TaqIB and severity of coronary artery disease in the Turkish population: a pilot study

Dilara Kaman[1*], Necip İlhan[1], Nevin İlhan[1], Mehmet Akbulut[2]

[1]Department of Biochemistry, Faculty of Medicine, Firat University, Elazig, Turkey. [2]Department of Cardiology, Faculty of Medicine, Firat University, Elazig, Turkey

ABSTRACT

The cholesteryl ester transfer protein (CETP) plays a crucial role in high-density lipoprotein (HDL) metabolism. Genetic variants that alter CETP concentration may cause significant alterations in HDL-cholesterol (HDL-C) concentration. In this case-control study, we analyzed the genotype frequencies of CETP Taq1B polymorphisms in coronary artery disease patients (CAD; n=210) and controls (n=100). We analyzed the role of the CETP Taq1B variant in severity of CAD, and its association with plasma lipids and CETP concentration. DNA was extracted from 310 patients undergoing coronary angiography. The Taq1B polymorphism was genotyped using polymerase chain reaction—restriction fragment length polymorphism (RFLP) analysis. Lipid concentrations were measured by an auto analyzer and CETP level by a commercial enzyme-linked immunosorbent assay (ELISA) kit. In our study population, the B2 allele frequency was higher in control subjects than patients with single, double or triple vessel disease. B2B2 genotype carriers had a significantly higher high-density lipoprotein cholesterol (HDL-C) concentration than those with the B1B1 genotype in controls (51.93±9.47 versus 45.34±9.93; p<0.05) and in CAD patients (45.52±10.81 versus 40.38±9.12; p<0.05). B2B2 genotype carriers had a significantly lower CETP concentration than those with the B1B1 genotype in controls (1.39±0.58 versus 1.88±0.83; p< 0.05) and in CAD patients (2.04±1.39versus 2.81±1.68; p< 0.05). Our data suggest that the B2 allele is associated with higher concentrations of HDL-C and lower concentrations of CETP, which confer a protective effect on coronary artery disease.

KEY WORDS: CETP; coronary artery disease; HDL-C
DOI: http://dx.doi.org/10.17305/bjbms.2015.157 *Bosn J Basic Med Sci. 2015;15(1):9-13. © 2015 ABMSFBIH*

INTRODUCTION

Cholesteryl ester transfer protein (CETP) is a crucial protein in high-density lipoprotein cholesterol (HDL-C) metabolism. The cholesteryl ester transfer protein mediates the transfer of cholesteryl ester from HDL-C to triglyceride-rich lipoproteins [1]. It is involved in modulating concentrations of HDL-C concentration [2,3] and may, therefore, alter susceptibility to coronary artery disease (CAD). Thus increased CETP activity is associated with lower HDL-C and higher low-density lipoprotein cholesterol (LDL-C) levels. The studies have demonstrated an inverse relationship between CETP activity and HDL-C [4–8]. Based on these findings, CETP has been proposed as a candidate locus for CAD, although its role is still debated [8].

*Corresponding author: Dilara Kaman, Fırat University Faculty of Medicine, Department of Biochemistry, 23119, Elazig, TURKEY, Phone: +90 424 2333555, 2260. E-mail: drdilara_76@hotmail.com

Submitted: 26 September 2014 / Accepted: 7 November 2014

Several polymorphisms have been reported in the CETP gene locus [9-11]. The most studied polymorphism to date has been TaqIB polymorphism, which has been shown to be a silent base change affecting the 277th nucleotide in the first intron of the gene [9,12]. The allele containing the restriction site for the Taq1 endonuclease is called B1, while the allele without the restriction site is called B2. Individuals with the B2 allele have been found to have higher plasma HDL-C concentrations [5, 13-17]. B2 allele is related to lower CETP activity and higher HDL-C level [16,17]. However, some other similar studies showed conflicting results [18-20]. The association with plasma HDL as well as with CAD may be population specific and influenced by environmental factors [18, 21-23].

The primary objective of the present study was to investigate a potential association between TaqIB polymorphism and lipid levels in Turkish participants. The secondary objective was to investigate the relationship between TaqIB and severity of coronary atherosclerosis with angiographically assessed in the Turkish population.

