Molecular Mechanisms of Cholesteryl Ester Transfer Protein Deficiency in Japanese

Makoto Nagano^{1,2}, Shizuya Yamashita², Ken-ichi Hirano², Mayumi Takano¹, Takao Maruyama², Mitsuaki Ishihara¹, Yukiko Sagehashi¹, Takeshi Kujiraoka¹, Kazuya Tanaka¹, Hiroaki Hattori¹, Naohiko Sakai², Norimichi Nakajima³, Tohru Egashira¹, and Yuji Matsuzawa⁴

¹ Department of Advanced Technology and Development, BML, Inc., Saitama, Japan.

² Department of Internal Medicine and Molecular Science, Graduate School of Medicine, Osaka University, Osaka, Japan.

³ Nakajima Clinic, Akita, Japan.

⁴ Sumitomo Hospital, Osaka, Japan.

Plasma cholesteryl ester transfer protein (CETP) facilitates the transfer of cholesteryl ester (CE) from high density lipoprotein (HDL) to apolipoprotein B-containing lipoproteins. Since CETP regulates the plasma levels of HDL cholesterol and the size of HDL particles, CETP is considered to be a key protein in reverse cholesterol transport (RCT), a protective system against atherosclerosis. The importance of plasma CETP in lipoprotein metabolism was demonstrated by the discovery of CETP-deficient subjects with marked hyperalphalipoproteinemia (HALP). Genetic CETP deficiency is the most important and common cause of HALP in the Japanese. Ten mutations of the CETP gene have been demonstrated as causes of HALP, including two common mutations: an intron 14 splicing defect (Int14 + 1 G → A) and an exon 15 missense mutation (D442G). The subjects with CETP deficiency show a variety of abnormalities in the concentration, composition, and function of both HDL and low density lipoprotein (LDL). CETP deficiency is considered a physiological state of impaired RCT, which may possibly lead to the development of atherosclerosis despite high HDL cholesterol levels. However, the pathophysiological significance of CETP in terms of atherosclerosis has been controversial. Epidemiological studies in Japanese-Americans living in Hawaii and Japanese in the Omagari area, where HALP subjects with an intron 14 splicing defect of the CETP gene are markedly frequent, have shown a relatively increased incidence of coronary atherosclerosis in CETP deficiency. On the other hand, the *TaqIB* polymorphism-B2 allele with low CETP mass and increased HDL cholesterol has been related to a decreased risk for coronary heart disease (CHD) in many studies, including the Framingham Offspring Study. The current review focused on the characterization of the Japanese subjects with CETP deficiency, including our recent findings. *J Atheroscler Thromb*, 2004; 11: 110–121.

Key words: Cholesteryl ester transfer protein deficiency, Hyperalphalipoproteinemia, HDL cholesterol, Atherosclerosis

Address for correspondence: Shizuya Yamashita, Department of Internal Medicine and Molecular Science, Graduate School of Medicine, Osaka University, 2-2 Yamada-oka, Suita, Osaka 565-0871, Japan.

E-mail: shizu@imed2.med.osaka-u.ac.jp

Received February 9, 2004.

Accepted for publication March 9, 2004.

Introduction

Many epidemiological studies have demonstrated a negative correlation of serum high density lipoprotein (HDL) cholesterol levels with the incidence of coronary heart disease (CHD), and subjects with genetic HDL deficiency often have accompanying atherosclerotic car-

diovascular diseases (1, 2). Therefore, HDL plays a crucial role in the protection of blood vessels from atherosclerosis. HDL functions to deliver excess cholesterol from peripheral tissues to the liver for excretion into the bile, a pathway designated as 'reverse cholesterol transport' (3, 4). In this system, small HDL or free apolipoprotein (apo) A-I removes free cholesterol (FC) from the peripheral cells, and FC in HDL is esterified by lecithin: cholesterol acyltransferase (LCAT) to cholesteryl ester (CE), which is subsequently transferred to very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and low density lipoprotein (LDL) by plasma cholesteryl ester transfer protein (CETP) (5–7). IDL and LDL are catabolized via the LDL receptor in the liver. Furthermore, the CE moiety of HDL is taken up selectively by the liver via scavenger receptor class B type I (SR-BI) (8). Thus, CETP is involved in the regulation of plasma HDL cholesterol levels, and facilitates the remodeling of HDL particles.

