



The Association Between Apolipoprotein E Genetic Polymorphisms and Multiple Sclerosis

Multipl Skleroz ile Apolipoprotein E Genetik Polimorfizmi Arasındaki İlişki

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ABSTRACT

Background: Although the etiology of multiple sclerosis (MS) has not yet been clearly elucidated, MS is a chronic inflammatory disease, in which genetic and environmental factors are involved. The association between apolipoprotein E (APOE) genetic polymorphisms and various neurodegenerative diseases, including MS, is controversial. In the current study, specific APOE genotypes were investigated in patients with MS.

Methods: Fifty patients clinically diagnosed with MS and 30 healthy volunteers were included in the study. The APOE genotype was identified via the polymerase chain reaction (PCR) method. The patient and control groups were compared in terms of the frequency of APOE genotypes.

Results: The APOE genotype distribution in the patient group was as follows: $\epsilon 3/\epsilon 3$, 82.0%; $\epsilon 3/\epsilon 4$, 12.0%; and $\epsilon 2/\epsilon 3$, 6.0%. There were no significant differences between the patient and control groups with respect to the frequency of APOE genotypes, and APOE $\epsilon 4$ allele carriage ($p=0.56$). However, the frequency of APOE $\epsilon 4$ allele carriers were significantly higher among male patients ($p=0.007$).

Conclusion: These findings suggest that the APOE genotype has no effect on susceptibility to MS. Further studies with larger sample sizes to be able to include all APOE genotypes are warranted. Identification of genetic factors that may have a role in the etiology of MS will make a substantial contribution to the knowledge of the prevention and treatment of MS. (*Archives of Neuropsychiatry* 2013; 50: 110-115)

Key words: Multiple sclerosis, apolipoproteins E, genotype, neurodegenerative disease

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ÖZET

Amaç: Multipl skleroz (MS) henüz nedeni çok iyi aydınlatılmamış olmakla birlikte genetik ve çevresel faktörlerin etkisiyle ortaya çıkan kronik inflamatuvar bir hastalıktır. MS dahil çeşitli nörodejeneratif hastalıklar ile apolipoprotein E (APOE) genetik polimorfizmi arasındaki ilişki tartışmalıdır. Bu çalışmada MS'li hastalarda belirli APOE genotipleri araştırıldı.

Yöntem: Klinik olarak MS tanısı almış 50 hasta ve sağlıklı 30 kişi çalışmaya alındı. APOE genotipi PCR yöntemiyle belirlendi. APOE genotiplerinin hasta ve kontrol grubunda görülme sıklıkları karşılaştırıldı.

Bulgular: APOE genotip dağılımı MS grubunda sırayla $\epsilon 3/\epsilon 3$ %82,0, $\epsilon 3/\epsilon 4$ %12,0, $\epsilon 2/\epsilon 3$ %6,0 idi. MS ve kontrol grubu arasında genotiplerin görülme oranlarında belirgin farklılıklar yoktu ve $\epsilon 4$ taşıyıcılığı açısından anlamlı fark saptanmadı ($p=0,56$). Ancak MS hastalarında erkek cinsiyette $\epsilon 4$ taşıyıcılığı anlamlı olarak yüksek bulundu ($p=0,007$).

Sonuç: Sonuçlarımız APOE genotipinin MS'a yatkınlık üzerinde etkisinin olmadığını düşündürmüştür. Daha fazla olgu ile tüm genotipleri kapsayacak şekilde geniş kapsamlı çalışmalara gerek vardır. Etiyolojide rolü olabilecek genetik faktörlerin aydınlatılması MS hastalığının önlenmesi ve tedavi edilmesinde yararlı bilgiler sağlayacaktır. (*Nöropsikiyatri Arşivi* 2013; 50: 110-115)

Anahtar kelimeler: Multipl Skleroz, apolipoproteins E, genotip, nörodejeneratif hastalık

Çıkar çatışması: Yazarlar bu makale ile ilgili olarak herhangi bir çıkar çatışması bildirmemişlerdir.

