

Cholesteryl ester transfer protein polymorphism associated with macroangiopathy in Japanese patients with type 2 diabetes

Shu Meguro^{a,*}, Izumi Takei^b, Mitsuru Murata^{a,b}, Hiroshi Hirose^a, Naoyuki Takei^a, Yasutaka Mitsuyoshi^b, Keiko Ishii^b, Shuji Oguchi^b, Junko Shinohara^b, Eiko Takeshita^b, Kiyooki Watanabe^b, Takao Saruta^a

^a Department of Internal Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan

^b Department of Laboratory Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan

Received 7 February 2000; received in revised form 23 June 2000; accepted 10 August 2000

Abstract

A polymorphism in the gene for cholesteryl ester transfer protein (CETP) has been reported to be associated with serum cholesterol levels and risk for atherosclerotic vascular diseases, and to clarify the relationship between the gene polymorphism for CETP and macroangiopathy in diabetes mellitus, a cross-sectional study was performed. The subjects of the study were 182 Japanese (age: 59.6 ± 8.6 years) with type 2 diabetes and no signs of renal dysfunction, 24 of whom had macroangiopathy, and 158 of whom did not. The genotype of the subjects for the TaqIB polymorphism of CETP in intron one was analyzed by using polymerase chain reaction — restriction fragment length polymorphism. Serum CETP levels were significantly higher in the B1/B1 genotype than in the other genotypes ($P < 0.05$). The serum CETP levels were correlated with the serum LDL cholesterol levels ($P < 0.01$), but not with the HDL cholesterol levels. Macroangiopathy was more frequently observed in subjects with the B1/B1 genotype than in the other genotypes (odds ratio = 2.953, 95% confidence interval = 1.250–6.977, $P = 0.0136$). Logistic regression analysis revealed that the CETP genotype was independently associated with macroangiopathy. The exact mechanism underlying the association remains unknown, but differences in serum CETP levels may be involved. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Type 2 diabetes mellitus; Atherosclerosis; Cholesteryl ester transfer protein; Polymorphism

1. Introduction

Type 2 diabetes mellitus is associated with a major risk factor for coronary heart disease, cerebral vascular disease, and arteriosclerosis obliterans [1–4]. In addition to the duration of the disease and the status of glycemic control, the atherosclerosis in type 2 diabetes mellitus is also related to a lipid profile characterized by a high triglyceride level and a low HDL cholesterol level [5], insulin resistance [6], glycosylation of proteins [7], and genetic factors. Various candidate genes have been investigated for their associations with increased susceptibility to macrovascular complications, since

knowledge in this area could lead to more efficient treatments for diabetes mellitus.

The cholesteryl ester transfer protein (CETP), a hydrophobic glycoprotein composed of 476 amino acids, is thought to contribute to atherogenesis [15,16]. CETP mediates the transfer of cholesteryl ester from HDL–LDL and VLDL in exchange for triglyceride [8,9] and plays an important role in the reverse cholesterol transport system. High plasma levels of CETP are associated with reduced HDL cholesterol levels [10–13] and increased LDL cholesterol levels [14]. Several studies have also shown high CETP levels to be atherogenic [15,16], while other studies have suggested that CETP might have an antiatherogenic effect in certain setting [10]. Thus the debate as to whether CETP is atherogenic is still open, but the transfer of cholesterol esters from ‘atheroprotective’ HDL to ‘atherogenic’ apo-B containing LDL and VLDL is thought to play some role.

* Corresponding author. Tel.: +81-3-53633797; fax: +81-3-52693219.

E-mail address: shu-meg@yb3.so-net.ne.jp (S. Meguro).

Several mutations in the gene for CETP have been reported, and most of them affect plasma HDL cholesterol levels [12,13,17–19], resulting in an increased risk for atherosclerosis [20]. One of these mutations, the common TaqIB polymorphism in intron 1, has been reported to be associated with lipid transfer activity [12,13,17] and serum HDL cholesterol levels [21]. Ukkola et al. have reported finding that the B1/B1 genotype of this mutation was associated with cerebrovascular disease in Finnish patients with type 2 diabetes mellitus [22]. They also showed that individuals with the B2/B2 genotype had a lower prevalence of signs of macroangiopathy. Kuivenhoven et al. reported a significant association between the CETP TaqIB polymorphism and the progression of coronary atherosclerosis, suggesting that the presence of the B1 allele might be a risk factor for atherosclerosis [23]. Recently Durlach et al. also reported that French type 2 diabetic patients with the B2/B2 genotype had a lower prevalence of coronary heart disease, but this genotypic effect was only seen in male patients [24]. Metabolic abnormalities arising from the CETP TaqIB polymorphism probably have a systemic effect on the susceptibility of an individual to atherosclerosis. We therefore employed a cross-sectional study to elucidate the effects of this polymorphism on serum CETP and cholesterol levels, and overall atherosclerotic macrovascular complications in Japanese patients with type 2 diabetes mellitus.