MATERIALS AND METHODS

The Patient Population and Documentation of Cad Severity

This was a case–control study. The cases and controls were the angiographically confirmed. The study sample comprised 310 persons who underwent coronary angiography for diagnostic purposes. The angiograms were assessed by two cardiologists who were unaware that the patients were to be included in the study. None of the patients was treated with thrombolytics, angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers. Using coronary angiography, the study population was divided into subjects without any angiographically detectable CAD or with coronary arterial stenoses less than 50% (no vessel disease; n=100) and individuals with single-vessel disease (n=72), double-vessel disease (n=75) or triple-vessel disease (n=63) (Table 1). Written informed consent was obtained from all subjects, and a local ethical committee approved the study protocol.

Blood Sample Collection

Patients were fasted for at least six hours before venous blood samples were taken into 10 mL EDTA vacuum tubes, and blood samples were separated in a refrigerated centrifuge within 15 min of collection. Plasma was divided into small aliquots, stored at –20°C until analysis. Serum lipids were determined immediately after collection by using the Advia 1800 (Siemens) autoanalyser and reagents.

Genotyping

DNA was extracted from EDTA anti-coagulated whole blood from all subjects (controls and patients) and was prepared from DNA isolation kit (Sigma-Aldrich, GenElute Blood genomic DNA Kit, Catalog no: NA2010, Germany). The Taq1B polymorphism was genotyped by restriction fragment length polymorphism(RFLP) assay. A 535 bp fragment in the intron 1 of the CETP gene was amplified by using the primers 5' CACTAGCCCAGAGAGAGGAGTGCC3' and 5'CTGAGCCCAGCCGCACACTAAC3' Amplification was carried out in a volume of 20 µL containing 1 - 10 µg of genomic DNA, 20 pmol of each primer, 0.5 mM of each dNTP, reaction buffer, 2% DMSO and 1.0 U of Taq DNA polymerase. Amplified products were digested overnight with 8 µL of Taq1 at 65°C. The resulting fragments were 174 and 361 bp for B1 allele and 535 bp for B2 allele.

Determination Of Plasma Cetp and High-Sensitivity C-Reactive Protein (Hscrp) Level

The plasma level of CETP and hsCRP was measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Cusabio; Catalog no: CSB-E08567h and Catalog no: CSB E08617h). The sensitivity of the CETP assay is 0.2 ng/mL, the range of detection is 0.78 to 50 ng/mL and inter-assay and intra-assay variation is 5% and the sensitivity of the hsCRP assay is 0.156 ng/mL, the range of detection is 0.625 to 40 ng/mL, and inter-assay and intra-assay variation is 10%.

Statistical Analysis

Statistical analyzes were performed by using the SPSS 12.0 statistical package. We have presented normally distributed data as mean ± SD. Categorical variables are presented by frequency of counts, and inter-group comparisons analyzed by a chi-squared analysis. ANOVA was used to test for overall differences in mean levels of total cholesterol (T-Chol), LDL-cholesterol, HDL-cholesterol, triglyceride, hsCRP and CETP between the groups. When we analyzed the quantitative relationships between CETP and lipids, bivariate correlation coefficients were calculated, using Pearson's for parametric data. Two-tailed p values are reported. For the comparisons of the values of different groups, $p<0.05$ was considered statistically significant. Logistic regression analyzes were performed to investigate the association between the environmental factors and CETP TaqIB genotypes with HDL-C levels.

RESULTS

Clinical Characteristics of Patients With Single, Double or Triple Vessel Disease and Controls

The clinical characteristics of the patients with single, double, triple vessel disease and controls are shown in Table 1. Total cholesterol and LDL cholesterol levels did not differ significantly between the groups. Levels of triglyceride, HDL cholesterol, CETP, hsCRP and Body Mass Index (BMI) were significantly different among groups (p=0.038, p=0.0005, p=0.0001, p=0.0001, and p=0.037, respectively). The higher levels of LDL cholesterol, CETP, hsCRP, BMI and the lowest levels of HDL cholesterol were shown in patients with triple vessel disease.