Introduction

Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS) that usually affects young adults, and is pathologically characterized by multiple discrete areas of white matter inflammation, demyelination, and glial scarring (sclerosis) (1). Although the etiology of MS has not yet been clearly elucidated, it has been accepted that MS is a cell-mediated autoimmune disease, in which genetic and environmental factors are involved.

Data obtained from the animals in which experimental allergic encephalomyelitis (EAE) is induced have revealed that immunologic mechanisms play an important role in the pathogenesis of MS (1).

The prevalence studies have led to the impression that MS is more common at northern latitudes, however, the prevalence of MS is much lower among some races living in the same latitude. This observation has increased the interest in the underlying genetic susceptibility to MS. The gypsies, who have migrated from northern India to the Balkans in the second century and now living in

Hungary, represent a conspicuous example of this issue. The prevalence of MS among the Caucasians in Hungary is approximately 37 per 100,000, whereas the prevalence of MS is 1.8 per 100,000 among gypsies (2). Despite the fact that there have been no official prevalence studies in Turkey, the prevalence of MS among the Turkish population living in the Hesse state of Germany is lower as compared to the native population (3). The prevalence of MS among the Turkish population migrated from Turkey to Cyprus is 24 per 100,000 (4). Screening studies conducted in different regions of Turkey revealed an MS prevalence of 101/100,000 in an urban area in Istanbul (41st northern latitude) (5), of 51/100,000 in Black Sea coast (42nd northern latitude) (6), and of 30/100,000 in a metropolitan area of Edirne city (7). The prevalence of MS is also low among Orientals. The prevalence of MS is 2 per 100,000 (8) in Sapporo (Japan, 43rd northern latitude), whereas the prevalence of MS is 41 per 100,000 in Boston, which is at the same latitude as Sapporo (USA, 42nd northern latitude; 9). In brief, epidemiologic studies have shown that MS is more common among Caucasians, and is encountered less among Orientals and gypsies, revealing that certain races are more susceptible to MS. This evidence indicates the presence of an underlying genetic susceptibility. It has been shown in many studies that MS is approximately 2 times more common in females than in males (10). Although the reason is not clear, it has been considered that hormonal factors play a role in the etiology of the disease. This finding also suggests that the genetic information carried on the X chromosome might play a role in the development of autoimmune diseases. At least one of the first-, second-, or third-degree relatives of approximately 15% of MS patients has a history of the disease (11,12). Despite the limited number of epidemiologic studies performed in Turkey, the frequency of familial MS is estimated to be 3%-4% (13). In the studies performed on twins, in whom the genetic effect could be put forward prominently, the rate of development of MS in both of a pair of twins is approximately 24% among monozygotic twins, ranging from 6% to 50%. This rate is approximately 4% among dizygotic twins (14). The fact that MS is 6 times more common in monozygotic twins than dizygotic twins clearly highlights the genetic characteristics of the disease. Additionally, as the disease does not occur simultaneously in individuals who share the same genetic background, it can be suggested that an environmental factor is present in the etiology of the disease.

Interest in genetic markers to predetermine the progress of MS has gradually been increasing. Genetic markers are superior to many other markers because they are not changed with the activity of the disease. Among these markers, apolipoprotein E (APOE) genotype is one of the most promising genetic markers (the gene is designated APOE; the protein is designated apoE). Although the function of apoE in the brain is not clear, apoE is thought to play a role in the maintenance and repair of neurons, in the regulation of cholesterol transport, and in the excretion of excess cholesterol from the body. Although the number of genes involved in the etiology of MS is unknown, MS is probably a polygenic disease. The polymorphic APOE gene is located on chromosome 19q13. When family-based studies are taken into consideration, the development of MS seems to be associated with chromosome region 19q13 (15).

Three common alleles of the APOE gene are ϵ 2, ϵ 3, and ϵ 4 (10%, 74%, and 16%, respectively), which encode the protein isoforms, known as E2, E3, and E4 that exhibit different receptor-binding affinities (16). Owing to the fact that E3 is the most common isoform, E3 is considered to be the normal form, whereas E2 and E4 are considered variants.

In the current study, the APOE polymorphisms were investigated in patients diagnosed with MS and the outcomes were compared with those of the control group.