2. Research design and methods

2.1. Subjects

The subjects were 182 Japanese patients with type 2 diabetes mellitus who attended the outpatient clinic at Keio University Hospital, Tokyo. Diabetes mellitus was diagnosed according to the criteria published by the World Health Organization in 1985 [25]. Subjects with overt nephropathy whose serum creatinine level was above 132 $\mu\text{mol/l}$ were excluded from the study, because overt nephropathy can be a significant factor leading to atherosclerosis. One hundred and four subjects were male, and the remaining 78 were female. Twenty-four patients exhibited macroangiopathy, defined as a clinical history of myocardial infarction, coronary stenosis of more than 75% in diameter (confirmed by a coronary angiography), cerebral infarction (confirmed by either brain magnetic resonance imaging or computed tomography), or a history of amputation. The remaining 158 patients exhibited no signs of macroangiopathy. Written informed consent was obtained from each participating subject.

2.2. Clinical analysis

The duration of diabetes mellitus was determined by reviewing the clinical history of the patient or, if possible, based on the plasma glucose data in their medical records. Hypertension was defined as a systolic blood pressure > 140 mmHg, a diastolic blood pressure > 90 mmHg, or the prescription of antihypertensive medication. Hyperlipidemia was defined as a total cholesterol (TC) serum value of over 5.69 mmol/l, a triglyceride (TG) count of over 1.72 mmol/l, or the prescription of lipid-lowering medication. The subjects were questioned as to their history of smoking and divided into smoking and non-smoking groups according to their current status. BMI was calculated by dividing the subject's weight in kilograms by the square of their height in meters. Family history for coronary artery disease was also determined.

2.3. Genotyping

The genotype of intron 1 of the CETP gene was determined by the direct polymerase chain reaction (PCR) amplification method using a direct amplification buffer kit (Shimadzu, Japan). In accordance with the manufacturer's instruction, 0.5 $\mu\text{mol/L}$ each of two primers (5' CAC TAG CCC AGA GAG AGG AGT GCC 3' and 5' CTG AGC CCA GCC GCA CAC ACT AAC 3'), 200 $\mu\text{mol/l}$ dNTP, 0.5 μl whole blood, 0.5 U thermostable DNA polymerase, and ready-made buffer were mixed in a total volume of 50 μl . After initial denaturation (15 min at 80°C followed by 5 min at 94°C), 40 amplification cycles at 94°C (5 min), 60°C (1 min) and 72°C (1 min) with a final extension step of 10 min were carried out. An 8 μl volume of the PCR product was used for digestion with 9 U of the restriction enzyme TaqI (Toyobo, Japan) in a total volume of 10 μl for 3 h at 65°C. After electrophoresis of the PCR product in 2% agarose containing ethidium bromide, DNA restriction fragments were visualized and analyzed on a transilluminator. The allele containing the restriction site for TaqI was designated 'B1', and the allele that did not contain the TaqI restriction site was designated 'B2'.

2.4. Biochemical analysis

Routine clinical biochemical analyses were performed in the hospital laboratory. Serum LDL cholesterol levels were measured by homogeneous assay. Plasma CETP levels were measured by ELISA and monoclonal antibodies (Chugai, Tokyo). Urinary albumin excretion (UAE) levels were obtained for a single sample obtained in the morning and measured by nephelometry. Serum advanced glycation end product (AGE) levels were measured by an ELISA and monoclonal antibod-

ies for carboxymethyl lysine [26] (SRL, Tokyo). Serum homocystein levels were analyzed by HPLC (Nihon Bunkoh, Tokyo), and aldose reductase levels were analyzed with a commercial ELISA kit and monoclonal antibodies (Mitsubishi Gas Chemical, Tokyo).

