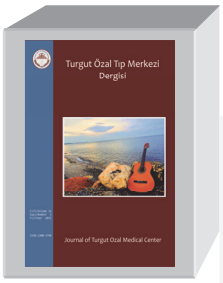

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# Apo E Genotyping İn Tuberculosis Patients From Malatya

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**Abstract**
**Objectives:** The aim of this study is to genotype the Apo E polymorphism and to show the variations in our tuberculosis patients and healthy volunteers who are from an area which has a high tuberculosis prevalance in Turkey. By comparing the data from patients and healthy individuals we aimed to understand whether host related factors like Apo E genotype of the patients are taking a role in the development of infection.
**Material and Methods:** In this study 60 Tuberculosis (TB) patients and 160 healthy individuals collected by Turgut Ozal Medical Center and Malatya Public Health Office were included. DNA was isolated from peripheral blood samples. Apo E genotyping was performed by using a commercial Real-Time PCR kit. The Fisher's exact test and Likelihood Ratio test was used to compare genotype and allele frequencies.
**Results:** Unlike others our study revealed no significant association between tuberculosis and Apo E genotypes. The data was analysed by comparing patient and healthy subject results.
**Conclusion:** This is the first study from Turkey that focuses on the association of Apo E and tuberculosis infection. Although others found, we couldn't find an association between tuberculosis and Apo E genotypes in our subject group. Although all the patients that could be reached in Malatya were included, the main limitation was the number of patients. By looking at bigger populations we may have more informative data.
**Key Words:** TB; SNP; Apo E; Genotyping.

## Malatya'daki Tüberkülozlu Hastalarda Apo E Genotiplendirmesi

**Özet**
**Amaç:** Bu çalışmadaki amacımız, ülke geneline göre tüberkülozlu hasta prevelansı yüksek olan bölgemizde, tüberküloz tanısı konmuş hasta populasyonunda ve sağlıklı gönüllülerde Apo E polimorfizmlerini genotiplendirmek ve varyasyonları göstermektir. Bu veriler ve normal bireylere ait veriler karşılaştırılarak olası Apo E-tübeküloz bağlantısına yönelik bir profil çıkarılması hedeflenmektedir.
**Gereç ve Yöntem:** Çalışmaya Turgut Özal Tıp Merkezi Mikrobiyoloji Anabilim Dalı ve Malatya İl Sağlık Müdürlüğü tarafından toplanan 60 tüberkülozlu (TB) hasta ve 160 sağlıklı birey dahil edildi. Periferal kan örneklerinden DNA izole edildi. Apo E genotiplendirmesi, Real-Time PCR temelli hazır kit kullanılarak gerçekleştirildi. Genotip ve allel frekanslarını karşılaştırmak için Fisher kesin ki-kare testi ve olabilirlik oranı kullanılmıştır.
**Bulgular:** Önceki çalışmaların aksine bizim çalışmamıza dahil ettiğimiz populasyonda tüberküloz ve Apo E genotipleri arasında bir bağlantı bulunamamıştır. Veriler hasta ve normallerin karşılaştırılması ve hasta ve sağlıklı bireylerin cinsiyetleri arasındaki farklılıkların karşılaştırılması ile analiz edilmiştir ve istatistiki açıdan anlamlı bir sonuca ulaşılamamıştır.
**Sonuç:** Bu çalışma, Apo E genotipleri ve tüberküloz arasındaki bağlantıyı araştıran Türkiye'de yapılmış ilk çalışmadır. Diğer populasyonlarda bağlantı bulunmuş olmasına rağmen biz tüberküloz ve Apo E allelleri ve genotipleri arasında bir bağlantı bulunamamıştır. Çalışmaya, Malatya ilinde ulaşılabilen tüm hastalar dahil edilmiş olmasına rağmen kısıtlayıcı etkenlerden bir tanesi hasta sayısının az olmasıdır. Daha büyük örnek grubu ile çalışarak daha bilgilendirici bir sonuca ulaşılabileceği düşünülmektedir.
**Anahtar Kelimeler:** TB; SNP; Apo E; Genotipleme.

## INTRODUCTION

Tuberculosis (TB) is one of the leading infectious diseases throughout the world and it is one of the significant mortality cause (1, 2). About one third of the world population is infected with *Mycobacterium tuberculosis* and about one tenth will develop active TB. According to World Health Organization (WHO) predictions by the year 2020 there will be 150 million new active tuberculosis cases and 37 million TB related deaths and each year 2 million people die due to tuberculosis (3, 4). For Turkey the number is 20,000 TB cases each year (4). Between 1996 and 2007, there was ~ 13% increase in TB cases in Turkey. Malatya is one of

the biggest cities in eastern Anatolia region of Turkey. Comparing to the other regions TB incidence rate is higher. Between 2003-2005 the TB cases increased ~9% and it has vital importance to understand the underlying reasons of that increase (1, 5).

