Apolipoprotein B gene polymorphisms in people in the East Mediterranean area of Turkey

L. Tamer,[1] K. Tanriverdi,[2] B. Ercan,[1] A. Unlu,[1] N. Sucu,[3] H. Pekdemir[4] and U. Atik[1]

تعدد أشكال جين الأبوليبوبروتين – ب لدى الأتراك في المنطقة المُطلّة على شر ق المتوسط

لوتوهر تامر، كهرمان تانريفردي، باهادير اركان، علي أونلو، نهير سوكو، حسن بكدمير، اوغور عتيق

الخلاصة: قد تزيد الطفرات النقطية في المجال الرابط للمستقبلات في الليبوبروتين المنخفض الكثافة من مستويات الكوليسترول في الدم. وقد أدت ثلاث طفرات في أبوليبوبروتين ب – 100 إلى عيب في الربط على الشكل التالي: (الأرجينين 3500 ← غليسين، أرجينين 3500 ← تريبتوفان والأرجينين 3531 ← سيستين). وقد قدرنا معدل تكرار الطفرات النقطية للأبوليبوبروتين – ب (الرامزة 3500) C9774T (الأرجينين 3500 ← تريبتوفان) وG9775A (الأرجينين 3500 ← غليسين)، وذلك في 179 من المصابين بالتصلب العصيدي و145 من المصابين بفرط شحميات الدم و272 من الأصحاء في المناطق التركية المُطلّة على شر ق المتوسط. وقد تم قياس مستويات الشحوم والبروتينيات الشحمية بجهاز اعتيادي للقياس الطيي البيولوجي، فيما تم كشف طفرة الأبوليبوبروتين – ب باستخدام التفاعل التسلسلي للبوليميراز ذي الزمن الحقيقي، ولم يمكن العثور بأي من هذه الوسيلتين على الطفرة. لذا فإن هذه المنطقة تندر فيها الطفرات النقطية للأبوليبوبروتين ب – 100 وقد لا تعود أسباب فرط شحميات الدم والتصلب العصيدي لتلك الطفرات.

ABSTRACT Point mutations in the receptor binding domain of low density lipoprotein may increase cholesterol levels in blood. Three mutations of Apo B-100 protein result in defective binding (Arg 3500 Gln, Arg 3500 Trp and Arg 3531 Cys). We estimated the frequency of Apo B point mutations (codon 3500) C9774T (Arg 3500 Trp) and G9775A (Arg 3500 Gln) in 179 atherosclerotic, 145 hyperlipidaemic individuals and 272 healthy individuals in the east Mediterranean region of Turkey. Lipid and lipoprotein levels were measured with routine biochemical analyser and Apo B mutation was detected using real-time PCR. Neither mutation was found. In this region, Apo B-100 protein mutations are rare and causes of hyperlipidaemia and atherosclerosis may therefore be unrelated to them.

Les polymorphismes du gLne de l apolipoprotØine B dans la population de la rØgion est-mØditerranØenne en Turquie

RESUME Les mutations ponctuelles sur le site de liaison du rØcepteur des lipoprotØines de basse densitØ peuvent faire augmenter le taux de cholestØrol sanguin. Trois mutations de la protØine apo B-100 entra nent une liaison dØfectueuse (Arg 3500 Gln, Arg 3500 Trp and Arg 3531 Cys). Nous avons estimØ la frØquence des mutations ponctuelles de l apo B (codon 3500) C9774T (Arg 3500 Trp) et G9775A (Arg 3500 Gln) chez 179 patients athØrosclØrotiques, 145 sujets hyperlipidØmiques et *272 sujets en bonne* santØ de la rØgion est-mØditerranØenne en Turquie. Le taux de lipides et de lipoprotØines a ØtØ mesurØ l aide d un analyseur biochimique et la mutation de l apo B a ØtØ recherchØe *en utilisant la PCR en temps rØel.* Aucune mutation n a ØtØ trouvØe. Dans cette rØgion, les mutations de la protØine apo B-100 sont rares et les causes de l hyperlipidØmie et de l athØrosclØrose peuvent donc *ne pas Œtre liØes ces mutations.*

[1]*Department of Biochemistry;* [3]*Department of Cardiovascular Surgery;* [4]*Department of Cardiology, Faculty of Medicine, Mersin University, Mersin, Turkey.*
[2]*Department of Biochemistry, Faculty of Medicine, Cukurova University, Adana, Turkey.*
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Introduction

Nearly two-thirds of all circulating cholesterol is transported by low density lipoprotein (LDL) particles. The plasma concentration of cholesterol is regulated by which circulating LDL is taken primarily into the hepatocytes. Apolipoprotein B-100 (Apo B-100) is the major protein associated with the LDL particle and contains the ligand that binds LDL to its receptor [*1*]. Human Apo B-100 is a large, hydrophobic protein of 4536 amino acids and a molecular weight of approximately 540.000 Da. It is synthesized in the liver [*2*]. Gene mutations in the LDL receptor or in the receptor-binding zone of the Apo B-100 can disrupt binding and impair removal of circulating LDL. More than 150 mutations have been identified in the LDL receptor gene, associated with familial hypercholesterolaemia (FH), an autosomal dominant inherited disorder characterized by severe hypercholesterolaemia, frequent presence of tendon xanthomas, and premature coronary heart disease [*3*].