Materials and Methods

Study groups

Fifty MS patients with a disease duration of less than 2 years presenting at the MS Outpatient Clinic of Dicle University Faculty of Medicine were included in the study. The patients had been clinically diagnosed with MS according to the McDonald diagnostic criteria (17). Patients with clinical, radiologic, pathologic, or serologic findings associated with neoplasia, infection, vascular or non-demyelinating pro-inflammatory etiology were excluded from the study. The control group consisted of 30 healthy volunteers with normal neurologic examination findings. After being informed about the study, consent of the patient and control groups was obtained. The study was initiated after obtaining approval of the Local Ethics Committee.

Assessment of the APOE Genotype

The whole blood samples obtained from the patient and control groups were treated with a hypotonic solution to lyse the erythrocytes, and then centrifuged to separate leukocytes. The leukocyte pellet was suspended using sodium chloride-Tris-EDTA (STE) solution. Cell membranes and proteins were hydrolyzed by addition of sodium dodecyl sulfate (SDS) and proteinase K. Protein and cell residues were removed using phenol-chloroform following an overnight incubation. Genomic DNA was obtained by precipitating the purified DNA in cold pure ethanol.

The APOE genotype was identified by using the APOE mutation detection kit (codons 112 and 158) as developed for the Light-Cycler instrument (Table 1). By using polymerase chain reaction (PCR), a 265-bp fragment, including codons 112 and 158 of the APOE gene, was amplified from the human genome DNA. The amplicon

Table 1. APOE genotypes

Codon 112 Genotype	Codon 158 Genotype	Allele type
TGC	TGC	ϵ 2/ ϵ 2
TGC	CGC	ϵ 3/ ϵ 3
CGC	CGC	ϵ 4/ ϵ 4
TGC	CGC/TGC	ϵ 2/ ϵ 3
CGC/TGC	CGC/TGC	ϵ 2/ ϵ 4
CGC/TGC	CGC	ϵ 3/ ϵ 4

genotype was determined with specific fluorophore-labeled probes through a fluorescent signal obtained after melting curve analysis following hybridization. Allele analysis was then performed using data analysis values to determine the allele types of the patients.

Statistical Analysis

Data were analyzed using Epi Info 2000 software (CDC, Atlanta, GA, USA). A Student's t-test was used to evaluate the differences between means and chi-square analysis was used to compare the distribution of frequencies. A p value less than 0.05 was considered statistically significant.

Results

The mean age of the patient group was 32.9±9.8 years; 33 (66%) of the patients were females and 17 were males. The mean age of the healthy control group was 30.5±3.6 years and 20 (66.6%) of the healthy volunteers were females. There were no significant differences between the groups with respect to age ($p=0.21$). The APOE genotype distribution for $\epsilon 3/\epsilon 3$, $\epsilon 3/\epsilon 4$, and $\epsilon 2/\epsilon 3$ was 82.0%, 12.0%, and 6.0%, respectively. None of the patients had the homozygous $\epsilon 4/\epsilon 4$ genotype. The homozygous $\epsilon 4/\epsilon 4$ genotype was determined in one healthy volunteer. None of the patients or healthy volunteers had the homozygous $\epsilon 2/\epsilon 2$ genotype. The most common genotypes in the control group were similar to those noted in the patient group. The most frequent genotype was $\epsilon 3/\epsilon 3$ (82.0% in the patient group, and 83.33% in the control group). The demographic data of the patient and control groups and the APOE genotype distribution are presented in Table 2.

The evaluation of the APOE genotype distribution with respect to gender in MS patients revealed that the homozygous $\epsilon 3/\epsilon 3$ genotype was the most common genotype among both gender groups. However, the frequency of APOE $\epsilon 4$ allele carriers were significantly higher among male patients ($p=0.007$), as 5 of the 6 MS patients with the $\epsilon 4$ allele were males. The results are presented in Table 3. Table 4 displays the distribution of APOE $\epsilon 4$ allele carriage in the patient and control groups. No significant difference existed

Table 2. Demographic characteristics and APOE genotypes of the patient and control groups