2.5. Statistical analysis

All data were analyzed with the statistical software StatView 5.0 (SAS Institute, Japan). Differences in continuous variables among the TaqIB subgroups were analyzed by one-way factorial ANOVA. Triglyceride and microalbumin were analyzed after transformation to their natural logarithm because of their skewed distributions. The PLSD Fisher test was used for posthoc analysis. The correlations between serum CETP levels and serum lipoproteins were calculated by simple linear regression analysis. The Hardy–Weinberg equilibrium or odds ratio (OR) and its 95% confidence interval (CI) for the presence of macroangiopathy within the CETP genotypes were analyzed by using the χ^2 -square test. Logistic regression analysis was performed to adjust the contribution of each risk factor for atherosclerosis.

3. Results

3.1. Frequency of CETP TaqIB polymorphism

The genotype frequencies were 39.6, 44.5, and 15.3% for B1/B1, B1/B2, and B2/B2, respectively. These frequencies did not differ significantly from those of 333 age-matched healthy Japanese controls

(31.2, 54.1, 14.7% for B1/B1, B1/B2, and B2/B2, respectively). The calculated frequencies of the B1 alleles and the B2 alleles were 0.62 and 0.38, respectively. The observed frequencies were in Hardy–Weinberg equilibrium ($P < 0.05$).

Comparison with other reported populations, although all Caucasian, showed results similar to those in our study in one population [23] but significantly lower frequency of the B1 allele in the other two populations [22,24].

3.2. Clinical characteristics and CETP genotype

When the patients were classified according to their TaqIB genotype, no statistically significant differences in clinical or metabolic variables were found among the TaqIB genotypes (Table 1). The proportion of the subjects taking statin medication was not significantly different among the genotypes (Table 1). Only two subjects were taking fibrates, and none were taking any other class of lipid-lowering medication. The groups were also similar with regard to known risk factors for atherosclerosis. The prevalence of diabetic retinopathy (data not shown), level of urinary albumin excretion (UAE), and prevalence of overt proteinuria were similar in all groups.

3.3. Association between CETP genotype and serum CETP levels

The B1 allele was significantly associated with a higher serum CETP level in this study group. The

Table 1
Clinical and metabolic characteristics according to CETP TaqIB genotype. Data shown are the means \pm 2 SD

Characteristics	B1/B1 (n = 72)	B1/B2 (n = 81)	B2/B2 (n = 29)
Age (y/o)	61.1 \pm 7.7	58.7 \pm 8.1	58.4 \pm 11.4
Sex (female/male)	33/39	38/43	7/22
BMI	21.8 \pm 4.7	23.1 \pm 4.9	22.7 \pm 2.5
Duration of diabetes mellitus (yearr)	10.6 \pm 7.8	9.3 \pm 7.2	12.2 \pm 8.7
Current smoker (%)	22	27	17
Hyperlipidemia (%)	29	33	21
Hypertension (%)	39	41	45
Family history of coronary artery disease (%)	18	27	40
Statin medication (%)	9.7	7.3	6.9
Fasting plasma glucose (mmol/l)	8.2 \pm 1.9	8.9 \pm 2.4	9.0 \pm 2.5
HbA1c (%)	7.1 \pm 1.2	7.4 \pm 1.3	7.6 \pm 1.6
Serum creatinine (μ mol/l)	66 \pm 19	63 \pm 14	67 \pm 14
Total cholesterol (mmol/l)	5.28 \pm 0.93	5.45 \pm 0.91	5.34 \pm 0.88
HDL cholesterol (mmol/l)	1.46 \pm 0.51	1.40 \pm 0.31	1.44 \pm 0.33
LDL cholesterol (mmol/l)	3.13 \pm 0.91	3.41 \pm 0.88	3.20 \pm 0.75
Triglyceride (mmol/l)	1.43 \pm 0.97	1.50 \pm 0.95	1.55 \pm 1.05
Overt proteinuria (%)	18	18	18
UAE (mg/g creatinin)	34.1 \pm 66.7	60.3 \pm 161	41.4 \pm 74.7
AGE (mU/ml)	3.5 \pm 1.9	3.6 \pm 2.3	3.4 \pm 1.2
Glycated albumin (%)	22.6 \pm 5.1	22.4 \pm 5.1	23.8 \pm 6.0
Homocysteine (nmol/ml)	9.5 \pm 2.8	10.1 \pm 4.2	10.2 \pm 2.9
Aldose reductase (ng/mg Hb)	11.5 \pm 3.3	10.9 \pm 2.2	12.5 \pm 2.5