To date, several point mutations of the putative receptor binding domain of Apo B-100 have been identified [*4,5*]. Only 3 of these mutations have been shown to produce binding-defective Apo B-100 by appropriate genetic and functional investigations [*6,7*]. The first substitution to be discovered, and apparently the most frequent one, is Apo B-100 (Arg 3500→Gln). The other 2 substitutions are Apo B-100 (Arg 3500→Trp) and Apo B-100 (Arg 3531 >Cys) and occur less frequently [*1,5,6*]. Compared with Apo B-100 (Arg 3500→Gln), B-100 (Arg 3531→Cys) is associated with a smaller increase in LDL. Consistently, LDL that contained Apo B 100 (Arg 3531→Cys) exhibits less reduction of LDL receptor binding *in vitro* than did LDL endowed with Apo B-100 (Arg 3500→Gln) [*6,8*].

In the present study, we aimed to estimate the frequency of Apo B point mutations (codon 3500) C9774T (Arg 3500→Trp) and G9775A (Arg 3500→Gln) in 179 patients with atherosclerosis and compare this with 272 healthy subjects and 185 hypercholesterolaemic patients in the people living on the east Mediterranean coast of Turkey.

Methods

Participants

A total of 596 people were included in the study from the people living on the East Mediterranean coast of Turkey. The study sample included 272 healthy controls (133 female, 139 male), 145 controls with hypercholesterolaemia (117 female, 28 male) and 179 patients with atherosclerotic coronary artery disease (69 female and 110 male). All participants were randomly recruited from 2 hospitals in Turkey: university hospitals in Mersin and Adana. The participants were aged between 40 and 60 years. Patients with coronary artery disease were classified according to their coronary angiographic evidence of ≥ 70% stenosis of a major coronary artery. They were attending the cardiology clinic and scheduled to undergo coronary by-pass. In all, 124 patients with atherosclerosis (69%) had a history of myocardial infarction and 90 patients (50%) had smoking history. Patients with diabetes were excluded from the study. The control group was selected from clinically healthy individuals whose lipid parameters were in the normal reference range. They had no history of coronary heart disease, diabetes or hypertension; 115 individuals (42 %) had a smoking

history in the control group. People who had cholesterol levels of 200 239 mg/dL were considered as having borderline high blood cholesterol and those ≥ 240 mg/dL cholesterol levels were considered as having high blood cholesterol [9]. Since both levels are undesirable for blood cholesterol, we considered that individuals with cholesterol levels ≥ 200 mg/dL had hypercholesterolaemia; 75 (52%) subjects had a smoking history in the group with hypercholesterolaemia. Whole blood (EDTA-anticoagulated) and serum were collected after an overnight fast.

DNA isolation

DNA samples were isolated from blood samples, collected by venepuncture in sterile siliconized EDTA 2-mL Vacutainer tubes, using High Pure PCR Template Preparation Kit and MagNA Pure LC DNA Isolation Kit I by MagNa Pure LC automated DNA isolation instrument (Roche Molecular Biochemicals, Mannheim, Germany).

Detection of Apo B mutation

The determination of Apo B mutation was done using Real-time PCR and the Apo B mutation detection kit (Roche Diagnostics, GmbH, Mannheim, Germany).

Real Time PCR Principle: a 207 bp fragment of the apolipoprotein B gene was amplified with specific primers from human genomic DNA. The amplicon was detected by fluorescence using a specific pair of hybridization probes. The hybridization probes consist of 2 different short oligonucleotides that hybridize to an internal sequence of the amplified fragment during the annealing phase of the PCR cycle. If a mutation is present, the mismatches of the mutation probe with the target destabilize the hybrid. With a wild type genotype, mismatches do not occur, and the hybrid has a higher T_M. The temperature is slowly increased and when the mutation probe melts off and the 2 fluorescent dyes are no longer in close proximity, the fluorescence decreases. For mutated genotypes, this occurs at lower temperatures than for the wild type genotype

In addition, we performed DNA sequencing of 5 randomly selected patients, 5 controls and 5 individuals with hypercholesterolaemia.

Measurement of lipids and lipoproteins

Apolipoprotein A (Apo A) and Apolipoprotein B (Apo B) were determined by immunoturbidometric methods. Triglyceride (TG), total cholesterol (TC) and high density lipoprotein (HDL) were analysed by GPO/PAP enzymatic colorimetric, CHOD/PAP enzymatic colorimetric and direct COHD/PAP enzymatic colorimetric methods, respectively. LDL content was calculated from the primary measurements using the empirical equation of Friedewald et al. [10]. All these parameters were determined by Cobas Integra 700 biochemical analyser (Roche Diagnostics, GmbH, Mannheim, Germany). The results were expressed in terms of arithmetic means ± standard deviation.