	Patient group (n=50)	Control group (n=30)
Gender (F/M)	33/17	20/10
Age (years). mean±SD	32.9±9.8	30.5±3.6
APOE genotype distribution (%)		
$\epsilon 3/\epsilon 3$	41 (82.0)	25 (83.3)
$\epsilon 3/\epsilon 4$	6 (12.0)	2 (6.6)
$\epsilon 2/\epsilon 3$	3 (6.0)	1 (3.3)
$\epsilon 2/\epsilon 2$	-	-
$\epsilon 2/\epsilon 4$	-	1 (3.3)
$\epsilon 4/\epsilon 4$	-	1 (3.3)

between the two groups in terms of APOE $\epsilon 4$ allele carriage. The distribution of APOE $\epsilon 4$ allele carriage in the control group according to gender is presented in Table 5. It was determined that none of the males in the control group was an $\epsilon 4$ allele carrier.

Discussion

Understanding the etiology of MS will make a substantial contribution to the knowledge of the prevention and treatment of the disease. Therefore, studies concerning the etiology of MS are of importance, and the interest in genetic studies has increased in the recent years (18). However, the number of genes and the other mechanisms involved in the etiology of MS have not been clearly elucidated (19,20,21). Meta-analyses of the raw data from eleven published studies, including a total of 719 multi-case MS families revealed evidence of linkage with genome-wide statistical significance in the Major histocompatibility complex (MHC) region on chromosome 6 (22). In the genetic studies performed in England and Canada, and in the study conducted by the MS Genetics Group, the outcomes were associated with the 3q21-25, 6p21, and 19q13

Table 3. The APOE genotype distribution in the patient group according to gender

	$\epsilon 3/\epsilon 3$ n (%)	$\epsilon 2/\epsilon 3$ n (%)	$\epsilon 3/\epsilon 4$ n (%)
Female	31 (93.9)	1 (3.0)	1 (3.0)
Male	10 (58.8)	2 (11.8)	5 (29.4)
Total	41 (82.0)	3 (6.0)	6 (12.0)

$\chi^2=9.62$ $p=0.005$, Chi-Square (Monte Carlo)

Table 4. The distribution of APOE $\epsilon 4$ allele carriage in the patient and control groups

	Allele 1 ($\epsilon 2/\epsilon 3$, $\epsilon 3/\epsilon 3$) n (%)	Allele 2 ($\epsilon 3/\epsilon 4$, $\epsilon 2/\epsilon 4$, $\epsilon 4/\epsilon 4$) n (%)
Patient group	44 (88.0)	6 (12.0)
Control group	26 (86.7)	4 (13.3)
Total	70 (87.5)	10 (12.5)

$p=1.000$, Fisher's Exact Test

Table 5. The distribution of APOE $\epsilon 4$ allele carriage in the control group according to gender

	Allele 1 ($\epsilon 2/\epsilon 3$, $\epsilon 3/\epsilon 3$) n (%)	Allele 2 ($\epsilon 3/\epsilon 4$, $\epsilon 2/\epsilon 4$, or $\epsilon 4/\epsilon 4$) n (%)
Female	16 (80.0)	4 (20.0)
Male	10 (100.0)	-
Total	26 (86.7)	4 (13.3)

Fisher exact test, $p=0.12$

chromosomes (15, 23-25). APOE gene is located on chromosome 19, the relationship of which with MS has been shown (15,26,27). The frequency of each APOE isoform varies with geographical region, race and ethnicity, and the relationship between APOE polymorphism and the disease is not always consistent (28,29).

