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Genetic variations of the apolipoprotein B gene in Turkish patients with coronary artery disease

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Abstract

Background: The results of studies that clarify the association of genetic markers at the apolipoprotein B (apo B) gene (EcoRI and XbaI polymorphisms) with coronary artery disease (CAD) are not consistent and suggest that the effect is context dependent (dependent on ethnicity and sex). The present study represents the first investigation of the apo B gene polymorphisms in Turkish patients with CAD and their influence on lipid levels.

Aim: The study investigated the association of apo B gene EcoRI and XbaI polymorphisms with CAD and with variation in lipid levels (total cholesterol (T-Chol), high-density lipoprotein cholesterol (HDL-Chol), low-density lipoprotein cholesterol (LDL-Chol), and triacylglycerol (TAG)).

Subjects and methods: The study group was composed of 150 individuals with angiographically documented CAD and 100 angiographically proven to be healthy controls. PCR-RFLP was used to determine the DNA polymorphisms of the apo B gene.

Results: The frequencies of apo B genotypes detected with EcoRI (AA, AG, GG) and XbaI (CC, CT, TT) did not differ significantly between case and control subjects. A significant association between EcoRI genotypes and T-Chol ($p \leq 0.05$), and LDL-Chol ($p \leq 0.001$) was observed only in CAD patients. Patients with the AA genotype had higher levels of serum T-Chol and LDL-Chol compared with AG. With logistic regression analysis the XbaI TT genotype was found to be associated with CAD prevention. However, no significant differences in lipid variables were determined for the XbaI polymorphisms in the patients with CAD.

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Conclusions: Apo B EcoRI genotypes were not found as risk factors for CAD, whereas XbaI TT genotype was detected to prevent against CAD in our study group.

Keywords: *Polymorphism, lipoproteins, coronary artery disease*

Introduction

Atherosclerotic cardiovascular diseases (CVD) have remained the main causes of mortality in the world. A number of risk factors identified for coronary artery disease (CAD) include positive family history of CAD, cigarette smoking, hypertension, dyslipidaemia, diabetes mellitus, and obesity (Chan and Dresel 1990).

Apolipoprotein B is the exclusive protein constituent and the ligand of low-density lipoprotein (LDL), and thus plays a central role in lipid metabolism. The apo B gene is highly polymorphic and several restriction fragment length polymorphisms (RFLPs) of the apo B gene have been shown to be associated with variation in serum lipid levels in different populations (Iso et al. 1996, Gylling et al. 1997, Delghandi et al. 1999, Korhonen et al. 1999, Guzman et al. 2000, Machado et al. 2001). The EcoRI polymorphism of the apo B gene detects a mutation in the coding region (exon 29) G12669A, replacing a Glu by Lys in the peptide, the main domain for recognition of the LDL-receptor (Berg et al. 1976, Ma et al. 1987). The apo B gene XbaI cutting site is located in the coding region (exon 26) and affects the third base of the codon for Thr-2488 of apo B-100 which is not associated with an amino acid change. The apo B gene XbaI polymorphism is probably in linkage disequilibrium with another functionally important mutation in apo B-100 that affects LDL metabolism (Chan and Dresel 1990, Iso et al. 1996, Delghandi et al. 1999).

The allele lacking the XbaI site (T) and/or its homozygous genotype (TT) have been reported as more common in survivors of myocardial infarction (Hegele et al. 1986, Tybjaerg-Hansen et al. 1991, Bohn et al. 1993) and in patients with CAD (Monsalve et al. 1988, Myant et al. 1989) than in controls. However, it has been shown that XbaI polymorphism in apo B gene does not significantly determine the plasma cholesterol levels, and is not related to the levels of serum lipids and lipoproteins in childhood (Hubacek et al. 1998). Apo B EcoRI RFLP is related to changes in low-density lipoprotein cholesterol (LDL-Chol) during low and high cholesterol intake (Gylling et al. 1997). The frequency of EcoRI GG (absence of the cutting site) varies in different ethnic groups (Hegele et al. 1986, Iso et al. 1996). Hypercholesterolaemic patients with EcoRI AA (presence of the cutting site) had lower total cholesterol (T-Chol), very low-density lipoprotein cholesterol (VLDL-Chol), low-density lipoprotein cholesterol (LDL-Chol) and slower fractional catabolic rate (FCR) for LDL, and their VLDL was richer in cholesterol than that of patients with EcoRI R+/R−. Thus, hypercholesterolaemia can be due to particle-related slow clearance of LDL in some patients (Korhonen et al. 1999). However, the EcoRI polymorphism was not associated with variation of lipid profile and risk for coronary heart disease in Brazilian individuals (Guzman et al. 2000, Machado et al. 2001), in Indians (Misra et al. 2001), and in multiethnic Asian populations (West et al. 1983).