Genotype Frequencies

Genotypic and allelic frequencies for the study groups are shown in Table 2. There was no deviation from Hardy–Weinberg equilibrium for the polymorphisms considered. Genotype frequencies of B2B2, B2B1 and B1B1 were respectively: 29%, 45% and 26% in controls, 18.1%, 50% and 31.9% in single vessel disease, 18.7%, 33.3% and 48% in double vessel disease and 27%, 31.7% and 41.3% in triple vessel disease. The difference of genotypes between groups were statistically significant (p=0.028). The B2 and B1 allele frequencies were 51.5%, 48.5% in controls, 43.1%, 56.9% in single vessel

TABLE 1. Clinical characteristics of individuals with single, double, triple vessel disease and controls

	Control n=100	Single vessel n=72	Double vessel n=75	Triple vessel n=63	*p*
Age (years)	58.11±7.68	60.36±11.90	60.18±10.52	61.33±5.86	0.15
Gender (F/M)	53/47	48/24	49/26	46/17	0.055
Total cholesterol (mg/dL)	203.81±50.01	200.47±48.54	197.50±47.45	211.98±66.11	0.42
Triglyceride (mg/dL)	148.74±94.23	172.29±78.65	166.54±75.99	187.00±80.93	0.038
HDL-cholesterol (mg/dL)	46.76±9.83	42.81±11.07	41.99±9.81	40.06±10.04	0.005
LDL-cholesterol (mg/dL)	131.07±39.82	127.27±38.21	124.48±36.85	140.44±59.75	0.16
CETP Level (ng/mL)	1.53±0.68	1.88±1.32	2.66±1.70	3.42±1.82	0.0001
hsCRP (ng/mL)	2.76±1.13	4.50±2.12	5.41±2.53	5.62±2.55	0.0001
BMI (kg/m^2)	24.98±1.98	25.45±1.97	25.20±2.08	25.88±1.76	0.037
Smokers (%)	21%	23.6%	28.8%	27.0%	0.70

disease, 35.3%, 64.7% in double vessel disease and 42.9%, 57.1% in triple vessel disease, respectively. The difference of alleles between groups were statistically significant (*p*=0.026).

Association of the CETP Taq1B allele with lipids and CETP levels

Table 3 and Table 4 show the association between the CETP Taq1B allele and lipid and CETP level in controls and patients with CAD, respectively. The mean HDL-cholesterol was higher in the B2B2 genotype in controls and patients, and it was significantly different among groups (*p*=0.002 and *p*=0.02 respectively). Serum level of CETP was lower in B2B2 genotype in controls and patients, and it was also significantly different among groups (*p*=0.012 and *p*=0.03 respectively). No other associations were detected between the CETP Taq1B allele and total cholesterol, LDL cholesterol, triglyceride, and hsCRP.

Correlations between lipid levels and CETP

Table 5 shows the correlations between lipid levels and CETP in the total population. There was a significant negative correlation between CETP and HDL cholesterol levels (r = -0.15, *p*=0.013) but a significant correlation between CETP and LDL cholesterol, triglyceride was not found.

Logistic regression analysis was performed to analyze the relevance of selected parameters (BMI, smoking, gender and-Taq1B2) to HDL cholesterol levels (Table 6). It was observed that BMI and smoking had no lowering effect on HDL-C levels. However, gender (male) and Taq1B was a strong risk factor on HDL-C levels.

DISCUSSION

In the present study, we found an association of CETP TaqIB gene polymorphism with severity of CAD in Turkish population. For CETP, we chose theTaq1B polymorphism in intron-1, because the Taq1B polymorphism has a much larger effect on plasma CETP levels [24].