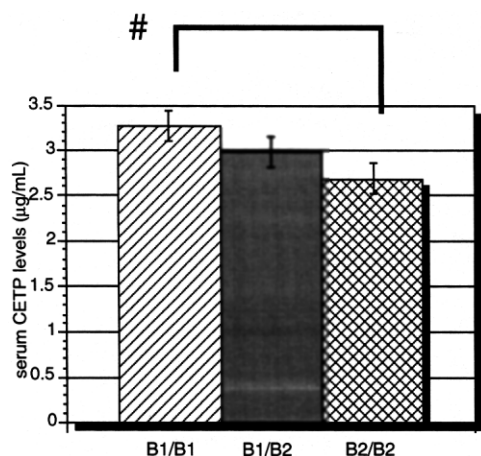


Fig. 1. Serum CETP levels according to CETP TaqIB genotype. #: $P < 0.05$ for the difference between B1/B1 and B2/B2.

difference in CETP levels between B1/B1 and B2/B2 was statistically significant ($P < 0.05$; see Fig. 1), and there seemed to be a gradual change in CETP levels according to the genotype.

3.4. Correlation between serum lipoprotein and serum CETP levels

Serum LDL cholesterol levels were positively correlated with serum CETP levels ($P < 0.01$). No significant inverse correlation between serum CETP and serum HDL cholesterol levels were demonstrated in this study (see Fig. 2).

3.5. Prevalence of macroangiopathy and CETP genotypes

The prevalences of macroangiopathy were 20.8,

Table 2

Association between CETP genotype and macroangiopathy. OR and 95% CI were tested by χ^2 -square analysis

Macroangiopathy	Genotype			Total
	B1/B1	B1/B2	B2/B2	
Positive	15 (20.8)	7 (8.6)	2 (6.9)	24
Negative	57 (79.2)	74 (91.4)	27 (93.1)	158
Total	72	81	29	182

8.6%, and 6.9% for B1/B1, B1/B2, and B2/B2, respectively (Table 2). Statistical analysis among the three genotypes was impossible because of the small number of macroangiopathy-positive subjects in the B2/B2 genotype. When we pooled the data for the B1/B2 and B2/B2 genotypes, the prevalence of macroangiopathy in the B1/B1 genotype was significantly higher than in the B1/B2 + B2/B2 genotype (OR = 2.953, 95%, CI = 1.250–6.977, $P = 0.0136$).

3.6. Logistic regression analysis

The B1/B1 genotype was significantly associated with the prevalence of macroangiopathy (OR = 5.38, $P = 0.009$), and the duration of diabetes mellitus was also associated with macroangiopathy (OR = 1.109, $P = 0.005$; see Table 3). When the HDL cholesterol levels were included as an independent variable, the B1/B1 genotype was still significantly associated with the prevalence of macroangiopathy (OR = 5.38, $P = 0.01$;

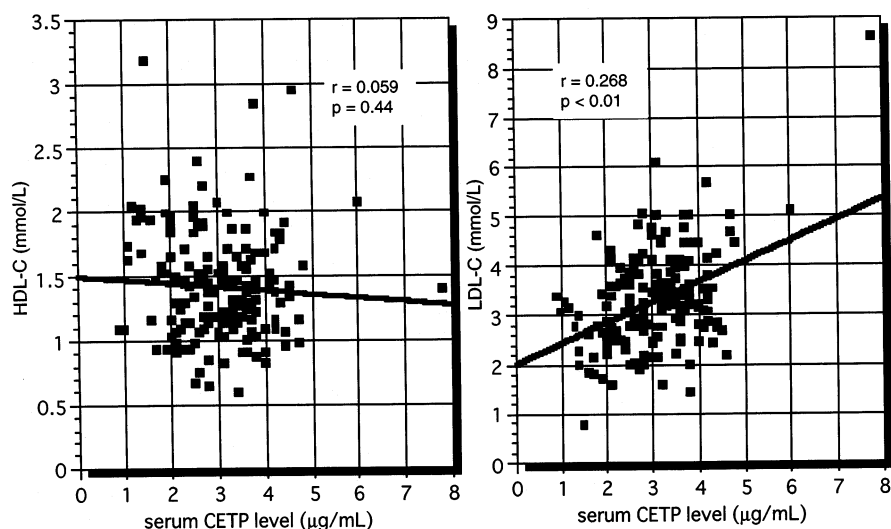


Fig. 2. Correlation between serum CETP and cholesterol levels. HDL-C: HDL cholesterol; LDL-C: LDL cholesterol.