Since the contribution of EcoRI and XbaI polymorphisms to the development of CAD differ among populations, the aim of the present study is to examine the effect of the EcoRI and XbaI polymorphisms over lipid parameters and their association with CAD by evaluating their frequency distributions in patients with CAD as compared with control patients.

Material and methods

Study subjects

We studied 150 angiographically proven CAD patients (77 males, 73 females) who were inpatients, in the Group Florence Nightingale Hospital (Istanbul, Turkey). Criteria for CAD was narrowing of artery 50% or more with angiography. Control patients, those who and whose first-degree relatives did not have CAD consisted of 100 angiographically normal people (52 male, 48 female). The absence of atherosclerosis and CADs has been shown by invasive and non-invasive methods. Conventional risk factors for CAD such as dyslipidaemia (high-density lipoprotein cholesterol (HDL-Chol) levels $< 45\,\mathrm{mg\,dl^{-1}}$, triglyceride levels $> 150\,\mathrm{mg\,dl^{-1}}$ and LDL-Chol levels $> 130\,\mathrm{mg\,dl^{-1}}$), hypercholesterolaemia (T-Chol $> 200\,\mathrm{mg\,dl^{-1}}$, and LDL-Chol levels $> 130\,\mathrm{mg\,dl^{-1}}$ or on antilipidaemic agents), hypertension (blood pressure > 130/80 or prior therapy), diabetes mellitus (fasting blood glucose of $> 126\,\mathrm{mg\,dl^{-1}}$ or prior therapy), obesity (BMI > 25) and smoking (current smokers) were obtained by viewing records and interviewing patients. Lipid-lowering drug use was not withheld before lipid measurements, because it would not be ethically suitable in patients who had CAD together with dyslipidaemia. The mean age of the patients was 60.51 ± 0.95, while that of controls was 59.23 ± 1.28 years. Written consent was taken from each patient following a full explanation of the study, which was approved by the Ethics Committee of the Kadir Has University. The study groups were matched for age, as well as social and economic status.

Subjects with secondary hypertension (renal artery stenosis, glomerulonephritis), diabetic nephropathy (Kimmelstiel–Wilson syndrome), hypertension with endocrinopathies (pheochromocytoma, Cushing syndrome, hyper and hypothyroidism), patients with pseudohypertension and those who take oral contraceptives were not included in the study.

Analytical methods

The plasma glucose concentration was measured by the glucose oxidase method using kit of Biotrol on Bayer/opeRA analyser. Serum T-Chol was measured using commercial kit of Biotrol; HDL-Chol was measured using commercial Randox's kit; LDL-Chol was calculated by the formula of Friedewald and triacylglycerol (TAG) determination was made by the method of lipase/glycerol kinase UV endpoint on opera analyser.

DNA analysis

Total genomic DNA was prepared from leucocytes of 10 ml blood after lysis of red blood cells (Miller et al. 1988). Polymorphic regions of apo B gene were amplified by polymerase chain reaction (PCR) from genomic DNA. Primer sequences and procedures for PCR amplification have previously been described (Iso et al. 1996). In brief, PCR analysis of the EcoRI and XbaI polymorphic sites was performed in a DNA thermocycler using 50 µl reactions with commercially available buffer composed of 25 mM $MgCl_2$, $300\,\mu\mathrm{mol\,l^{-1}}$ of deoxynucleotide triphosphates (dNTPs), $10\,\mathrm{pmol\,\mu l^{-1}}$ of forward and reverse primers, and 1.25 units of thermostable DNA polymerase from *Thermus aquaticus* and $20\,\mathrm{ng\,\mu l^{-1}}$ DNA. Ten microlitre PCR products of apo B gene were digested with 2 units EcoRI and XbaI enzymes for at least 3 h at 37°C. Restriction fragments were separated in 1.5% agarose gel electrophoresis, visualized under a UV lamp, and their sizes were estimated according to DNA marker (pBR322-HaeIII). Alleles of EcoRI polymorphic site were classified as A or G, while alleles of XbaI polymorphic site were classified as C or T according to the respective mutated or non-mutated bases.