TABLE 2. Genotype and allele frequencies among groups

	Control n: 100 (%)	Single vessel n: 72 (%)	Double vessel n: 75 (%)	Triple vessel n: 63 (%)	*p*
Genotypes					
B2B2	29 (29)	13 (18.1)	14 (18.7)	17 (27.0)	0.028
B2B1	45 (45)	36 (50.0)	25 (33.3)	20 (31.7)	
B1B1	26 (26)	23 (31.9)	36 (48.0)	26 (41.3)	
Alleles					
B2	51.5	43.1	35.3	42.9	0.026
B1	48.5	56.9	64.7	57.1	

TABLE 3. Biochemical markers of the control subjects according to Taq1B genotypes

Variables	B1B1	B1B2	B2B2	*p*
Total cholesterol (mg/dL)	195.80±32.63	204.61±60.32	209.79±46.01	0.58
Triglyceride (mg/dL)	127.73±55.51	157.88±114.94	153.72±86.42	0.41
HDL-cholesterol (mg/dL)	45.34±9.93	44.19±8.85	51.93±9.47	0.002
LDL-cholesterol (mg/dL)	126.00±28.44	131.88±47.62	134.41±36.19	0.73
CETP Level (ng/mL)	1.88±0.83	1.41±0.56	1.39±0.58	0.012
hsCRP (ng/mL)	3.00±1.13	2.71±1.21	2.63±1.02	0.44

TABLE 4. Biochemical markers of the patients with CAD according to Taq1B genotypes

Variables	B1B1	B1B2	B2B2	*p*
Total cholesterol (mg/dL)	210.57±44.17	197.13±48.19	198.52±76.93	0.23
Triglyceride (mg/dL)	187.08±86.88	165.07±67.56	168.27±78.64	0.16
HDL-cholesterol (mg/dL)	40.38±9.12	40.99±10.89	45.52±10.81	0.02
LDL-cholesterol (mg/dL)	134.83±35.36	127.58±39.09	126.20±69.01	0.48
CETP Level (ng/mL)	2.81±1.68	2.83±1.68	2.04±1.39	0.034
hsCRP (ng/mL)	5.26±2.44	4.85±2.20	5.53±2.80	0.30

The role of CETP in coronary artery disease is controversial, particularly with respect to the effect of genetic variants that alter the activity and concentration of this protein and its regulation of HDL-C. Studies of these genetic variants may be significant because CETP inhibitors are still being tested as potential therapeutic agents for raising plasma HDL concentrations.

Epidemiological evidence strongly favors the notion that the risk of cardiovascular disease (CVD) is inversely related to the plasma HDL cholesterol concentration [25]. Low HDL cholesterol is still predictive of high CVD risk in subjects with low LDL cholesterol [26]. The CETP mediates the exchange of

TABLE 5. Correlations between lipid levels and CETP in total population

	CETP	T- Chol	HDL-C	LDL-C	TG
CETP					
r	-	0.016	–0.15	0.063	0.015
p		0.78	0.013	0.29	0.80
T-Chol					
r	0.016	-	0.183	0.924	0.495
p	0.78		0.001	0.0001	0.0001
HDL-C					
r	–0.148	0.183	-	0.104	–0.156
p	0.013	0.001		0.067	0.006
LDL-C					
r	0.063	0.924	0.104	-	0.259
p	0.294	0.0001	0.067		0.0001
TG					
r	0.015	0.495	–0.156	0.259	-
p	0.807	0.0001	0.006	0.0001	

TABLE 6. Logistic regression analyses for serum HDL-C levels with environmental factors and TaqIB genotypes

Independent variables	β	S.E.	*p*	OR (95% CI)
BMI	0.236	0.248	0.342	0.778-2.058
Smoking	0.318	0.291	0.275	0.777-2.431
Gender (Male)	–0.860	0.250	0.001	0.259-0.690
Taq1B2	–0.846	0.265	0.001	0.255-0.721

lipids between lipoproteins, resulting in the net transfer of cholesteryl ester from HDL to other lipoproteins. Thus, the CETP lowers HDL-C and increases non-HDL-C, resulting in a lipoprotein distribution predisposing to atheroma formation [17].

The frequencies of the genotypes B1B1, B1B2 and B2B2 in our population with coronary artery disease were 40.5, 38.6 and 21%, respectively, with a B2 allele frequency of 0.29 (data not shown). These results are similar to those reported in a Turkish population report [27]. There have been reports of differences in the frequencies of the B2 allele in other ethnic groups: the frequency of the B2 allele was found to be 0.43, 0.44, 0.43 and 0.42 in Europeans, Americans, Israelis and Taiwanese, respectively [6,15,17].