The risk for developing active TB disease based on both inherited and acquired factors. There are complex interactions between host and bacterium in order to develop active TB. Among several factors in 2008 Martens et al reported that hypercholesterolemia impairs immunity to tuberculosis (3). Earlier studies showed that *Apo E* $^{-/-}$ mice have impaired defense against different pathogens like *Klebsiella penumoniae*, *Candida albicans*, *Listeria monocytogens* (6, 7, 8).

Susceptibility to different infectious agents in hypercholesterolemic mice is diverse and the reason for that may be the increased availability of lipids as a nutrient source for microbes or impaired cytotoxic C lymphocyte activation or decreased capacity for phagocytosis (3). Apo E is synthesized mainly by the liver. Apo E's main function is to act as a regulator in steps of lipid and lipoprotein metabolism (9). Apo E acts as a very high affinity ligand for members of LDL receptor the family and regulates the cholesterol and lipid exchange to target cells (10). A number of studies showed that certain Apo E alleles are associated with host response to different infectious agents like hepatitis C virus (HCV) or associated with treatment response of patients. But in literature there is a lack about the association of Apo E alleles and TB.

In literature there are only two studies related with the association of tuberculosis with Apo E alleles. A study performed by Farivar et al in 2008 showed that Apo E E4 allele frequency was higher in patients and in controls E3 was higher (11). And also E2 allele was higher in patients as well. On the other hand Wozniak et al studied with a population from Indian subcontinent and they revealed that E2 allele was a strong risk factor for women (12).

Here we aimed to investigate the relationship between Apo E genotypes and TB in patients from Malatya. 60 patients and 160 controls were the subjects of this study. They were genotyped and analyzed to get the Apo E genotype profile of that population.

## MATERIALS and METHODS

The present study was performed in the Department of Microbiology and Department of Molecular Biology and Genetics of Inonu University and was carried out in accordance with the Declaration of Helsinki guidelines and was approved by the Local Ethics Committee. All subjects were recruited from the same geographical location. Written informed consent was obtained from all patients and controls prior to the study. 60 TB patients and 160 normal healthy volunteers were included to the study. Tuberculin skin test was performed. The diagnosis was confirmed by MTB culture and radiological and histological findings. Only patients with pulmonary tuberculosis were included in the study.

Genomic DNA was extracted from anticoagulated venous blood by using a commercial kit (QIAamp DNA Blood Mini Kit) according to the manufacturer's protocol. All blood and DNA samples were coded using a coding system in order to protect the confidentiality of the subjects. Samples were genotyped by using a commercial Real-Time PCR kit ready (ApoE genotyping kit to Euroclon, Italy) using Rotorgene (QIAGEN, Germany) Real-Time PCR instrument.

### Statistical Analysis

This is a case-control study. The distributions of the allele and genotype frequencies are represented by count and percentage. The Fisher's exact test and Likelihood Ratio test was used to compare genotype and allele frequencies between cases and control group, and between genders. The level of significance was set at $p<0.05$.

## RESULTS

60 patients and 160 healthy volunteers were the subjects of this study. Our study revealed that the most common genotype both in healthy and normal subjects was E3/E3 (88% of patients and 81 % of controls) as expected. The comparison of genotypes and alleles of patients and the controls are shown in Table 1. Our analyses found no significant association to any of the genotypes that were found in patients and normals. The analyses were also performed by grouping subjects according to their genders but this subgrouping gave no association either. The comparison of different gender genotypes and allele frequencies are given for controls in Table 2 and for patients in Table 3.

**Table 1.** The distribution of genotypes and allele frequencies in patients and controls.

| Group | E2/E2 | E2/E3 | E3/E3 | E3/E4 | E4/E4 | p | E2 | E3 | E4 | p |
|---|---|---|---|---|---|---|---|---|---|---|
| **Patient N(%)** | 0 (%0) | 0 (%0) | 53 (%88,3) | 7 (%11,7) | 0 (%0) | 0,315 | 0 (%0) | 113 (%94,2) | 7 (%5,8) | |
| **Control N(%)** | 2 (%1,3) | 1 (%0,6) | 130 (%81,3) | 22 (%13,8) | 5 (%3,1) | | 5 (%1,6) | 282 (%88,4) | 32 (%10,0) | 0,067 |

