistent evidence of involvement in MS. In a cross-sectional study, to show the APOE genotype and allele frequency in the MS population of Iran in comparison with the control group, we genotyped its polymorphisms (ε2, ε3 and ε4 alleles). The authors investigated 81 patients with clinically definite MS and 93 asymptomatic elderly volunteers. The frequency of the APOE allele in the MS population in comparison with controls was 9.3% vs. 0.5% for ε4, 44.4% vs. 51.6% for ε3, and 46.3% vs. 47.8% for ε2. The highest frequency of APOE genotype was from ε2/ε3 with 66.7% vs. 94.6% and the lowest, ε4/ε4 genotype with 2.5% vs. 0%. The authors found significant differences in the distribution of ε4 allele between patients with MS and controls (9.3% vs. 0.5%; χ^2=15.2; df=2; p<0.001). The highest frequency of ε4 allele in MS patients was in Pure Turkish (25.0% vs. 5.3%) ethnicity. There was no significant relation between ethnicity and genotype. In the present study ε2/ε4, ε3/ε4 and ε4/ε4 genotypes were more common in bout-onset cases compared to primary progressive cases, and the secondary progressive disease was higher in carriers of ε4 allele. Also, the ε2 allele was higher in relapsing remitting disease.

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INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory disorder of the Central Nervous System (CNS) characterized by destruction of the myelin sheath, gliosis, varying degrees of axonal pathology, and progressive neurological dysfunction.[1] MS is a major cause of morbidity and disability in young adults, with a prevalence of 0.1% in individuals of northern European origin.[2,3] Genetic factors have been implicated by numerous studies, with an estimated sibling-recurrence risk of 20%-40% and a

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greatly increased concordance rate in MZ, compared with DZ twins.[4,5]

One of several candidate genes in the 19q13 region is the APOE gene, which codes for a major lipid carrier protein (apoE) in the brain. It has been suggested that apoE is the major apolipoprotein which redistributes lipids, participates in cholesterol homeostasis in the brain and is involved in the growth and repair of the nervous system.[6]

In addition, this protein is related to immunoregulation and neurobiological routes (neuronal repair, remodeling, and protection). Apolipoprotein E is mainly synthesized in the liver (<90%), but also in other tissues, such as the bowel, brain, lungs, kidney, and macrophages, and secreted as a glycosylated protein. Apolipoprotein E polymorphism is located in the long arm of chromosome 19. Point changes are the molecular features of this polymorphism in the nitrogen bases, causing the replacement of 1 amino acid in the 112 and 158 positions of the peptide chain. The 3 most common isoforms are as follows: E2 (cysteine/cysteine), E3 (cysteine/arginine), and E4 (arginine/arginine). Of the 3 isoforms, E3 is the most frequently reported in the literature in the general population and E4 is rare in the centenarian population. The allelic frequency of APOE varies in the populations because the polymorphism of its locus ranges from 16% to 53% according to the population. Therefore, different phenotypes of APOE exist, and they result from 6 genotypes as follows: 3 homozygous (E2E2, E3E3, and E4E4), and 3 heterozygous (E2E3, E3E4, and E2E4). As a result of the lower frequencies of the (4 and (2 alleles, the phenotypes E-4/4, E-4/2, and E-2/2 are relatively rare.[7,8]

Initially, the prominence of apo E-3 suggested that it was the parent ("wild-type") form of the protein and that apo E-4 and apo E-2 were variants. However, it now appears that ε4 is most likely the ancestral allele. Almost all animals, including the higher primates, such as the baboon, possess the equivalent of apo E-4 homozygosity (arginine at the residue corresponding to amino acid 112 in the sequence). In addition, aboriginal human groups, such as the Huli of the Papua New Guinea highlands, have ε4 as the most common allelic form. The evolution of the allelic forms of apo E is of considerable interest to population geneticists and suggests that there is a selective advantage to the occurrence of apo E-3, and possibly apo E-2, in humans.

Corbo and Scacchi (1999) analyzed the APOE allele distribution in the world. They pointed out that the APOE3 allele is the most frequent in all human groups, especially in populations with a long-established agricultural economy such as those of the Mediterranean basin, where the allele frequency is 0.849-0.898.[9]

Because MS is a disorder with repeated damage to the CNS followed by attempts of repair, it has been speculated that the APOE genotype could also affect the prognosis of MS. To detect the genotype and allele frequencies in MS patients in comparison with healthy controls in Iran, which had not been studied before, the goal of the present study was to examine the APOE polymorphisms and compare the allele frequencies in the ethnical groups.