It was already suggested by others [28] that there may be a link between CETP polymorphisms and severity of CAD. Several studies have reported the B2B2 genotype to be associated with high HDL-C levels [15-17,29] this effect being looked at as a consequence of the lower CETP activity associated with this genotype [15,16,29]. The association of the B2 allele with higher HDL-C levels was confirmed in our population controls and patients with single, double or triple vessel disease. Our study is one of the few which have investigated the association between CETP Taq 1B genotype and the severity of CAD. In the present study, the highest frequency of B2 allele was shown in control subjects (51.5%) and the highest frequency of B1 allele was shown in double/triple vessel disease (61.2%).

Although Taq1B polymorphism has been the most studied RFLP to date, these studies have yielded conflicting results. Although some studies have confirmed this association, some have not [30-32]. This discrepancy in previous studies may be the result of influences of other factors on HDL-C, such as gender, BMI, smoking, and alcohol consumption. In our study, we reported that the B2 allele of the CETP gene Taq1B polymorphism was associated with a significant increase in HDL-C concentration and reduction in the severity of coronary artery disease. There are several well-known environmental factors influencing HDL-C levels that act through the activities of lipases or lipid transfer proteins. In our study male sex and-Taq1B2 decrease HDL-C levels. However, the BMI and smoking did not reach the level of significance in our study group.

In summary, CETP gene Taq 1B genotype was associated with HDL-C concentration and the severity of coronary artery disease in Turkish population. The B2 allele has a protective effect on coronary artery disease by increasing HDL-C concentration. CETP is expected to be a potential target for the development of new pharmacological agents that may raise serum HDL-C concentrations. It will therefore be interesting to examine the relationship between responses to such drugs and the genotypes of the ***CETP*** gene.

DECLARATION OF INTERESTS

The authors declare no conflicts of interests.

REFERENCES

[1] White DA, Baxter M. Hormones and Metabolic Control. 2nd ed. London: Arnold; 1994.

[2] Inazu A, Brown ML, Hesler CB, Agellon LB, Koizumi J, Takata K,et al. Increased high-density lipo protein levels caused by a common cholesteryl-ester transfer protein gene mutation. N Engl J Med 1990;323:1234-1238. http://dx.doi.org/10.1056/NEJM199011013231803.

[3] Inazu A, Jiang XC, Haraki T, Yagi K, Kamon N, Koizumi J,et al. Genetic cholesteryl ester transfer protein deficiency caused by two prevalent mutations as a major determinant of increased levels of high density lipoprotein cholesterol. J Clin Invest 1994;94:1872-1882. http://dx.doi.org/10.1172/JCI117537.

[4] Freeman DJ, Packard CJ, Shepherd J, Gaffney D. Polymorphisms in the gene coding for cholesterol ester transfer protein are related to plasma high-density lipoprotein cholesterol and transfer pr-tein activity. Clin Sci 1990;79:575–581. http://dx.doi.org/10.1161/01.ATV.17.3.560.

[5] Kuivenhoven JA, de Knijff P, Boer JM, Smalheer HA, Botma GJ, Seidell JC, et al. Heterozygosity at the CETP gene locus. Influence on plasma CETP concentrations and HDL cholesterol levels. Arterioscler Thromb Vasc Biol 1997; 17: 560–568. http://dx.doi.org/10.1161/01.ATV.17.3.560.

[6] Kark JD, Sinnreich R, Leitersdorf E, Friedlander Y, Shpitzen S, Luc G. Taq1B CETP polymorphism, plasma CETP, lipoproteins, apolipoproteins and sex differences in a Jewish population sample characterized by low HDL-cholesterol. Atherosclerosis 2000;151: 509–518.http://dx.doi.org/10.1016/S0021-9150(99) 00408-6.

[7] Agellon LB, Walsh A, Hayek T, Moulin P, Jiang XC,Shelanski SA,et al. Reduced high density lipoprotein cholesterol in human

cholesteryl ester transfer protein transgenic mice. J Biol Chem 1991;97:2917–2923.

[8] Inazu A, Koizumi J, Mabuchi H. Cholesteryl ester transfer protein and atherosclerosis. Curr Opin Lipidol 2000;11(4):389–396. http://dx.doi.org/10.1097/00041433-200008000-00008.