**Table 2.** Gender differences in the distribution of genotypes and allele frequencies of control group

| Control group | E2/E2 | E2/E3 | E3/E3 | E3/E4 | E4/E4 | p | E2 | E3 | E4 | p |
|---|---|---|---|---|---|---|---|---|---|---|
| Female N(%) | 0 (%0) | 0 (%0) | 35 (%87,5) | 5 (%12,5) | 0 (%0) | 0,376 | 0 (%0) | 75 (%93,8) | 5 (%6,3) | 0,087 |
| Male N(%) | 2 (%1,3) | 1 (%0,6) | 95 (%81,3) | 17 (%14,2) | 5 (%4,2) | | 5 (%2,1) | 208 (%86,7) | 27 (%11,3) | |

**Table 3.** Gender differences in the distribution of genotypes and allele frequencies of patient group

| Control | E3/E3 | E3/E4 | p | E3 | E4 | p |
|---|---|---|---|---|---|---|
| Female N(%) | 21 (%84,0) | 4 (%16,0) | 0,436 | 46 (%92,0) | 4 (%8,0) | 0,449 |
| Male N(%) | 32 (%91,4) | 3 (%8,6) | | 67 (%95,7) | 3 (%4,3) | |

## DISCUSSION

The purpose of this study was to find out the relationship of Apo E genotypes with TB. In order to figure out the genotypes and most common alleles of the patients we have collected 60 TB patients that were under control in Turgut Ozal Medical Center and Malatya Public Health Office. The data about the possible effect of Apo E in infectious diseases lead us to focus on Apo E genotypes. Metabolic and immune systems are interacting together to response the pathogenic invasions. It is known that by disrupting the function of leukocytes, a metabolic syndrome with elevated triglycerides, low levels of HDL and insulin increases the risk of infections (13-15). Low levels of HDL and high levels of LDL are seen in metabolic syndrome are related with inflammation. Cholesterol in blood is complexed with lipoproteins and Apo E regulates lipid transport by binding to LDLR and regulates the cholesterol efflux pathway (16). Earlier studies showed that hypercholesterolemia due to Apo E or LDLR deficiency causes a disruption in the immune response to different infectious agents. And in 2008 Martens et al revealed that Apo E-/- hypercholesterolemic mice can't resist *Mycobacterium tuberculosis* infection (3). In the light of this information we decided to investigate the Apo E genotypes in our group of patients and healthy volunteers.

As far as we know Apo E genotypes are studied in a wide range of diseases including Alzheimer's disease, diabetes mellitus, brain tumours, glaucoma, atherosclerosis, cancer susceptibility. On the other side many pathogens like HCV, *herpes labialis, herpes simplex encephalitis* and etc. are related with Apo E since they bind one or more molecules that Apo E uses for attachment or for entry into the cell (12).

Heparin sulphate proteoglycans (HSPG) and low density lipoprotein receptor family members are the molecules that Apo E is in contact with. *M. tuberculosis* enters in to the cells by using HSPG path. Most probably pathogens and Apo E are getting into competition for these molecules. So having a specific isoform of Apo E may be a protective factor in developing TB infection. But up to now in literature there are only two reports about the relationship between Apo E genotypes and *Mycobacterium tuberculosis* infection. In 2008 Farivar et al. analyzed 250 tuberculosis patients and they used 250 age and sex matched controls. Their results showed that E4 genotype was higher in patients and in normal controls E3 was higher and E2 was higher in patients as well. They were suggesting that large population based studies were needed (11).

After that in 2009 Wozniak et al. published their results about a population from Indian subcontinent. They worked with 54 pulmonary TB cases and 75 healthy controls. They couldn't find a significant association when they analyzed the whole group as in our study. But when they separated the group by gender they have seen that Apo E2 allele frequency was significantly higher in female TB patients. Apo E2/E3 genotype was higher amongst female patients but the results were not statistically significant. On the other hand Apo E3 allele and E3/E3 genotype was higher in male patients (12).

Comparing to others, our study revealed that in our group of population Apo E3/E3 was the highest genotype seen both in patients and healthy controls (88,3% and 81,3% respectively, Table 1). Apo E3 allele was the most common allele in both groups as well (94,2 % and 88,4 % respectively, Table 1). When we separate the groups by gender again we had the results of 84% Apo E3/E3 genotype in female patients and 91,3% in male patients (Table3). In control group we didn't have any E2/E2 or E2/E3 genotypes in females and for males the frequencies were 1,3% and 0,6% respectively (Table2).

On the other side for patient group we only had E3/E3 and E3/E4 genotypes and E3/E3 was the dominant genotype in both genders. The main limitation of our study was the number of patients that we had in this study. We believe it is an important fact to work with high number of patients and controls in order to understand clearly the nature of the association. This type of association studies are needed to be done on different populations from different part of the world with high number of subjects.

## CONCLUSION

Even though it may seem an important factor in development of TB infection there appears no association between Apo E genotypes and TB infection in our group of patients. The study should be replicated in different populations with different ethnic origins and with high number of subjects.

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