data not shown). When we included the statin medication as an independent variable, the B1/B1 genotype was still significant (OR = 3.65, $P = 0.01$; data not shown).

4. Discussion

The results of this cross-sectional study suggest the possibility that the TaqIB polymorphism of the CETP gene is associated with macroangiopathy in Japanese Type 2 diabetic patients.

We observed a different genotype frequency in this study compared with some of the previous studies reported [22–24]. This difference could not be the explanation for the variance of the prevalence of atherosclerosis throughout the world, because the population with the higher prevalence of atherosclerosis, like the Finnish population, had a lower frequency of the risky B1/B1 genotype than the population with the lower prevalence of atherosclerosis, like the Japanese population. The fact that similar results for atherosclerosis of this polymorphism were observed in various populations instead suggested that the polymorphism might be related to the more essential pathology of atherosclerosis.

A significant association between serum CETP and LDL cholesterol levels was found, but an inverse correlation between serum CETP and HDL cholesterol levels was not. Most studies [12,13] have shown that higher CETP levels are related to lower serum HDL cholesterol levels. Another study has shown an association between serum CETP and LDL cholesterol levels in healthy Japanese subjects [14], but whether this relationship is specific to the Japanese population or attributable to environmental factors, including dietary differences, remains unclear. A previous study [27] on CETP gene deficiency in Japanese–Americans in Hawaii, however, suggests that environmental factors, and not race, may influence the relationship between serum CETP and lipoprotein levels. Theoretically, the

decreased affinity of LDL cholesterol for its receptors in the liver due to their degeneration and resultant prolongation of LDL cholesterol clearance may influence the serum lipoprotein profile. Diabetes mellitus is one of the states known to degenerate LDL cholesterol. Another possibility is that LDL cholesterol oxidation or glycation and its delayed clearance from serum influences the relation between CETP activity and serum lipoprotein profile. Although this is merely a speculation, a recent report by Talmud et al. suggested that a novel tetranucleotide repeat within the CETP promoter which had strong allelic association with the CETP TaqIB polymorphism was related to the LDL particle size [28].

In this study, neither LDL cholesterol nor HDL cholesterol levels differed significantly between the genotype groups. Thus, the higher prevalence of macroangiopathy in the B1/B1 genotype cannot be attributed to the effect of CETP on serum lipoprotein levels. The results of logistic regression analysis in this study showed that the CETP B1/B1 genotype was a significant risk factor for macroangiopathy in Japanese Type 2 diabetes mellitus, even if HDL cholesterol levels were included as an independent variable. The difference in serum CETP levels among the CETP genotypes seemed to imply that the CETP levels account for the genotypic effect on macroangiopathy in patients with diabetes mellitus. However, the underlying mechanism of how CETP affects atherogenesis is unclear. Recently, the accumulation of atherogenic substances, such as oxidized LDL cholesterol, in the subendothelium [29,30] and an inflammation-like reaction to them [31,32] is thought to be the main cause of accelerated plaque formation and vulnerability. CETP may affect cholesterol metabolism by reducing the amount of cholesterol ester extraction from atherosclerotic lesions as a result of reduced HDL function or increasing the influx of cholesterol ester into the lesion as a result of an increase in atherogenic Apo-B-containing lipoprotein. However, this polymorphism may be a non-functional marker in linkage disequilibrium with functional variants of the CETP gene or other closely linked genes, such as lecithin cholesterol acyltransferase.

A recent study performed by Durlach et al. suggested a sex-dependent effect of CETP polymorphism for coronary heart disease. The low prevalence of macroangiopathy in our study, mainly because of the low prevalence of atherosclerosis in the Japanese population, made gender separated analysis difficult, and thus this issue was not reconfirmed by this study.