The significance of plasma CETP in lipoprotein metabolism was highlighted by the discovery of CETP deficiency with marked hyperalphalipoproteinemia (HALP). Genetic CETP deficiency is the most important and common cause of HALP in the Japanese. In the current review, we will focus on the findings obtained on Japanese CETP deficiency.

CETP gene mutations and their frequency in the Japanese

Human CETP cDNA, cloned by Drayna *et al.* (9), encodes a very hydrophobic glycoprotein with an *Mr* of 74,000, containing 476 amino acid residues (4, 10). The human CETP gene is mapped in chromosome 16 (16q13) near the LCAT gene and spans over 25k base pairs, including 16 exons (11). Since genetic deficiency of CETP activity was first described by Kurasawa *et al.* and

Koizumi *et al.* in 1985 (12, 13), to date, genetic CETP deficiency has been the most important and frequent cause of HALP in the Japanese (14–19). Ten mutations in the CETP gene have so far been identified Japanese CETP deficiency, including 3 splicing site, 3 nonsense, 1 promoter, and 3 missense mutations (14, 17, 18, 20–25) (Table 1). Of the 10 mutations, it has been demonstrated that a G-to-A substitution at the 5' splice donor site of intron 14 (Int14 + 1 G → A) and a missense mutation of exon 15 (D442G) in the CETP gene are common mutations associated with HALP in the Japanese. Furthermore, the prevalences of the Int14 + 1 G → A and D442G mutations are extremely high in the general Japanese population, with heterozygote frequencies of 1% and 7%, respectively (15, 18, 26, 27). Other mutations are less frequent than the two common mutations in the CETP gene, but 4 mutations including –69 G → A, L151P, G181X and Int14 + 3 T ins have been found in unrelated Japanese subjects with HALP. Some studies including ours have demonstrated that CETP deficiency contributed to about half of HALP subjects in the Japanese (18, 20–22). By using the Invader[®] assay (28) for 9 CETP gene mutations, we studied their frequency among 466 unrelated Japanese subjects (163 men and 303 women) with HALP (HDL cholesterol ≥ 2.07 mmol/l = 80 mg/dl) excluding primary biliary cirrhosis, nephrotic syndrome, thyroid dysfunction and massive alcohol drinkers (≥ 80 g/day), and demonstrated that the frequency of CETP deficiency was 61.7% and 31.4% for marked HALP (HDL cholesterol ≥ 2.59 mmol/l = 100 mg/dl) and moderate HALP in the Japanese, respectively (Fig. 1) (20, 21).

CETP gene mutations and its functional impairment

Seven mutations including splicing and nonsense mutations are known to be null type mutations of the CETP

Table 1. Mutations of the CETP gene identified in Japanese subjects with hyperalphalipoproteinemia.

Exon/Intron	Mutation	Position	Nucleotide change	Codon	Amino acid change	Reference
Promoter	– 69 G → A	– 69	gccc(g/a)gaag	NA	NA	20
Exon 5	L151P	8030	CTG C(T/C)C CTG	151	Leu → Pro	21
Exon 6	G181X	9150	AAG (G/T)GA CAG	181	Gly → Stop	22
	Q182X	9153	GGA (C/T)AG GTG	182	Gln → Stop	23
Exon 9	R282C	11528	GGC (C/T)GC CTC	282	Arg → Cys	21
Exon 10	Q309X	13199	TTC (C/T)AA GAG	309	Gln → Stop	24
Intron 10	Int10 + 2 T → G	13206	GAGg(t/g)aact	NA	NA	25
Intron 14	Int14 + 1 G → A	20291	CTC(g/a)taagt	NA	NA	14
	Int14 + 3 T ins	20293	CTCgt(-/t)aagt	NA	NA	18
Exon 15	D442G	21433	TTC G(A/G)C ATC	442	Asp → Gly	17

The position is relative to the GenBank sequence AC010550 with the start of transcription denoted as position +1. NA indicates not applicable.

