Absence of apolipoprotein B-3500 mutation in Turkish patients with coronary and cerebrovascular atherosclerosis

Koroner ve serebrovasküler aterosklerozlu Türk hastalarda apolipoprotein B-3500 mutasyonu yokluğu

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ABSTRACT

Objective: The arginine- to-glutamine change at codon 3500 of the apolipoprotein B-100 (apo B) is a well-known genetic cause of hypercholesterolemia. Since increased cholesterol levels lead to atherosclerosis, identification of the carries of the apo B-3500 mutation is an important step in the risk stratification of individuals and families with hypercholesterolemia. The prevalence of this mutation in Turkish population is not well known. We aimed to investigate the frequency of apo B-100 mutations (codon 3500) C9774T (Arg 3500→Trp) and G9775A (Arg 3500→Gln) in patients with atherosclerosis in comparison with healthy subjects.

Methods: This cross-sectional study included 442 unrelated subjects living on the West coast of Turkey. Subgroups consisted of 165 patients with coronary artery disease, 163 patients with ischemic stroke, and 114 healthy control subjects.

Results: We did not find any apo B-100 mutation both in the patient and control groups.

Conclusion: As it is hypothesized that this mutation arose within the Central European region from a common ancestor approximately 7000 years ago and spread across Europe, our result of the absence of the R3500Q mutation in Turkish patients give an important information about the geographical distribution of the apo B-R3500Q, that the mutation has not reached to Anatolia. (Anadolu Kardiyol Derg 2008; 8: 7-9)

Key words: Apolipoprotein B-100, hypercholesterolemia, atherosclerosis

ÖZET

Amaç: Apolipoprotein B-100 (apo B)’ün 3500. kodonunda arginin yerine glutamin de¤iflimi bilinen bir genetik hiperkolesterolemi nedenidir. Yüksek kolesterol düzeyleri aterosklerozla yol açt›¤›ndan, apo B-100 mutasyonunu tafl›yan bireylerin saptanmas› hiperkolesterolemili aile ve kiﬂilerin risk mücadeleinde önemli bir basama¤› te¤kil etmektedir. Bu mutasyonun Türk toplumundaki prevalans› iyi bilinmemektedir. Bu çal›ﬂmada 2 farkl› apo B-100 mutasyonunun (3500 kodondaki C9774T (Arg 3500→Trp) ve G9775A (Arg 3500→Gln)) aterosklerozlu hastalardaki sikl›¤› s›¤l›kli bireylerle karﬂ›laﬂt›rmal› olarak araﬂt›rma amaçland›k.

 Yöntemler: Bu kesitsel çal›ﬂmada Türkiye’nin bat› k›y›s›nda yaﬂayan ve kan ba¤› olmayan 442 kifli incelenmiﬂtir. Koroner hastal›¤› olan 165 kifli, iskemik inme öyküsü olan 163 kifli ve 114 s›¤l›kli çal›flman›n alt gruplar›n› oluﬂturdu.

 Bulgular: Hem hastalarda, hem de kontrol grubunda hiç apo B-100 mutasyonu sa¤t›lmad›k.

 Sonuç: Bu mutasyonun yaklaﬂık 7 bin yil öncesi Avrupa’da ortak bir atadan ç›k›p tüm Avrupa’ya yayıldığı hipotezinden harekete Türk hasta- larda R3500Q apo B mutasyonuna saﬂt›rma olmaz bu mutasyonun co¤rafik dağılımı hakk›nda önemli bir bilgi vermektedir: Mutasyon henüz Anadolu’ya ulaﬂmam›ﬂt›r. (Anadolu Kardiyol Derg 2008; 8: 7-9)

 Anahtar kelimeler: Apolipoprotein B-100, hiperkolesterollemi, ateroskleroz

Introduction

Apolipoprotein B-100 (apo B-100) is the major protein component of the circulating atherogenic low-density lipoprotein (LDL) particle and serves as the ligand for the LDL receptor (1). Similar to LDL receptor defects, gene mutations in the receptor-binding zone of apo B-100 can disrupt binding and impair removal of circulating LDL. Several point mutations of the LDL receptor binding domain of apo B-100 leading to familial defective apo B-100 (FDB) disorder have been identified (2, 3). Familial defective apo B-100 disease, a genetic disorder of LDL metabolism characterized by hypercholesterolemia and premature atherosclerosis (3), is estimated to occur in one of 500 to one in 700 people in several Caucasian populations. In
most cases, it results from the mutations (C9774T and G9775A) in the codon for amino acid 3500 leading to the substitution of glutamine for arginine. Identification of the apo B-3500 mutation positive individuals is an important step in risk stratification of patients with hypercholesterolemia. The prevalence of these two mutations in Turkish population is not well known.

In the present study, we aimed to investigate the frequency of apo B-100 mutations (codon 3500) C9774T (Arg 3500 → Trp) and G9775A (Arg 3500 → Gln) in patients with atherosclerosis in comparison to healthy subjects living in Aegean coast of Turkey.