Many studies have been published concerning the APOE polymorphisms in MS, however, the results are controversial. In a study in which the frequency of APOE $\epsilon 4/\epsilon 4$ homozygotes was significantly higher among MS patients as compared to controls, the APOE $\epsilon 4/\epsilon 4$ genotype was associated with increased risk of developing MS (30). The APOE gene consists of multiple alleles, leading to the occurrence of the three common apoE isoforms. The frequencies in the general population have been reported to be 10%, 74%, and 16% for $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$, respectively (31). Owing to the fact that the E3 isoform was the most common form in various studies investigating the relationship between the general population and APOE, as well as between MS and APOE, E2 and E4 are considered variants (28,30,32,33,34,35,36,37,38,39,40,41,42,43). The prevalence of MS in Turkey is between that of the European countries in which MS is common and the Asian countries in which MS is less common. Although the findings of the current study concerning the most common genotypes were similar to those of studies conducted in European countries, the $\epsilon 3/\epsilon 3$ genotype was more common as compared to the other countries. The fact that none of the patients in the current study had the $\epsilon 2/\epsilon 4$, $\epsilon 2/\epsilon 2$, and $\epsilon 4/\epsilon 4$ genotypes might be attributed to the small sample size of the current study. On the other hand, Kuwait differs from other countries in terms of the frequency of the $\epsilon 4/\epsilon 4$ genotype (29). In the current study, in which the relationship of the APOE polymorphisms with susceptibility to MS was investigated, we determined that the patient and the control groups were similar with respect to the most common genotypes.

In a meta-analysis conducted by Pinholt et al. (44), 26 case-control studies were reviewed. The genotype distribution of 36-428 patients, who participated in these studies, was identified, and compared to that of the healthy controls. The only different outcome was obtained from the study performed by Hógh et al. (30), in which the frequency of the homozygous $\epsilon 4/\epsilon 4$ genotype was higher among 240 MS patients than the healthy controls ($n=361$), however, the frequencies of other genotypes were not different from those of the healthy controls. MS has not been associated with heterozygosity or homozygosity for APOE $\epsilon 4$ in any of the other studies (28,32,33,34,35,36,38,42,45,46,47,48,49,50,51,52). Although a relatively high frequency of the homozygous $\epsilon 4/\epsilon 4$ genotype has been noted in some studies, no significant relationship has been established (29,33,36,46,49). In their meta-analysis, Burwick et al. (53) reviewed 22 studies of MS and APOE, and concluded that the existing findings did not support a role for APOE in MS. The findings of the current study were in accordance with the existing data in the literature, with the exception of the study conducted by Hógh et al. (30), which is the only study reporting different findings. In accordance with the literature, the frequencies of APOE genotypes were similar in the patient and control groups, suggesting that there was no relationship of APOE genotype with susceptibility to the disease.

We evaluated a total of 50 MS patients (33 females, 66%; 17 males, 34%) in the current study investigating the APOE polymorphisms among MS patients. The control group consisted of 20 (66.6%) healthy female and 10 (33.3%) healthy male volunteers. No significant differences were noted between the two groups in terms of age. There were no significant differences between the patient and control groups with respect to APOE $\epsilon 4$ allele carriage, when the two groups were compared without taking the gender differences into consideration. However, intra-group analyses revealed that the $\epsilon 3/\epsilon 4$ genotype was significantly more common among male patients ($p=0.007$). In contrast, no male volunteer in the control group carried the APOE $\epsilon 4$ allele. Previous studies have established no relationship between the APOE $\epsilon 4$ allele and gender (28,33,34,35,45,46,47,48,50,51,52,54,55), with the exception of the study conducted by Al-Shammri et al. (29) on 39 MS patients. In that particular study, the authors divided the patients into two subgroups (the APOE $\epsilon 4$ category [$n=8$] and the APOE non- $\epsilon 4$ category [$n=31$]), and found a significantly higher frequency of the APOE $\epsilon 4$ allele among female patients.

In contrast to the literature, the frequency of the APOE $\epsilon 4$ allele was significantly higher among male patients in the current study. This is an observation that has not been reported before and that should be investigated among a larger population. Small number of cases for a genetic study is the major limitation of our study. Differently from the literature, the frequency of the APOE $\epsilon 4$ allele was higher in male patients in our study; this may be due to the small number of cases.

In conclusion, the frequency of the APOE $\epsilon 3/\epsilon 3$, $\epsilon 3/\epsilon 4$, and $\epsilon 2/\epsilon 3$ genotype was 82.0%, 12.0%, and 6.0%, respectively, and these findings are in agreement with the general population in terms of frequency. Owing to the fact that the frequency of the APOE $\epsilon 3/\epsilon 3$ genotype was high (82.0% in the patient group, and 83.33% in the control group) in the patient and control groups in agreement with the general population, it was concluded that the APOE genotype was not a risk factor for developing MS.

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