Statistical analysis

Statistical analyses were conducted using Unistat 5.1 software. A comparison of variables between two groups was performed using the unpaired *t*-test. Hardy–Weinberg equilibrium was tested by chi-square test. Genotype frequencies were estimated by chi-square test. A comparison of variables between two groups or among three groups was performed using the unpaired *t*-test or one-way ANOVA, respectively. Since the observed number of CC and GG genotypes in the control group were low, they were not taken into account during the comparison of lipid profiles as a function of apo B XbaI and EcoRI genotypes.

Obesity, hypertension, diabetes mellitus, dyslipidaemia, smoking habit, and the XbaI and EcoRI genotypes of the apo B gene were selected as potential risk factors for CAD. The predictors of CAD were determined by using multivariate logistic regression analysis. Odds ratios (OR) with two-tailed *p*-values were calculated as a measure of the association of the selected parameters with CAD. *p*-values ≤ 0.05 were considered significant.

Results

The comparison of some clinical characteristics in patients with CAD and controls is shown in Table I. The mean T-Chol, HDL-Chol, LDL-Chol, body mass index (BMI), systolic blood pressure levels of patients between CAD and controls were not significantly different. However, TAG, fasting glucose, and diastolic blood pressure levels were higher in patients as compared with controls (Table II). The genotype frequency distributions for the CAD and control groups with respect to EcoRI and XbaI polymorphisms are presented in Table III. None of the differences in genotype frequencies between the CAD and control groups was statistically significant. Association of the apo B gene EcoRI and XbaI polymorphisms with respect to lipid parameters are shown in Tables IV and V, respectively. In patients with CAD, the apo B gene EcoRI genotypes were not found to have significantly different HDL-Chol and TAG levels when compared with each other. Whereas T-Chol and LDL-Chol levels were found to differ between apo B gene EcoRI AG, AA and GG genotypes ($p \leq 0.05$). With Bonferonni test the EcoRI AA carriers had significantly higher ($p \leq 0.05$) LDL-Chol levels than that of AG genotype carriers. Among the controls, no such association was found between EcoRI AG and AA genotypes for the analysed lipid parameters. There was no significant difference in the levels of any lipids either in the CAD or in control groups when different apo B XbaI genotypes were compared.

Obesity, hypertension, diabetes mellitus, dyslipidaemia, smoking habit and apo B EcoRI (AG, AA) and XbaI (TT, CT) genotypes were selected as conventional risk factors to be analysed in multiple logistic regression analysis (Table VI). Hypertension, diabetes mellitus, dyslipidaemia and smoking were found to be as risk factors for CAD in our study. We observed that the apo B XbaI TT genotype was found to be associated with CAD prevention, whereas EcoRI genotypes were not associated with either protective or increased risk with CAD.

Discussion

The entire lipid levels were higher in patients as compared to controls in the present study; however, only TAG levels reached statistical significance. One possible explanation for this could be that lipid-lowering drugs were not withheld prior to lipid testing for this study as this would not have been ethically justifiable in patients who had angiographically proven CAD with dyslipidaemia and were already on lipid lowering drugs.

Table I. Clinical characteristics of CAD patients and control subjects.

	Patients ($n=150$) n (%)	Controls ($n=100$) n (%)	$p\leq$
Dyslipidaemia	109 (72)	34 (32)	0.001
Hypertension	97 (66.7)	24 (24)	0.001
Diabetes mellitus	56 (37)	8 (8)	0.001
Obesity	100 (67.3)	58 (58)	NS
Smokers	33 (22)	2 (2)	0.01

The variables were compared with χ^2 test among groups. NS, statistically not significant.

Table II. Clinical and biochemical characteristics of CAD patients and control subjects.

	Patient ($n=150$)	Control ($n=100$)
BMI ($kg\,m^{-2}$)	26.88 ± 0.44	25.89 ± 0.56
SBP (mmHg)	129.37 ± 2.11	128.20 ± 2.05
DBP (mmHg)	77.62 ± 0.97*	74.50 ± 0.95
T-Chol ($mg\,dl^{-1}$)	198.63 ± 4.69	191.14 ± 5.27
HDL-Chol ($mg\,dl^{-1}$)	43.29 ± 2.27	47.78 ± 1.19
LDL-Chol ($mg\,dl^{-1}$)	132.47 ± 3.89	130.32 ± 5.33
TAG ($mg\,dl^{-1}$)	143.38 ± 7.06**	112.16 ± 6.80
Fasting glucose ($mg\,dl^{-1}$)	116.62 ± 4.84***	84.08 ± 2.11

Values are represented as means $\pm$ SE. NS, statistically not significant. SBP, systolic blood pressure. DBP, diastolic blood pressure. Groups were compared with Student's t-test for the variables. *$p\leq0.05$, **$p\leq0.01$, ***$p\leq0.001$.

