Association of apolipoprotein E polymorphism with susceptibility to multiple sclerosis

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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 contribution of the major histocompatibility complex (MHC) has been established in numerous genetic linkage and association studies. In addition to the MHC, 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 differences in APOE allele frequencies in multiple sclerosis compared with controls, we genotyped polymorphisms in four alleles namely; ε2, ε3 and e4 alleles. This study was carried out on 81 patients with clinically definite MS and 93 asymptomatic, randomly selected elderly volunteers. A significant difference was observed in the distribution of e4 allele between patients with MS and controls (9.3% vs. 0.5%; χ^2=15.2; df=2; p<0.001). This provides strong support for the association of MS with APOE ε4 allele.

Keywords: *Multiple Sclerosis, Apolipoprotein E, Polymorphism, Iran.*

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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 xonal pathology, and progressive neurological dysfunction (Hauser *et al*., 2000). MS is a major cause of morbidity and disability in young adults, with a prevalence of 0.1% in individuals of Northern European origin (Kurtzke, 1983; Rosati, 1994). Genetic factors have been implicated by numerous studies, with an estimated sibling-recurrence risk of 20-40% and a greatly increased concordance rate in MZ, compared with DZ twins (Sadovnick *et al*., 1993; Ebers *et al*., 1995).

Earlier, clinical studies and recent genomic screens have found the strongest and most widely replicated evidence for an MS susceptibility gene at the major histocompatibility complex (MHC) region on chromosome 6p21.3 (Haines *et al*., 1996; Ebers *et al*., 1996). One of the first non-MHC regions of interest identified through linkage analysis was chromosome 19q13, near the apolipoprotein E locus (Haines *et al*., 1996; Haines *et al*., 1993). Additional evidence for this region came from allelic association studies (Barcellos *et al*., 1997; Zouali *et al*., 1999; D'Alfonso *et al*., 2000) and, more recently, from follow-up analyses by the Multiple Sclerosis Genetics Group (MSGG) in both North American (Pericak-Vance *et al*., 2001) and San

Marinese populations (Haines *et al*., 2000). As with most complex diseases, the data are not entirely consistent; not all studies have shown evidence of linkage (Chataway *et al*., 1998; D´Alfonso *et al*., 1999), and association results were based on polymorphisms in different markers.

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 (Weisgraber *et al*., 1994).

Three common variants of apoE are present in the general population: apoE ε2, contains cysteine residues at amino acids 112 and 158; apoE ε3 which contains a cysteine residue at 112 and an arginine residue at 158, and apoE ε4, contains arginine at 112 and 158. The single amino acid substitutions may yield differences in the lipid binding characteristics and receptor interactions (Mahley, 1988).

Both coding-region polymorphisms and promoter-region variants, that modify *APOE* expression levels are plausible MS candidate genes. In clinical and epidemiologic studies, the role of *APOE* in MS has been somewhat controversial, because of reports of both the presence (Dousset *et al*., 1998; Evangelou *et al*., 1999; Lucotte *et al*., 2000) and the absence (Ferri *et al*., 1999; Pirttila, 2000; Weatherby *et al*., 2000a; Weatherby *et al*., 2000b) of an association between the *APOE*-4 allele with susceptibility to or severity of the disease. The limited sample size of many of these earlier studies may partly explain the inconsistent findings.

Given the strong evidence of a potential role of *APOE* in MS, the goal of the present study was to examin if *APOE* polymorphism (alleles ε2, ε3, and ε4) for association with MS susceptibility.

MATERIALS AND METHODS

Subjects

In collaboration between Tehran University of Medical Science, National Research Center for Genetic Engineering and Biotechnology, Iranian MS Society and six hospitals in Tehran, 81 patients suffering from MS were identified who volunteered for cross-sectional study. All the patients and controls were first given a consent form to be signed to make these analyses authorized according to our institutional ethical guidelines. All patients had their medical records reviewed by neurologist. Patients were asked to participate in this study if they had clinically definite MS according to Poser's criteria (Poser *et al*., 1983). In total, this population study consisted of 20 men and 61 women and had a mean age of 31.5 ± 10.0 years. All patients were white. In the patients group 12.3% (n=10) had one or more affected family members and the others (87.7%, n=71) were sporadic patients.

All ethnically matched healthy controls (n=93) were asked about their age, ethnicity and whether affected with MS. We selected age ≥ 50 for controls to lower the probability of getting MS in future. Their mean age was 64.45 ± 9.7 years, and there were 42 men and 51 women.