Results

The levels of TC, HDL, LDL, VLDL, TG, Apo A and Apo B are given in Table 1. Our results show that there was an increase in the levels of TC, LDL, VLDL, TG and Apo B and a decrease in Apo A levels in individuals with hypercholesterolaemia and patients with atherosclerosis compared to normolipidaemic controls. On the other hand, neither C9774T (Arg 3500→Trp) nor G9775A (Arg 3500→Gln) mutations for Apo B codon 3500 was detected in the individuals by Real-Time PCR.

Table1 **Mean cholesterol, triglyceride, lipoprotein and apoprotein levels of individuals with atherosclerosis, hypercholesterolaemia and normolipidaemia**

Individuals with:	TC (ref < 200 mg/dL)	HDL (ref > 65 mg/dL)	LDL (ref < 130 mg/dL)	VLDL (ref < 40 mg/dL)	TG (ref < 200 mg/dL)	APO A (ref 180–225 mg/dL)	APO B (ref 60–133 mg/dL)
Hypercholesterolaemia (*n* = 185)	263.3 ± 27.0	43.8 ± 10.2	183.1 ± 43	35.3 ± 17.4	187.4 ± 55	144.1 ± 18.7	147.5 ± 23.1
Normolipidaemia (*n* = 272)	182.3 ± 28.0	47.5 ± 15.2	113.6 ± 26.0	22.5 ± 12.4	105.9 ± 42	153.3 ± 29.2	96.9 ± 22.2
Atherosclerosis (*n* = 179)	202.2 ± 45.6	37.5 ± 10.8	119.0 ± 36.6	30.1 ± 19	150.3 ± 45	114.9 ± 20.2	101.83 ± 26.4

Results expressed as the arithmetic mean ± standard deviation.
n = number of sample.

Discussion

The Apo B arginine-to-glutamine change at codon 3500 by been established as a cause of the failure of the LDL particle to bind to its receptor and the consequent hypercholesterolaemia of familial defective Apo B-100 [*5*]. The Apo B (Arg 3500→Gln) mutation has been identified in the USA, Denmark, Germany, Italy, France, Austria, Australia, the Netherlands, South Africa, Switzerland and indirectly in Norway and Sweden since some of the probands from the Oregan study were of Norwegian and Swedish descent [*2*]. However, the mutation has not been detected in either Finland or former Soviet Union republics. The Apo B-100 (Arg 3500→Gln) mutation has been observed with an approximate frequency of 1 in 500 to 1 in 700 in populations of European (Caucasian) origin [*2,8*].

Several point mutations of the putative receptor binding domain of Apo B-100 have been identified. Only 3 of these mutations have been shown to produce defective binding Apo B-100 by appropriate genetic and functional investigations [*3–6,10,12*]. The first substitution to be discovered, and apparently the most frequent one, was Apo B-100 (Arg 3500→Gln). The other 2 substitutions, Apo B-100 (Arg 3500→Trp) and Apo B-100 (Arg 3531→Cys), occur less frequently [*8,13*].

Teng et al. showed that the prevalence of heterozygote in 373 Chinese individuals with hyperlipidaemia was 0.3% for the Arg 3500→Gln mutation and 2.4 % for the Arg 3500→Trp mutation [*13*]. When 373 unrelated individuals with hyperlipidaemia were screened for the presence of Apo B-100 mutations, Tai et al. found 9 Arg 3500→Trp index cases, 7 classified as having type IIa and 2 as having type IIb hyperlipidaemia [*14*]. The authors reported that one of them was of Scottish descent

and the others were of Asian descent. They suggested that Arg 3500→Trp alleles were inherited from a common ancestor in Asian populations.

Fisher et al. investigated 297 consecutive individuals with LDL concentrations > 155 mg/dL and triglycerides < 200 mg/dL for Apo B mutation. Apo B-100 (Arg 3500→Trp) was described in just 1 family of European origin, in 2 families with a mixed Chinese and Malayan descent, in 1 family of Asian descent living in the Glasgow region and in another 9 unrelated individuals from Taiwan [15].

In our study we investigated the frequency of Apo B point mutations (codon 3500) C9774T (Arg 3500→Trp) and G9775A (Arg 3500→Gln) in 596 individuals (272 healthy controls, 185 controls with hypercholesterolaemia and 179 patients with atherosclerosis). Neither C9774T (Arg 3500→Trp) nor G9775A (Arg 3500→Gln) mutation was found in the people living on the East Mediterranean coast of Turkey. As can be seen in our study, the mutations of Apo B-100 protein are very rare. As regards this population, they were not observed in our sample.

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A practical guide for health researchers

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