Table III. Apolipoprotein B gene EcoRI and XbaI genotype frequencies in CAD patients and control subjects.

	EcoRI genotypes; n (%)			XbaI genotypes; n (%)		
	AG	GG	AA	CT	TT	CC
CAD	33 (22.2)	11 (7.1)	106 (70.7)	85 (56)	47 (32)	18 (12)
Control	22 (22)	6 (6)	72 (72)	46 (46)	46 (46)	8 (8)

Table IV. Associations of apo B gene EcoRI polymorphism with lipid profile in CAD patients and control subjects.

	Apo B gene EcoRI polymorphism			
CAD	AG ($n=33$)	AA ($n=106$)	GG ($n=11$)	ANOVA, $p\leq$
T-Chol ($mg\,dl^{-1}$)	178.90 ± 7.15	202.69 ± 6.01	206.57 ± 8.30	0.05
HDL-Chol ($mg\,dl^{-1}$)	36.65 ± 1.96	44.16 ± 3.05	49.14 ± 2.98	NS
LDL-Chol ($mg\,dl^{-1}$)	111.98 ± 6.45	137.99 ± 4.89	131.86 ± 6.65	0.05
TAG ($mg\,dl^{-1}$)	169.75 ± 15.22	138.45 ± 8.20	126.71 ± 30.06	NS
Control	AG ($n=22$)	AA ($n=72$)	GG ($n=6$)	Student's t-test, $p\leq$
T-Chol ($mg\,dl^{-1}$)	194.43 ± 10.11	191.92 ± 6.39	ND	NS
HDL-Chol ($mg\,dl^{-1}$)	44.35 ± 1.98	48.58 ± 1.50	ND	NS
LDL-Chol ($mg\,dl^{-1}$)	134.66 ± 10.28	128.42 ± 6.36	ND	NS
TAG ($mg\,dl^{-1}$)	136.26 ± 17.43	108.00 ± 7.05	ND	NS

ND, statistical analysis not performed because of low sample number. NS, statistically not significant.

Table V. Associations of apo B gene XbaI polymorphism with lipid profile in CAD.

	Apo B gene XbaI polymorphism			
CAD	CT ($n=85$)	TT ($n=47$)	CC ($n=18$)	ANOVA, $p\leq$
T-Chol (mg dl^{-1})	202.29 ± 6.39	195.53 ± 9.23	189.33 ± 7.25	NS
HDL-Chol (mg dl^{-1})	44.43 ± 3.42	42.80 ± 3.72	39.17 ± 1.99	NS
LDL-Chol (mg dl^{-1})	137.35 ± 5.25	125.30 ± 7.49	127.00 ± 7.63	NS
TAG (mg dl^{-1})	133.62 ± 7.77	162.93 ± 16.07	139.73 ± 18.21	NS
Control	CT ($n=46$)	TT ($n=46$)	CC ($n=8$)	Student's t-test, $p\leq$
T-Chol (mg dl^{-1})	182.56 ± 4.31	196.13 ± 8.15	ND	NS
HDL-Chol (mg dl^{-1})	46.03 ± 1.22	47.43 ± 1.46	ND	NS
LDL-Chol (mg dl)	118.67 ± 5.87	137.22 ± 7.59	ND	NS
TAG (mg dl)	105.54 ± 5.43	121.56 ± 12.60	ND	NS

ND, Statistical analysis not performed because of low sample number. NS, statistically not significant.

Table VI. Risk factors identified to be associated with CAD by multiple logistic regression analysis.

		Parameter estimates			
		β	SE	OR	$p=$
Obesity		0.072	0.232	1.070	0.758
Hypertension		0.841	0.229	2.320	0.002
Diabetes mellitus		0.824	0.322	2.279	0.010
Dyslipidaemia		0.663	0.226	1.942	0.003
Smoking habit		1.311	0.627	3.711	0.037
Apo B EcoRI	AG	0.307	0.497	1.359	0.537
	GG	1.102	0.437	1.107	0.816
	AA	−0.409	0.738	0.665	0.579
Apo B XbaI	CT	0.142	0.326	1.152	0.663
	TT	−0.759	0.366	0.468	0.038
	CC	0.617	0.484	1.854	0.202

The multivariate logistic regression model contained obesity, hypertension, diabetes mellitus, dyslipidaemia, smoking habit, and apo B EcoRI and XbaI genotype variables. β indicates estimated coefficient. SE, standard error. OR, adjusted odds ratio.