APOE genotyping

APOE genotyping was done according to the standard procedures. Extraction of high molecular weight DNA from peripheral whole blood, PCR amplification, and *Hin*6I restriction enzyme digestion was done. For each of the samples a two-stage PCR protocol was employed to amplify the target DNA encompassing codons 112 and 158 of the *APOE* gene. The first PCR was carried out with the outer primer sequences (Ape-1: 5´-TCC AAG GAG CTG CAG GCG GCG CA-3´ and Ape-3: 5´-ACA GAA TTC GCC CCG GCC TGG TAC ACT GCC A-3´) according to the protocol described by Wenham *et al*. (1991), amplifying a 227 bp DNA product. One microlitter of 10-100 times diluted aliquot of the initial PCR product was used as a template for the second PCR (semi-nested PCR) with the nested primer R-1: 5´-CTG GGC GCG GAC ATG GAG-3´ reported elsewhere (Egensperger *et al*., 1995), and the outer primer Ape-3, and gave rise to a 197 bp DNA product. The semi-nested PCR was performed in a 50 µl reaction mixture containing 25 pmol of each primer, 1.5 mmol/l $MgCl_2$, 200 µmol/l of each dNTPs, 50 mmol/l KCl, 10 mmol/l Tris-HCl (pH 9), 0.1% Triton X-100, and 1.4 unit *Thermus aquaticus* (*taq*) polymerase. One cycle of denaturation at 95°C for 3 min, followed by 35 cycles at 95°C for 30 s , 68°C for 30 s, and 72°C for 40 s. The reaction was terminated with a 10 min extension step at 72°C. An aliquot (25 µl) of the PCR-amplified product was digested with restriction enzyme *Hin*6I. For genotype analysis, the small fragment-sized cleavage products of *Hin*6I (48, 72, 81 and 91 bp) were electrophoresed through 20% polyacrylamide gels and stained with $AgNO_3$.

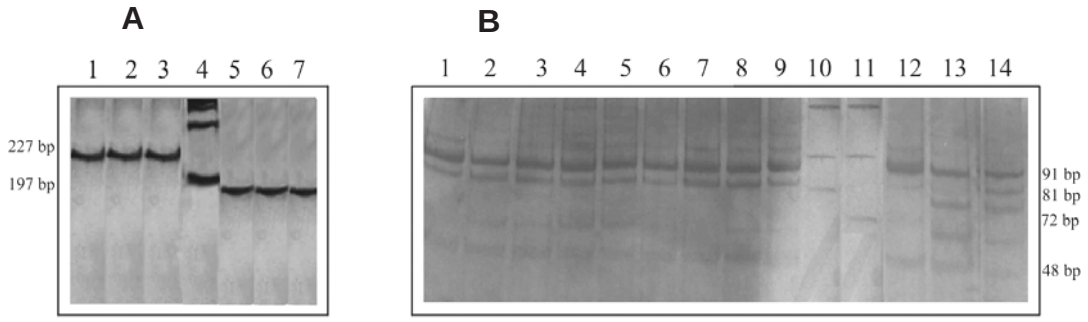

Figure 1. (A) 20% polyacrylamide gel showing different APOE genotypes. Lanes 1, 2 and 3: PCR products (227 bp) from first round PCR; Lane 4: a molecular weight marker 100 bp DNA ladder. Lanes 5, 6 and 7: PCR products (197 bp) from second round PCR (semi-nested PCR). (B) Lane 1-9, 12, 13, and 14: *APOE* also ε2/ε3, ε3/ε3, ε3/ε4 and ε2/ε4 genotypes, respectively; Lane 10 and 11: molecular marker 100 bp and 50 bp, respectively.

Statistical analysis

The distribution of *APOE* genotypes in patients with MS and controls was compared by means of χ^2 statistics. The level of statistical significance was set at $p<0.05$. All tests were performed with the statistical package for social sciences (SPSS/PC+) version 10.0.

RESULTS

In the principle of the amplification refractory mutation system as applied for detection of six common *APOE* genotypes, three specific primers are used in two PCR reactions. A common primer is located downstream of the mutation.

Figure 1 shows the results obtained by *APOE* genotyping. The use of a semi-nested PCR with PCR products from the first round PCR as a starting template produced acceptable results (Fig. 1A). Figure 1B shows gel-separated, *Hin*6I-digested products of semi-nested PCR.

The digested PCR product of allele ε3 was characterized by the presence of 91 and 48 bp products. Electrophoresis of the ε2 allele product showed 91 and 81 bp and that of ε4 allele showed 72 and 48 bp products. Thus, the presence of 91 (only), 81, and 72 bp products indicated the presence of ε3, ε2, and ε4 alleles, respectively. A combination of these electrophoretic patterns was seen in heterozygous persons. As indicate in figure 1, the first round PCR resulted in production of 227 bp fragment and the product of the second round PCR was a 197 bp. Digestion with *Hin*6I produced different fragments with 91, 81, 72 and 48 bp in length.