[9] Drayna D, Lawn R. Multiple RFLPs at the human cholesteryl ester transfer protein (CETP) locus. Nucleic Acids Res 1987;15(11):4698. http://dx.doi.org/10.1093/nar/15.11.4698.

[10] Freeman D, Shepherd J, Packard CJ, Humphries SE, Gaffney D. An StuI RFLP at the human cholesteryl ester transfer protein (CETP) locus. Nucleic Acids Res 1989;17(7):2880. http://dx.doi.org/10.1093/nar/17.7.2880.

[11] Zuliani G, Hobbs HH. EcoN I polymorphism in the human cholesteryl ester transfer protein (CEPT) gene. Nucleic Acids Res 1990;18(9):2834.http://dx.doi.org/10.1093/nar/18.9.2834-a.

[12] Yamashita S, Hirano K, Sakai N, Matsuzawa Y. Molecular biology and pathophysiological aspects of plasma cholesteryl ester transfer protein. Biochim Biophys Acta 2000;1529(1-3):257-275. http://dx.doi.org/10.1016/S1388-1981(00) 00164-5.

[13] Bernard S, Moulin P, Lagrost L., Picard S, Elchebly M, Ponsin G, et al. Association between plasma HDL-cholesterol concentration and Taq1B CETP gene polymorphism in non-insulin-dependent diabetes mellitus. J Lipid Res 1998;39(1):59-65.

[14] Fumeron F, Betoulle D, Luc G, Behague I, Ricard S, Poirier O, et al. Alcohol intake modulates the effect of a polymorphism of the cholesteryl ester transfer protein gene on plasma high-density lipoprotein and the risk of myocardial infarction. J Clin Invest 1995;96(3):1664-1671.http://dx.doi.org/10.1172/JCI118207.

[15] Gudnason V, Kakko S, Nicaud V, Savolainen MJ, Kesäniemi YA, Tahvanainen E, et al. Cholesteryl ester transfer protein gene effect on CETP activity and plasma high-density lipoprotein in European populations. The EARS Group. Eur J Clin Invest 1999;29(2):116-128. http://dx.doi.org/10.1046/j.1365-2362.1999.00412.x.

[16] Kuivenhoven JA, Jukema JW, Zwinderman AH, de Knijff P, McPherson R, Bruschke AV, et al. The role of a common variant of the cholesteryl ester transfer protein gene in the progression of coronary atherosclerosis. The Regression Growth Evaluation Statin Study Group. N Engl J Med 1998;338(2):86-93. http://dx.doi.org/10.1056/NEJM199801083380203.

[17] Ordovas JM, Cupples LA, Corella D, Otvos JD, Osgood D, Martinez A, et al. Association of cholesteryl ester transfer protein TaqIB polymorphism with variations in lipoprotein subclasses and coronary heart disease risk: the Framingham study. Arterioscler Thromb Vasc Biol 2000;20(5):1323-1329.http://dx.doi.org/10.1161/01.ATV.20.5.1323.

[18] Corbex M, Poirier O, Fumeron F, Betoulle D, Evans A, Ruidavets JB, et al. Extensive association analysis between the CETP gene and coronary heart disease phenotypes reveals several putative functional polymorphisms and gene–environment interaction. Genet Epidemiol 2000;19(1):64–80.http://dx.doi.org/10.1002/1098-2272(200007) 19:1<64::AID-GEPI5>3.0.CO;2-E.

[19] Arca M, Montali A, Ombres D, Battiloro E, Campagna F, Ricci G, et al. Lack of association of the common TaqIB polymorphism in the cholesteryl ester transfer protein gene with angiographically assessed coronary atherosclerosis. Clin Genet 2001;60(5):374–380. http://dx.doi.org/10.1034/j.1399-0004.2001.600510.x.

[20] Cuchel M, Wolfe ML, deLemos AS, Rader DJ. The frequency of the cholesteryl ester transfer protein-TaqI B2 allele is lower in African Americans than in Caucasians. Atherosclerosis 2002;163(2):169-174. http://dx.doi.org/10.1016/S0021-9150(01) 00769-9.