MATERIAL AND METHODS

Subjects

In collaboration between Tehran University of Medical Sciences, National Research Center for Genetic Engineering and Biotechnology and Iranian MS Society and six hospitals in Tehran, 81 patients with MS were identified who volunteered for cross-sectional study. All patients had their medical records reviewed by a neurologist. Patients were asked to participate in this study if they had clinically definite MS according to Poser's criteria.[10] In total, the MS study population consisted of 20 men and 61 women and had a mean age of 31.5±10.0 years. All patients were white. In patients group 12.3% (n=10) had one or more affected family members and the others (87.7%, n=71) were sporadic patients. The classification of ethical groups of MS patients and controls was based on ancestral origin. The majority of cases and controls were from Fars and Pure Turks. However, the main population group in Tehran is Persian and Turkish and the differences in ethnical groups was not valuable.

The control sample was 93 Iranian adolescents, 42 males (M) and 51 females(F), all unrelated and healthy. We selected age ≥50 for samples to lower the probability of getting coronary artery disease, multiple sclerosis and Alzheimer's disease in future. Their mean age was 64.45±9.7 years. The four classification of samples were based on language and ethnicity of the patients and volunteers (Pure Turk, Pure Shomali, Fars and others).

APOE genotyping

ApoE genotyping was done according to standard procedures of extraction of high molecular weight DNA from peripheral whole blood, semi-nested PCR amplification, and Hin6I restriction enzyme digestion.[11]

Statistical analysis

Comparison of the distribution of APOE genotypes in ethnical groups were analyzed by means of contingency $\chi 2$ tests. The level of statistical significance was set at $p<0.05$. All statistical tests were performed with the statistical package for social sciences (SPSS/PC+) version 10.0.

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RESULTS

In this cross-sectional study, multiple sclerosis was more common in women (M: F ratio 1: 2.1) with peak incidence at age 27. The mean age of female patients is less than males (30.3±9.25 vs. 34.3±11.0). About 71.5% had started having symptoms from 21-40 years; 6.1%, at ages 16 to 20; 16.0%, at ages 41 to 50, and 2.5% at ages >50, which has the same pattern of distribution of MS patients in age groups throughout the world. In MS patients 12.3% (n=10) had one or two affected family members and the others (87.7%, n=71) were sporadic patients.

In the patients, the frequency of the relapsing remitting was higher (50.6%) in comparison with secondary progressive forms and primary progressive with 42.0% and 7.4%, respectively. The maximum of relapses was 12 and the range in disease duration was 0.08 to 25 years. At least 79% of patients had disease duration less than 10 years.

In total, 40.7% of patients had no interval treatment and 39.5% with interferon therapy (29 Avonex; 3 Betaseron) and 19.8% had other interval treatment such as IVIG, methotrexate, cyclophosphamide, dexamethasone, and azathioprine (Table I). The authors found significant differences in the distribution of ε4 allele between patients with MS and controls (9.3% vs. 0.5%; (2=15.2; df=2; p<0.001).

In the MS cohort, the frequencies were as follows: ε2/ε2-7 (8.6%), ε2/ε3-54 (66.7%), ε2/ε4-7 (8.6%), ε3/ε3-7 (8.6%), ε3/ε4-4 (4.9%), ε4/ε4-2 (2.5%). Also, in control group the frequencies were: ε2/ε3-88 (94.6%), ε2/ε4-1 (1.1%), ε3/ε3-4 (4.3%), and there was no ε2/ε2, ε3/ε4, and ε4/ε4 genotype detected.

In the investigation of 162 chromosomes from MS patients and 186 chromosomes from controls, the distribution of frequency for ε2 allele was 75(46.3%) vs. 89(47.8%), ε3 72(44.4%) vs. 96(51.6%), and ε4 15(9.3%) vs. 1(0.5%). The allele frequencies of three polymorphisms observed in the ethnical groups of MS patients and controls are reported in Table II. In MS patients and controls the highest frequency of ε2 allele was in Fars and Pure Turk, ε3 in Fars, and ε4 in Pure Turk and Fars groups.

Although assumption of Chi-square were not met in this Table, after several recode and collapses in Table and after increasing expected counts, there was no significant relation between ethnicities and genotypes (Table II).

The highest homozygosity in ε2 allele was in Pure Turks with 16.7%, in ε3 allele, Pure Shomali with 16.7%, and in ε4 allele was in other ethnical groups that were from two patients with Pure Lor ethnicity (p=0.66).

Table I. Frequency of treatment in MS patients

Treatment status	N	%
No interval treatment	33	40.7
Interferon therapy	32	39.5
Other interval treatment	16	19.8

DISCUSSION

In MS the CNS tissue involvement is probably due to an autoimmune reaction to myelin, and the degree of myelin degradation is variable. In addition, during the course of the disease, axons mat or may not be involved and gliotic scars may or may not develop. MS is therefore characterized by a variety of clinical courses as primary progressive, relapsing remitting, secondary progressive and a variety of clinical forms from benign to severe or even to life-threatening. Unfortunately, to date, no laboratory marker has been able to predict the course of the disease. Therefore, the prognosis is still based on poorly reliable clinical and magnetic resonance imaging (MRI) parameters. The identification of a laboratory marker predictive of the clinical outcome in non-disabled

Table II. Distribution of allele ε2, ε3 and ε4 in four ethnic groups category in MS patients and controls.