The genetic variation of apo B has been studied extensively. The allelic variation of apo B gene polymorphisms may have some association with various ethnic groups. The EcoRI AA genotype has been reported to be the most frequent one in Koreans (Hong et al. 2001), Japanese (Iso et al. 1996) and multiethnic asian populations (Misra et al. 2001), whereas the EcoRI GG genotype has been shown to be the major genotype in Finns (Gylling et al. 1997) and west Eurasians (Hegele et al. 1986, Tybjaerg-Hansen et al. 1991). The frequency of XbaI T allele has been found to be high in North Indians (Misra et al. 2001), Koreans (Hong et al. 2001) and multiethnic Asian populations (Choong et al. 1999). In our study, the AA and CT genotypes were found to be the highest in frequency when compared to AG, GG and TT, CC, respectively in both patients and controls except that the XbaI CT and TT genotype frequencies were equal in control subjects. No significant differences were observed in genotype frequencies at the EcoRI and XbaI polymorphic sites in the apo B gene between CAD patients and controls in the present study.

Apo B EcoRI polymorphism has been widely studied in a number of populations (West et al. 1983, Genest et al. 1990, Siest et al. 1995, Iso et al. 1996, Gylling et al. 1997, Delghandi et al. 1999, Guzman et al. 2000, Machado et al. 2001, Misra et al. 2001), and data about the effect of EcoRI polymorphism on cholesterol level differ among these ethnic groups. Apo B EcoRI polymorphism has been found to affect the cholesterol levels in Norwegians (Howard et al. 1996), in Japanese (Iso et al. 1996) and west Eurasians (Chan and Dresel 1990). There was no association between apo B EcoRI polymorphism and cholesterol level in multiethnic Asian populations (West et al. 1983), Finns (Korhonen et al. 1999), west Eurasians (Iso et al. 1996), Asian Indians (Misra et al. 2001). In the present study we show the clear association of the EcoRI polymorphism in the apo B gene on the variation of plasma levels of LDL-Chol and T-Chol in the CAD patients, in which the AA genotype carriers had higher levels of LDL-Chol and T-Chol as compared to the AG genotype carriers.

Some reports failed to show any relation between XbaI polymorphism and serum lipid levels in atherosclerotic patients (Delghandi et al. 1999) while other studies determined association between XbaI TT genotype and low total and LDL-Chol levels in hyperlipidaemic subjects and concluded a contribution of XbaI TT genotype to the susceptibility for the development of coronary heart disease (Korhonen et al. 1999, Machado et al. 2001). In the present study, although T-Chol and LDL-Chol levels were lower in the TT genotype when compared with CT, the difference did not reach significance either in the CAD or in the control group. As a possible explanation for the differences of allele frequency and lipid association of the apo B polymorphisms among populations studied, the differences in the genetic background may be a more important factor than environmental variations, such as diet or lifestyle. Another possibility is that they may be due to the differences in linkage disequilibria between the two polymorphic sites of the apo B gene among populations. A meta-analysis has demonstrated positive associations for EcoRI and CAD, with odds ratio (OR) of 1.73 (95% CI 1.19–2.50) for carriers of AA (Chiodini et al. 2003). Another meta-analysis on west Eurasians concluded that homozygotes for the XbaI C allele had significantly elevated levels of LDL-Chol and apoB, but a decreased risk of ischaemic heart disease (IHD). In subjects homozygous for the EcoRI A allele had significantly decreased levels of total and LDL-Chol, but unaltered risk of IHD (Boekholdt et al. 2003).

In conclusion, there was no significant difference in genotypic frequencies of the EcoRI and XbaI sites of the apo B gene between the patient and control groups in our study. The presence of the EcoRI cutting site of apo B gene is associated with higher serum T-Chol and LDL-Chol levels whereas the XbaI genotypes were not found to be associated with the lipid variables in this sample of Turkish CAD patients. The protective effect of XbaI TT genotype was determined against CAD, whereas neither a protective nor an increased risk was shown for EcoRI genotypes. The limits in power of the present study due to small sample size means that larger studies are required.