[21] Eiriksdottir G,Bolla MK, Thorsson B, Sigurdsson G, Humphries SE, Gudnason V. The -629C>A polymorphism in the CETP gene does not explain the association of TaqIB polymorphism with risk and age of myocardial infarction in Icelandic men. Atherosclerosis 2001;159(1):187-192. http://dx.doi.org/10.1016/S0021-9150(01) 00489-0.

[22] Park KW, Choi JH, Kim HK, Oh S, Chae IH, Kim HS, et al. The association of cholesteryl ester transfer protein polymorphism with high-density lipoprotein cholesterol and coronary artery disease in Koreans. Clin Genet 2003;63(1):31-38.http://dx.doi.org/10.1034/j.1399-0004.2003.630105.x.

[23] Radeau T, Vohl MC, Houde I, Lachance JG, Noël R,Després JP,et al. HDL cholesterol and TaqIB cholesteryl ester transfer protein gene polymorphism in renal transplant recipients. Nephron 2000;84(4):333-341.http://dx.doi.org/10.1159/000045608.

[24] Blankenberg S, Rupprecht HJ, Bickel C, Jiang XC, Poirier O, LacknerKJ, et al. Common genetic variation of the cholesteryl ester transfer protein gene strongly predicts future cardiovascular death in patients with coronary artery disease. J Am Coll Cardiol 2003;41(11):1983-1989.http://dx.doi.org/10.1016/S0735-1097(03) 00408-X.

[25] Prospective Studies Collaboration, Lewington S, Whitlock G, Clarke R, Sherliker P, Emberson J, et al. Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. Lancet. 2007;370:1829–1839.http://dx.doi.org/10.1016/S0140-6736(07) 61778-4.

[26] Barter P, Gotto AM, LaRosa JC, Maroni J, Szarek M, Grundy SM, et al. HDL cholesterol, very low levels of LDL cholesterol, and cardiovascular events. N Engl J Med.2007;357(13):1301–1310. http://dx doi.org/10.1056/NEJMoa064278.

[27] Yilmaz H, Isbir T, Agachan B, Karaali ZE. Effects of cholesterol ester transfer protein Taq1B gene polymorphism on serum lipoprotein levels in Turkish coronary artery disease patients. Cell Biochem Funct 2005;23(1):23–28.http://dx.doi.org/10.1002/cbf.1124.

[28] Chen SN,Cilingiroglu M,Todd J,Lombardi R,Willerson JT,Gotto A M Jr,et al. Candidate genetic analysis of plasma highdensity lipoprotein-cholesterol and severity of coronary atherosclerosis. BMC Med Genet 2009; 10:111.http://dx.doi.org/10.1186/1471-2350-10-111.

[29] Kuivenhoven JA, de Knijff P, Boer JM, Smalheer HA, Botma GJ, Seidell JC, et al. Heterozygosity at the CETP gene locus. Influence on plasma CETP concentrations and HDL cholesterol levels. Arterioscler Thromb Vasc Biol 1997;17:560–568.http://dx.doi.org/10.1161/01.ATV.17.3.560.

[30] Kolovou GD, Anagnostopoulou KK, Karyofillis P, Salpea KD, Yiannakouris N, Zarkalis D, et al. Cholesteryl ester transfer protein gene polymorphisms and severity of coronary stenosis. Clin Invest Med 2006;29(1):14–19.http://dx.doi.org/10.1016/j.atherosclerosis.2007.12.038.

[31] Tsai MY, Johnson C, Kao WH, Sharrett AR, Arends VL, Kronmal R, et al. Cholesteryl ester transfer protein genetic polymorphisms, HDL cholesterol, and subclinical cardiovascular disease in the Multi-Ethnic Study of Atherosclerosis. Atherosclerosis 2008;200(2):359–367.http://dx.doi.org/10.1016/j.atherosclerosis.2007.12.038.

[32] Doğru-Abbasoğlu S,Parildar-Karpuzoğlu H, Depboylu B, Cine N, Uysal M, Aykaç-Toker G.I405V and TaqIB polymorphisms of the cholesteryl ester transfer protein and their relation to serum lipid and lipoprotein levels in a Turkish population. Cell Biochem Funct 2009; 27(2):76–80.http://dx.doi.org/10.1002/cbf.1536.