Eighty one patients, all white, who fulfilled the clinical criteria of definite MS were examined and genotyped at the *APOE* locus. Figures 2 and 3 show the distribution of *APOE* genotype and allele frequencies in MS patients and controls.

In the MS cohort, the frequencies were as follow: 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%). Comparison of the percentage frequencies showed that the ε4/ε4 (2.5% vs. 0.0%), ε3/ε4 (4.9% vs. 0.0%) and ε2/ε4 (8.6% vs. 1.1%) genotype was higher in MS patients than that of controls ($\chi^2=25.75$, $p<0.001$, exact test) (Fig. 2). As illustrated in figure 3, ε4 allele was more common in cases than in controls (9.3 vs. 0.5%, respectively). This difference was statistically significant ($\chi^2=15.2$, df=2; $p=0.001$).

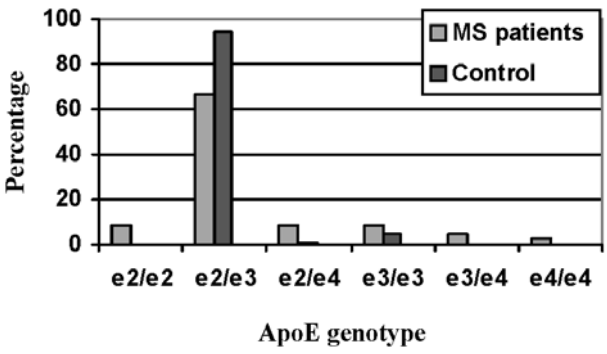

Figure 2. Distribution of *APOE* genotypes among MS patients and controls.

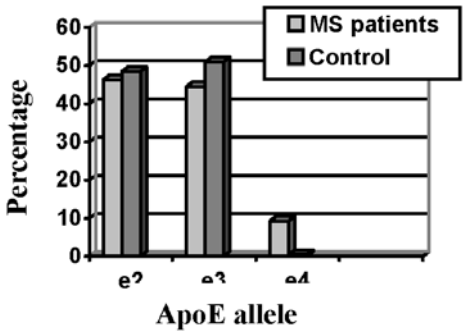

Figure 3. Distribution of *APOE* allele frequencies in MS patients and controls.

DISCUSSION

Previous studies indicated that the *APOE* genotype frequency distribution in multiple sclerosis population was not significantly different from control populations (Ballerini *et al*., 2000; Chapman *et al*., 1999; Evangelou *et al*., 1999; Gervais *et al*., 1998). Fewer studies have suggested *APOE* effects on MS susceptibility, although one case-control study reported a higher risk of developing MS for *APOE*-4/4 homozygotes (Schmidt *et al*., 2002).

Some clinical support for the *APOE*-4 association with disease progression was suggested by an MRI study of brain lesions among patients with MS, which revealed more-extensive tissue destruction or less efficient repair in *APOE*-4 carriers (Chapman *et al*., 2001). However, several studies failed to show any *APOE*-4 association with MS (Ferri *et al*., 1999; Pirttila *et al*., 2000; Weatherby *et al*., 2000a; Weatherby *et al*., 2000b). In addition to the obvious influence of sample size, other factors that may contribute to the lack of consistency across studies include, differences between study population, with respect to proportion of mild and severe MS, or distribution of disease duration.

The results of this study provide strong support for MS susceptibility by the ε4 allele of the *APOE* gene. Our findings clearly show significant differences between cases and controls, with cases having higher ε4 allele as compared to the controls. Recently, *APOE* polymorphism has been investigated in MS patients and an association emerged between the *APOE* ε4 allele and a more rapid progression of disability in MS (Schmidt *et al*., 2002; Chapman *et al*., 2001; Fazekas *et al*.,2000, 2001 and Hogh *et al*., 2000). Low levels of apoE occur in (Cerebrospinal fluid) CSF of MS patients that could result from decreased production of apoE. The association of ε4 allele with MS may suggest a genetically determined diminution of apoE secretion.

In regard to the gene and genotypic frequencies related to *APOE* polymorphism, it is important to emphasize that these frequencies vary according to the population studied. It has been suggested that the gene frequencies related to this polymorphism are extremely variable, mainly in regard to the ε4 allele frequency (Mahley, 1988; Curtiss, 2000). In Europe, this gene has a gradient distribution, the ε4 allele being more frequent in the northern region and less frequent in the southern region. In Asian populations, this allele has a low frequency, unlike that of the African and New Guina populations (Kurtzke, 1983; Rosati, 1994; Mahley, 1988). However, specific studies on the possible causes of this variation are still in their infancy. In general, the ε4 allele frequency observed in the control study were similar to those reported by other authors and provide an evidence for random selection of the control samples. Therefore, further studies that include detailed clinical measurements of an even larger number of patients and controls are needed to dissect the role of the 19q13 region in MS.

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