References

Berg K, Hames C, Dahlen G, Frick MH, Krishan I. 1976. Genetic variation in serum low density lipoproteins and lipid levels in man. Proc Natl Acad Sci USA 73:937–940.

Boekholdt SM, Peters RJ, Fountoulaki K, Kastelein JJ, Sijbrands EJ. 2003. Molecular variation at the apolipoprotein B gene locus in relation to lipids and cardiovascular disease: a systemic meta-analysis. Hum Genet 113:417–425.

Bohn M, Bakken A, Erikssen J, Berg K. 1993. XbaI polymorphism in DNA at the apolipoprotein B locus is associated with myocardial infarction (MI). Clin Genet 44:241–248.

Chan L, Dresel H. 1990. Genetic factors influencing lipoprotein structure: implications for atherosclerosis. Lab Invest 62:522–537.

Chiodini BD, Barlera S, Franzosi MG, Beceiro VL, Introna M, Tognoni G. 2003. apo B gene polymorphisms and coronary artery disease: A meta-analysis. Atherosclerosis 167:355–366.

Choong ML, Srthi SK, Koay ES. 1999. Effects of intragenic variability at 3 polymorphic sites of the apolipoprotein B gene on serum lipids and lipoproteins in a multiethnic Asian population. Hum Biol 71:381–397.

Delghandi M, Thangarajah R, Nilsen M, Grimsgaard S, Bonaa KH, Tonstad S, Jorgensen L. 1999. DNA polymorphisms of the apolipoprotein B gene (XbaI, EcoRI, and MspI RFLPs) in Norwegians at risk of atherosclerosis and healthy controls. Acta Cardiol 54:215–225.

Genest JJ, Ordovas JM, McNamara JR, Robbins AM, Meade T, Cohn SD, Salem DN, Wilson PW, Masharani U, Frossard PM, Schaefer EJ. 1990. DNA polymorphisms of the apolipoprotein B gene in patients with premature coronary artery disease. Atherosclerosis 82:7–17.

Guzman EC, Hirata MH, Quintao EC, Hirata RD. 2000. Association of the apolipoprotein B gene polymorphisms with cholesterol levels and response to fluvastatin in Brazilian individuals with high risk for coronary heart disease. Clin Chem Lab Med 38:731–736.

Gylling H, Kontula K, Koivisto UM, Miettinen HE, Miettinen TA. 1997. Polymorphisms of the genes encoding apoproteins A-I, B, C-III, and E and LDL receptor, and cholesterol and LDL metabolism during increased cholesterol intake. Common alleles of the apoprotein E gene show the greatest regulatory impact. Arterioscler Thromb Vasc Biol 17:38–44.

Hegele RA, Huang LS, Herbert PN, Blum CB, Buring JE, Hennekens CH, Breslow JL. 1986. Apolipoprotein B-gene DNA polymorphisms associated with myocardial infarction. N Engl J Med 315:1509–1515.

Hong SH, Song J, Kim JQ. 2001. The haplotype analyses using multiple markers of the apolipoprotein B gene in patients with coronary artery disease. J Korean Med Sci 16:719–724.

Howard BV, Lee ET, Fabsitz RR, Robbins DC, Yeh JL, Cowan LD, Welty TK. 1996. Diabetes and coronary heart disease in American Indians: The Strong Heart Study. Diabetes 45:Suppl. 3, S6–S13.

Hubacek JA, Pistulkova H, Pisa Z, Valenta Z, Skodova Z, Poledne R. 1998. Lack of an association between apolipoprotein B XbaI polymorphism and blood lipid parameters in childhood. Physiol Res 47:89–93.

Iso H, Harada S, Shimamoto T, Folsom AR, Koike K, Sato S, Iida M, Komachi Y. 1996. Polymorphism of the apolipoprotein B gene and blood lipid concentrations in Japanese and Caucasian population samples. Atherosclerosis 126:233–241.

Korhonen T, Savolainen MJ, Kesaniemi YA. 1999. Variation of apolipoprotein B as a possible cause of decreased low density lipoprotein clearance and hypercholesterolemia. Atherosclerosis 146:1–10.

Ma YH, Schumaker VN, Butler R, Sparkes RS. 1987. Two DNA restriction fragment length polymorphisms associated with Ag(t/z) and Ag(g/c) antigenic sites of human apolipoprotein B. Arteriosclerosis 7:301–305.