gene in the Japanese. The homozygous subjects with an Int14 + 1 G → A, G181X, Q182X or G309X mutation had no detectable levels of CETP mass and activity in the plasma (14–16, 22–24). Furthermore, Int10 + 2 T → G and Int14 + 3 T ins mutations are also thought to be null type mutations, because compound heterozygotes with Int14 + 1 G → A had no CETP mass and activity in the plasma (18, 25). The Int14 + 1 G → A mutation causes exon 14 skipping of mRNA in monocyte-derived macrophages (29). The exon-skipping deletion causes a frameshift and introduces a premature termination codon downstream at codon 403. It decreases the stability of mRNA and produces a truncated protein that is rapidly degraded intracellularly in the transfected COS-1 cells. These observations clearly explain the molecular basis of the subjects with the common Int14 + 1 G → A splicing mutation but also explain the subjects with the other nonsense mutations that lead to a larger truncation of the carboxyl terminus of CETP. In the Int10 + 2 T → G mutation, which was identified in subjects with the Int14 + 1 G → A mutation, the sequence analysis of CETP mRNA in the proband's monocyte-derived macrophages demonstrated abnormal splicing with the deletion of exon 10 as well as alternative splicing at a native AG site located 31 nucleotides 5' of the normal splice acceptor in intron 13 (25). Thus, the Int10 + 2 T → G mutation causes exon 10 skipping and the insertion of a 31-bp fragment between exon 13 and exon 14, which contains an in-

frame stop codon. On the other hand, the L151P mutation is the only missense mutation of the CETP gene, which has a null allelic effect (21). The CETP mass levels in two subjects with the L151P mutation were similar to those in subjects with the Int14 + 1 G → A mutation. Moreover, no CETP protein was detected in the media of the COS-7 cells transfected with the L151P mutant, although a comparable expression of CETP protein was detectable in the cells. The leucine at codon 151 is likely to be structurally important because this residue is homologously conserved among the lipid transfer/lipopopolysaccharide binding protein family members, and located within one of the β -sheets (β B7) in the NH₂-terminal domain (30–32).

A promoter mutation and 2 other missense mutations identified in the Japanese HALP subjects are thought to cause partial CETP deficiency. The – 69 G → A mutation is a G-to-A substitution at the – 69 nucleotide of the promoter region, corresponding to the second nucleotide of the PEA3/ETS binding site (CGGAA) located upstream of the putative TATA box (20, 33). A reporter gene assay has shown that this mutation markedly reduced the transcriptional activities in HepG2 cells (8% of wild type). The importance of the PEA3/ETS binding site in transcriptional regulation has also been supported by Gaudet and Ginsburg (34). Plasma CETP levels in 6 subjects with the – 69 G → A mutation suggested that this mutation might have a null allelic effect rather than partial defi-

Fig. 1. Frequencies of CETP gene mutations in the 270 Japanese subjects with moderate hyperalphalipoproteinemia (HALP) ($2.07 \text{ mmol/l} \leq \text{HDL cholesterol} < 2.59 \text{ mmol/l}$) (A) and the 196 subjects with marked HALP ($\text{HDL cholesterol} \geq 2.59 \text{ mmol/l}$) (B). HE: heterozygote, HO: homozygote, Int14 + 1 G → A HE/D442G HE, compound heterozygote for Int14 + 1 G → A and D442G. The others have at least one of the low prevalence mutations: – 69 G → A, L151P, G181X, R282C and Int14 + 3 T ins.

ciency *in vivo*. The D442G mutation was first identified in two probands, although heterozygous for this mutation, which had a 3-fold increase in HDL cholesterol and markedly decreased plasma CETP activity and mass (17). These data suggested that the D442G mutation might have some dominant effects on CETP and HDL *in vivo*. This was confirmed *in vitro* by the expression of wild type and mutant proteins in COS-7 cells. Cellular expression of mutant cDNA results in secretion at only 30% of wild type CETP activity. Moreover, co-expression of wild type and mutant cDNA leads to inhibition of the secretion of wild type CETP and activity. However, in the latter studies, the D442G heterozygous subjects had relatively high CETP activity and mass and a wide range of HDL cholesterol levels (18). Moreover, the specific activity of plasma CETP in the homozygous subjects with the D442G mutation was similar to that of the wild type (21). Therefore, as the authors explained in the discussion, other unknown factors contributed to the increased HDL in the two probands. The R282C mutation was found in only one compound heterozygote with the D442G mutation, whose plasma CETP levels were significantly lower when compared with those in D442G heterozygous sub-

jects (21). The mutant CETP showed a marked reduction in the secretion of CETP protein into media from transfected COS-7 cells (39% of wild type). Arginine at codon 282, a positively charged residue, is thought to be in the vicinity of the C-terminal pocket and interacts with the phosphate group of a bound phospholipid (31). Arginine at codon 282 is likely to be structurally rather than functionally important because the specific activity of the R282C mutant was similar to that of the wild type in the transfection experiment.