Although further investigations are needed to elucidate the mechanism of CETP's effect on atherosclerosis, our study suggests that the TaqIB polymorphism of the CETP gene may be a genetic risk factor for clinical macroangiopathy in Japanese Type 2 diabetes mellitus.

Table 3
Logistic regression analysis on multiple risk variables for macroangiopathy

	OR	<i>P</i> value
Age	1.07	0.131
Male sex	0.67	0.552
BMI	1.00	0.993
Hypertension	1.73	0.388
Hyperlipidemia	1.22	0.751
Smoking	2.69	0.201
Duration of diabetes mellitus	1.11	0.005
HbA1c	1.12	0.609
CETPTaq1 B1/B1 genotype	5.38	0.009

References

- [1] Pyorala K, Laakso M, Uusitupa M. Diabetes and atherosclerosis: an epidemiologic view. *Diabetes Metab Rev* 1987;3:463–524.
- [2] Laakso M, Lehto S. Epidemiology of macrovascular disease in diabetes. *Diabetes Reviews* 1997;5:294–315.
- [3] Haffner SM, Lehto S, Ronnema T, Pyorala K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction [see comments]. *New Engl J Med* 1998;339:229–34.
- [4] Laakso M. Hyperglycemia and cardiovascular disease in type 2 diabetes. *Diabetes* 1999;48:937–42.
- [5] Lehto S, Ronnema T, Haffner SM, Pyorala K, Kallio V, Laakso M. Dyslipidemia and hyperglycemia predict coronary heart disease events in middle-aged patients with NIDDM. *Diabetes* 1997;46:1354–9.
- [6] Reaven GM. Pathophysiology of insulin resistance in human disease. *Physiol Rev* 1995;75:473–86.
- [7] Vlassara H. Recent progress in advanced glycation end products and diabetic complications. *Diabetes* 1997;46(Suppl 2):S19–25.
- [8] Hesler CB, Tall AR, Swenson TL, Weech PK, Marcel YL, Milne RW. Monoclonal antibodies to the Mr 74 000 cholesteryl ester transfer protein neutralize all of the cholesteryl ester and triglyceride transfer activities in human plasma. *J Biol Chem* 1988;263:5020–3.
- [9] Yen FT, Deckelbaum RJ, Mann CJ, Marcel YL, Milne RW, Tall AR. Inhibition of cholesteryl ester transfer protein activity by monoclonal antibody: effects on cholesteryl ester formation and neutral lipid mass transfer in human plasma. *J Clin Invest* 1989;83:2018–24.
- [10] Tall AR. Plasma cholesteryl ester transfer protein. *J Lipid Res* 1993;34:1255–74.
- [11] Tall AR. Plasma high density lipoproteins: metabolism and relationship to atherogenesis. *J Clin Invest* 1990;86:379–84.
- [12] Hannuksela ML, Liinamaa MJ, Kesaniemi YA, Savolainen MJ. Relation of polymorphisms in the cholesteryl ester transfer protein gene to transfer protein activity and plasma lipoprotein levels in alcohol drinkers. *Atherosclerosis* 1994;110:35–44.
- [13] Kuivenhoven JA, de KP, Boer JM, Smalheer HA, Botma GJ, Seidell JC, Kastelein JJ, Pritchard PH. Heterogeneity at the CETP gene locus. Influence on plasma CETP concentrations and HDL cholesterol levels. *Arterioscler Thromb Vasc Biol* 1997;17:560–8.
- [14] Kinoshita M, Teramoto T, Shimazu N, Kaneko K, Ohta M, Koike T, Hosogaya S, Ozaki Y, Kume S, Yamanaka M. CETP is a determinant of serum LDL-cholesterol but not HDL-cholesterol in healthy Japanese. *Atherosclerosis* 1996;120:75–82.
- [15] Bhatnagar D, Durrington PN, Channon KM, Prais H, Mackness MI. Increased transfer of cholesteryl esters from high density lipoproteins to low density and very low density lipoproteins in patients with angiographic evidence of coronary artery disease. *Atherosclerosis* 1993;98:25–32.
- [16] Foger B, Luef G, Ritsch A, Schmidauer C, Doblinger A, Lechleitner M, Aichner F, Patsch JR. Relationship of high-density lipoprotein subfractions and cholesteryl ester transfer protein in plasma to carotid artery wall thickness. *J Mol Med* 1995;73:369–72.