Machado MO, Hirata MH, Bertolami MC, Hirata RD. 2001. Apo B gene haplotype is associated with lipid profile of higher risk for coronary heart disease in Caucasian Brazilian men. J Clin Lab Anal 15:19–24.

Miller SA, Dykes DD, Polesky HF. 1988. A simple salting out procedure for extracting DNA from nucleated cells. Nucleic Acids Res 16:1215.

Misra A, Nishanth S, Pasha ST, Pandey RM, Sethi P, Rawat DS. 2001. Relationship of Xba1 and EcoR1 polymorphisms of apolipoprotein-B gene to dyslipidemia and obesity in Asian Indians in North India. Indian Heart J 53:177–183.

Monsalve MV, Young R, Jobsis J, Wiseman SA, Dhamu S, Powell JT, Greenhalgh RM, Humphries SE. 1988. DNA polymorphisms of the gene for apolipoprotein B in patients with peripheral arterial disease. Atherosclerosis 70:123–129.

Myant NB, Gallagher J, Barbir M, Thompson GR, Wile D, Humphries SE. 1989. Restriction fragment length polymorphisms in the apo B gene in relation to coronary artery disease. Atherosclerosis 77:193–201.

Siest G, Henny J, Galteau MM, Schiele F, Steinmetz J, Visvikis S. 1995. Lipid and lipoprotein genetic variability: an important contribution from the French health examination centers. Clin Biochem 28:31–38.

Tybjaerg-Hansen A, Nordestgaard BG, Gerdes LU, Humphries SE. 1991. Variation of apolipoprotein B gene is associated with myocardial infarction and lipoprotein levels in Danes. Atherosclerosis 89:69–81.

West KM, Ahuja MM, Bennett PH, Czyzyk A, De Acosta OM, Fuller JH, Grab B, Grabauskas V, Jarrett RJ, Kosaka K, et al. 1983. The role of circulating glucose and triglyceride concentrations and their interactions with other 'risk factors' as determinants of arterial disease in nine diabetic population samples from the WHO multinational study. Diabetes Care 6:361–369.

Résumé. *Arrière plan:* Les résultats des études qui clarifient l'association des marqueurs génétiques du gène B (apo B) de l'alipoprotéine (polymorphismes EcoRI et XbaI) avec la

maladie coronarienne (MC), ne sont pas satisfaisants et suggèrent que l'effet est dépendant du contexte (dépendance par rapport au sexe et à l'ethnicité). Cette étude est la première recherche sur les polymorphismes EcoRI et XbaI du gène apo B chez des patients turcs souffrant de MC et sur leur influence sur les niveaux lipidiques.
But: L'étude explore l'association des polymorphismes EcoRI et XbaI du gène apo B avec la MC et avec la variation des niveaux lipidiques : cholestérol total (Chol-T), cholestérol de lipoprotéines de haute densité (Chol-LHD), cholestérol de lipoprotéines de basse densité (Chol-LBD) et glycéroltriacyl (GTA)
Sujets et méthodes. Le groupe étudié est composé de 150 individus présentant une angiographie de MC et de 100 individus a angiographie saine. La technique de RFLP-PCR a été utilisée pour déterminer les polymorphismes d'ADN du gène apo B.
Résultats: Les fréquences des génotypes de apo B détectées avec EcoRI (AA, AG, GG) et XbaI (CC, CT, TT) ne diffèrent pas significativement entre patients et contrôles. Une association significative entre génotypes EcoRI et Chol-T ($p \leq 0{,}05$) ainsi qu'avec Chol-LBD ($p \leq 0{,}001$) n'a été observée que chez les patients à MC. Les patients de génotype AA ont un niveau plus élevé que ceux de génotype AG pour les niveaux de Chol-T et de Chol-LBD dans le sérum. Par analyse de régression logistique, on trouve que le génotype XbaI TT est associé à la prévention de la MC. On n'a cependant pas trouvé de différence significative des variables lipidiques qui serait déterminée par les polymorphismes XbaI chez les patients MC.
Conclusion: Les génotypes apo B EcoRI n'apparaissent pas être des facteurs de risque pour la MC, tandis qu'il apparaît que le génotype XbaI TT protège de la MC dans le groupe étudié.