Ethnicity	Allele ε2*		Allele ε3#		Allele ε4+	
	MS	Control	MS	Control	MS	Control
Pure Turk	20	37	19	38	5	0
Pure Shomali	10	6	13	6	3	0
Fars	30	35	27	41	3	0
Others	15	11	13	11	4	1
Total	75	89	72	96	15	1

*MS: χ^2=3.01; df=3; p= 0.39, C: χ^2=2.21; df=3; p= 0.52
#MS: χ^2=3.38; df=3; p= 0.33, C: χ^2=1.46; df=3; p= 0.69
+MS: χ^2=1.71; df=3; p= 0.63, C: χ^2=1.44; df=3; p= 0.69

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or partially disabled patients would greatly contribute to the clinical management of MS patients.

In Olmsted County[12] ε2/ε4 and ε3/ε4 genotypes were more common (28 out of 100) in bout-onset cases compared to primary progressive cases (1 out of 12). Hamilton et al.[13] also reported that carriers of ε4 allele in the recent study. The ε2 allele was associated with relapsing remitting disease.

In the present study ε2/ε4 and ε3/ε4 and ε4/ε4 genotypes were more common (17 out of 75) in bout-onset cases compared to primary progressive cases (1 out of 6), and the secondary progressive disease was higher in carriers of (4 allele (7 for SP vs. 5 for RR and 1 for PP). Also, the ε2 allele was higher in relapsing remitting disease (36 for RR vs. 28 for SP and 4 for PP).

There was no data on APOE allele distribution in Iran for comparison. As Table III illustrates, the pattern of the APOE genotype in the present study was different from others in Europe and Australia. As indicated in Table III, the ε2/ε3 genotype was the most frequent form (66.7%) in MS patients of this study, but in the other studies in which data were available (Table III), the most frequent form was the ε3/ε3 genotype. Data from Turkey, Iraq, ... that are neighboring countries to Iran and Asian MS patients were not available to compare. More studies of APOE allele frequencies with larger sample size are needed in developing countries to complete the pattern in MS patients.

Allelic frequency has interesting variations in different ethnic groups. The APOE gene frequency in most European and North American populations is rather homogeneous (ε3, 76 to 79 percent; ε4, 11 to 15 percent; and ε2, 6 to 9 percent). There is a clear decreasing north/south gradient for the frequency of the ε4 allele in Europe. An obvious exception is the Finnish population, in which ε4 is much more prevalent (23 to 24 percent). There are no regional differences in the apoE allele frequencies in Finland with the exception of a very high frequency of the ε4 allele (0.30) in Saami. On the other hand, Asians (specifically Chinese and Japanese) have a relatively higher frequency of ε3(~82 to 85 percent) and, interestingly, the Turks of Central Asian origin have the highest ε3 frequency (86 percent). In contrast, African nationals from the Sudan and Nigeria have lower ε3 (~61 percent) and higher ε4 (~30 percent). A study comparing the APOE genotype in Black and Caucasian men in the United States revealed that Blacks resembled the Sudanese and Nigerians (ε3, 80.3 percent in Caucasians vs. 65.3 percent in Blacks; ε4, 11.9 percent in Caucasians vs. 23.2 percent in Blacks; ε2, 7.7 percent in Caucasians vs. 11.5 percent in Blacks). One of the interesting population studies compares the apoE phenotypes of two isolated cultural groups in Papua New Guinea-the Huli and the Pawaia. The Huli are characterized by an extremely high frequency of the ε4 allele and low frequency of the ε3 allele (ε3, 35.6 percent; ε4, 49.0 percent; ε2, 15.4 percent). The Pawaia have a very different apoE phenotype pattern, more similar to that of Blacks (ε3, 60.3 percent; ε4, 25.9 percent; ε2, 13.8 percent).

In the present study, the frequency of the APOE allele in the control population was 0.5% for ε4, 51.6% for ε3, and 47.8% for ε2 that has no complete similarity to the pattern of the world populations.

Although ethnicity in the population was not comparable to the general population, we saw the same pattern in each major ethnic group. For example, the frequency of 3/2 genotype in Pure Turks, Pure Shomali and Fars were: 95, 100, and 92%, respectively. It is clear that any weighing of these values would give results in a number between 92% and 100%.

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Table III. Comparison of APOE genotype in various MS patients populations.

APOE genotype	England (n=31)	France (n=129)	Italy (n=161)	Austria (n=374)	Iran (n=81)
2/2	0	0	0	1.12	8.6
2/3	2.8	8.5	10.4	12.6	66.7
2/4	2.8	1.6	0.6	3.2	8.6
3/3	72	69.0	80	63.6	8.6
3/4	22.2	19.4	9	19.0	4.9
4/4	0	1.6	0	0.5	2.5
Reference	14	15	16	17	Present study

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