Methods

Study population and design

The study design was cross-sectional and observational. We enrolled 442 unrelated subjects living on the West coast of Turkey. These patients constituted three study groups.

One hundred and sixty-five of the patients were classified as having coronary artery disease (CAD group). The diagnosis of CAD was based on the case history of past coronary revascularization procedure or was confirmed by coronary angiography (ie they had stenosis with greater than 50% narrowing in the cross-sectional area of one of the major coronary arteries).

Cerebrovascular disease (CVD) group constituted of 163 patients with a history of ischemic stroke. The CAD and CVD groups were randomly recruited from the Cardiology and Neurology departments of Ege University Medical School. We did not use any exclusion criteria.

One hundred and fourteen healthy volunteers among the medical and paramedical staff (sixty-eight males and forty-six females) served as the control group. None of the control subjects had prior history of CAD or CVD and all had normal resting electrocardiogram.

Study protocol was in agreement with the guidelines of our Institutional Review Board and written informed consent was obtained from all subjects for the use of their blood samples for the study after the nature of the study had been explained.

Lipid and lipoprotein analysis

Blood lipid parameters were available for only the CAD and control groups. The CVD group characteristics were obtained from hospital charts. Blood samples were collected after an overnight fasting by using antecubital vein. Total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides were assessed enzymatically by auto-analyzer (Bayer Diagnostics Dax 48, Toshiba, Japan). The LDL cholesterol was calculated by the Friedewald formula (4).

DNA analysis and mutation detection

Genomic DNA was extracted from peripheral leukocytes of the subjects using the High Pure PCR Template Preparation Kit (Roche Applied Science). All experiments were carried out on the LightCycler™ Instrument (Roche Applied Science) according to the protocols provided by the manufacturer. The polymerase chain reaction (PCR) and melting curve determination were performed in 20-μl volumes in glass capillaries (Roche Applied Science) polymorphic, mutated and wild type alleles were identified by the specific melting temperature (Tm) of the resulting amplicons. For the detection of the apo B codon 3500 mutations the LightCycler apo B (codon 3500) Mutation Detection kit was used (Roche Applied Science). The temperature of the wild type allele of apo B was 64.0°C, C9774T allele - 58.4°C, and G9775A allele - 54.6°C.

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<th>Variables</th>
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<td>114</td>
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<td>151.00±13.45</td>
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<td>Male</td>
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<td>LDL, mg/dl</td>
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Data are represented as numbers and Mean±SD
* - Comparisons are made by one-way ANOVA test
HDL - high density lipoprotein, LDL - low density lipoprotein

Table 1. Clinical characteristics of the study groups
The FDB is a disorder of LDL metabolism characterized by hypercholesterolemia and premature atherosclerosis (2, 3). The FDB phenotype closely resembles the familial hypercholesterolemia phenotype (5, 6). The main genetic cause of FDB is an apo B gene mutation that substitutes a glutamine for an arginine at position 3500 of the apo B protein. This abnormal apo B protein cannot bind well to the LDL receptor leading to the accumulation of LDL in plasma. In addition to the R3500Q mutation, other forms were described (R3531C, R3480W, and R3500W) with low rates of occurrence. Among these, the R3500Q and R3531C mutations are frequent in Caucasians (0.08%), whereas the R3500W mutation is very rare in that population, but more frequent in the South Asian population (7). The frequency of R3500Q mutation in hypercholesterolemic subjects largely differs across Europe: populations with highest frequencies cluster in Central Europe, and the mutation’s frequency decreases with increasing distance from the Central Europe (8-13). As almost all subjects with the mutation carry the same haplotype in Europe, it is hypothesized that this mutation arose within the Central European region from a common ancestor approximately 7000 years ago, and spread across Europe (14-15). Recent studies from different European populations also suggest that clear distribution gradients could be tracked from Central Europe in all directions, including southeast (16-20).

We did not detect the apo B-R3500Q mutation in any of our patients. This finding is in agreement with the previous observations that the R3500Q mutation had not been found in hyperlipidemic patients in Turkey (21-22). Tamer et al. (21) failed to identify the mutation in 596 people (272 healthy controls, 145 hypercholesterolemic patients, and 179 patients with atherosclerotic coronary artery disease) living on the east Mediterranean coast of Turkey. Mahley et al. (22) also did not detect the R3500Q mutation in the survey of 2,450 participants in the Turkish Heart Study. The absence of the R3500Q mutation also supports the hypothesis of a common origin of the mutation.

Conclusion

Our results, being in agreement with previous studies (21, 22) give an important information about the geographical distribution of the apo B-R3500Q mutation whereby the mutation has reached to Balkans but not to Anatolia, provide further evidence to Rosser’s (23) suggestion: “populations such as the Hungarians and Turks are unlikely to be separated from surrounding populations by genetic barriers”.

References