Zusammenfassung. *Hintergrund:* Die Ergebnisse von Studien, die die Beziehung zwischen genetischen Markern auf dem Apolipoprotein B (Apo B)-Gen und koronarer Herzkrankheit (coronary artery disease, CAD) klären, sind nicht vereinbar und legen nahe, dass der Einfluss vom Zusammenhang abhängt (je nach ethnischer Zugehörigkeit und Geschlecht). Die vorliegende Studie ist die erste Untersuchung von Apo B-Gen-Polymorphismen und ihrem Einfluss auf Lipidspiegel bei Türkischen Patienten mit CAD.
Ziel: Die Studie untersuchte die Beziehung von Apo B-Gen EcoRI- und XbaI-Polymorphismen mit CAD und mit der Schwankung der Lipidspiegel (Gesamtcholesterin (total cholesterol, T-Chol), High-density lipoprotein Cholesterin (HDL-Chol), Low-density lipoprotein Cholesterin (LDL-Chol) und Triacylglycerol (TAG)).
Probanden und Methoden: Die Studiengruppe bestand aus 150 Personen mit angiographisch dokumentierter CAD und 100 angiographisch gesicherten gesunden Kontrollen. PCR-RFLP wurden benutzt um DNS-Polymorphismen des Apo B-Gens zu bestimmen.
Ergebnisse: Die Häufigkeiten der Apo B-Genotypen, die mit EcoRI (AA, AG, GG) und XbaI (CC, CT, TT) bestimmt wurden, unterschieden nicht signifikant zwischen Patienten und Kontrollpersonen. Eine signifikante Beziehung zwischen EcoRI-Genotypen und T-Chol ($p \leq 0{,}05$) und LDL-Chol ($p \leq 0{,}001$) wurde nur bei CAD-Patienten beobachtet. Patienten mit dem Genotyp AA hatten höhere Serumspiegel von T-Chol und LDL-Chol, verglichen mit AG. Unter Verwendung einer logistischen Regressionsanalyse fand sich, dass der XbaI TT-Genotyp vor CAD schützt. Allerdings wurden keine signifikanten Unterschiede bei den Lipidvariablen hinsichtlich von XbaI-Polymorphismen bei Patienten mit CAD gefunden.
Zusammenfassung: Es wurde nicht gefunden, dass Apo B EcoRI-Genotypen Risikofaktoren für das Auftreten einer CAD darstellen, allerdings zeigte sich in unserer Studiengruppe, dass der XbaI TT-Genotyp gegen CAD schützt.

Resumen. *Antecedentes:* Los resultados de los estudios que tratan de aclarar la asociación de los marcadores genéticos en el gen de la apolipoproteína B (apo B) (polimorfismos EcoRI y XbaI) con la enfermedad arterial coronaria (EAC), no son consistentes y sugieren que el efecto depende del contexto (es dependiente de la etnicidad y del sexo). El presente estudio constituye la primera investigación sobre los polimorfismos del gen apo B en pacientes turcos con EAC y su influencia sobre los niveles lipídicos.
Objetivo: El estudio investigó la asociación de los polimorfismos EcoRI y XbaI del gen apo B con la EAC y con la variación en los niveles lipídicos (colesterol total (Col-T), colesterol asociado a lipoproteínas de alta densidad (Col-HDL), colesterol asociado a lipoproteínas de baja densidad (Col-LDL) y triacilglicerol (TAG)).
Sujetos y Métodos: El grupo estudiado estaba compuesto por 150 individuos con EAC documentada angiográficamente y 100 controles sanos, comprobados mediante un angiograma. Se utilizó la PCR-RFLP para determinar los polimorfismos del ADN del gen apo B.
Resultados: Las frecuencias de los genotipos apo B detectados con EcoRI (AA, AG, GG) y XbaI (CC, CT, TT) no diferían significativamente entre los casos y los controles. Se observó una asociación significativa entre los genotipos EcoRI y los niveles de Col-T ($p \leq 0{,}05$) y Col-LDL ($p \leq 0{,}001$), sólo en pacientes con EAC. Los pacientes con el genotipo AA tenían niveles más altos de Col-T y de Col-LDL séricos comparados con los de genotipo AG. Mediante un análisis de regresión logística se encontró que el genotipo XbaI TT estaba asociado con la prevención de la EAC. Sin embargo, en los pacientes con EAC no se encontraron diferencias significativas en las variables lipídicas para los polimorfismos XbaI.
Conclusiones: No se ha encontrado que los genotipos apo B EcoRI sean factores de riesgo para la EAC, mientras que se detectó que el genotipo XbaI TT prevenía contra la EAC en el grupo estudiado.