- [17] Freeman DJ, Griffin BA, Holmes AP, Lindsay GM, Gaffney D, Packard CJ, Shepherd J. Regulation of plasma HDL cholesterol and subfraction distribution by genetic and environmental factors. Associations between the TaqI B RFLP in the CETP gene and smoking and obesity. *Arterioscler Thromb* 1994;14:336–44.
- [18] Tamminen M, Kakko S, Kesaniemi YA, Savolainen MJ. A polymorphic site in the 3' untranslated region of the cholesteryl ester transfer protein (CETP) gene is associated with low CETP activity. *Atherosclerosis* 1996;124:237–47.
- [19] Bu X, Warden CH, Xia YR, De MC, Puppione DL, Teruya S, Lokensgard B, Daneshmand S, Brown J, Gray RJ, et al. Linkage analysis of the genetic determinants of high density lipoprotein concentrations and composition: evidence for involvement of the apolipoprotein A-II and cholesteryl ester transfer protein loci. *Hum Genet* 1994;93:639–48.
- [20] Hill SA, McQueen MJ. Reverse cholesterol transport — a review of the process and its clinical implications. *Clin Biochem* 1997;30:517–25.
- [21] Bernard S, Moulin P, Lagrost L, Picard S, Elchebly M, Ponsin G, Chapuis F, Berthezene F. Association between plasma HDL-cholesterol concentration and Taq1B CETP gene polymorphism in non-insulin-dependent diabetes mellitus. *J Lipid Res* 1998;39:59–65.
- [22] Ukkola O, Savolainen MJ, Salmela PI, von DK, Kesaniemi YA. DNA polymorphisms at the locus for human cholesteryl ester transfer protein (CETP) are associated with macro- and microangiopathy in non-insulin-dependent diabetes mellitus. *Clin Genet* 1994;46:217–27.
- [23] Kuivenhoven JA, Jukema JW, Zwinderman AH, de KP, McPherson R, Bruschke AV, Lie KI, Kastelein JJ. 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 [see comments]. *New Engl J Med* 1998;338:86–393.
- [24] Durlach A, Clavel C, Girard-Globa A, Durlach V. Sex-dependent association of a genetic polymorphism of cholesteryl ester transfer protein with high-density lipoprotein cholesterol and macrovascular pathology in type II diabetic patients. *J Endocrinol Metab* 1999;84:3656–9.
- [25] World Health Organization; Diabetes Mellitus: Report of a WHO Study Group. Geneva, World Health Org, 1985 (Tech. Rep. Ser. no 727).
- [26] Ono Y, Aoki S, Ohnishi K, Yasuda T, Kawano K, Tsukada Y. Increased serum levels of advanced glycation end-products and diabetic complications. *Diabetes Res Clin Pract* 1998;41:131–7.
- [27] Zhong S, Sharp DS, Grove JS, Bruce C, Yano K, Curb JD, Tall AR. Increased coronary heart disease in Japanese-American men with mutation in the cholesteryl ester transfer protein gene despite increased HDL levels [see comments]. *J Clin Invest* 1996;97:2917–23.
- [28] Talmud PJ, Edwards KL, Turner CM, Newman B, Palmen JM, Humphries SE, Austin MA. Linkage of the cholesteryl ester transfer protein (CETP) gene to LDL particle size: use of a novel tetranucleotide repeat within the CETP promoter [in process citation]. *Circulation* 2000;101:2461–6.
- [29] Burchfiel CM, Curb JD, Rodriguez BL, Yano K, Hwang LJ, Fong KO, Marcus EB. Incidence and predictors of diabetes in Japanese-American men: the Honolulu Heart Program. *Ann Epidemiol* 1995;5:33–43.
- [30] Davies MJ. Stability and instability: two faces of coronary atherosclerosis: the Paul Dudley White Lecture 1995. *Circulation* 1996;94:2013–20.
- [31] Falk E, Shah PK, Fuster V. Coronary plaque disruption. *Circulation* 1995;92:657–71.
- [32] Watanabe T, Haraoka S, Shimokama T. Inflammatory and immunological nature of atherosclerosis. *Int J Cardiol* 1996;54:S51–60 suppl.