Association of plasma CETP levels and lipid profiles with CETP mutations

Table 2 shows the lipid profiles and plasma CETP levels of the HALP subjects with the common CETP gene mutations (HDL cholesterol ≥ 2.07 mmol/l = 80 mg/dl) (20, 21). The plasma CETP concentrations determined by sandwich ELISA in 211 normolipidemic male subjects and female subjects without the known CETP gene mutations were 1.93 ± 0.50 μ g/ml (mean $\pm$ SD) and 2.36 ± 0.66 μ g/ml, respectively. The plasma CETP mass and activity were not detected in the homozygous subjects with the Int14 + 1 G $\rightarrow$ A mutation. The subjects with the

Table 2. Lipid profiles and plasma CETP levels in HALP subjects with the common mutations of the CETP gene.

Mutation	Int14 + 1 G $\rightarrow$ A HE (n = 58)	Int14 + 1 G $\rightarrow$ A HO (n = 29)	D442G HE (n = 84)	D442G HO (n = 12)	Int14 + 1 G $\rightarrow$ A HE / D442G HE (n = 24)	Controls (n = 90)
Sex	M/F 22/36	M/F 17/12	M/F 25/59	M/F 3/9	M/F 10/14	M/F 53/37
Age, years	41 $\pm$ 15*	37 $\pm$ 20	57 $\pm$ 11**	55 $\pm$ 16**	52 $\pm$ 10**	32 $\pm$ 6
Total cholesterol, mmol/l	5.92 $\pm$ 1.01**	7.01 $\pm$ 1.42**	6.15 $\pm$ 1.16**	5.74 $\pm$ 0.57**	5.92 $\pm$ 1.03**	4.42 $\pm$ 0.59
HDL cholesterol, mmol/l	2.72 $\pm$ 0.52**	4.03 $\pm$ 0.83**	2.51 $\pm$ 0.36**	2.74 $\pm$ 0.49**	3.21 $\pm$ 0.80**	1.55 $\pm$ 0.34
Triglyceride, mmol/l	1.01 $\pm$ 0.60	1.29 $\pm$ 0.66*	0.89 $\pm$ 0.51	0.82 $\pm$ 0.37	0.94 $\pm$ 0.45	1.03 $\pm$ 0.67
LDL cholesterol, mmol/l	2.74 $\pm$ 1.06	2.35 $\pm$ 0.78	3.23 $\pm$ 1.14**	2.66 $\pm$ 0.70	2.56 $\pm$ 0.98	2.48 $\pm$ 0.62
Apo A-I, mg/dl	195 $\pm$ 32**	224 $\pm$ 56**	190 $\pm$ 37**	205 $\pm$ 26**	206 $\pm$ 52**	123 $\pm$ 16
Apo A-II, mg/dl	36.6 $\pm$ 8.2	40.9 $\pm$ 10.3**	35.4 $\pm$ 7.3	38.0 $\pm$ 9.8*	40.7 $\pm$ 10.4**	33.6 $\pm$ 5.1
Apo B, mg/dl	85 $\pm$ 22	77 $\pm$ 29	93 $\pm$ 27**	77 $\pm$ 18	81 $\pm$ 25	79 $\pm$ 17
Apo C-II, mg/dl	5.0 $\pm$ 1.4**	7.5 $\pm$ 2.3**	4.6 $\pm$ 1.3**	4.5 $\pm$ 1.3*	5.4 $\pm$ 1.6**	3.7 $\pm$ 1.2
Apo C-III, mg/dl	12.2 $\pm$ 3.4**	22.2 $\pm$ 10.0**	12.0 $\pm$ 3.2**	11.5 $\pm$ 2.1*	15.0 $\pm$ 5.8**	8.9 $\pm$ 4.0
Apo E, mg/dl	5.3 $\pm$ 1.6*	9.9 $\pm$ 4.3**	5.9 $\pm$ 2.2**	6.6 $\pm$ 2.2**	7.5 $\pm$ 2.9**	4.6 $\pm$ 0.9
CETP mass, μ g/ml	1.4 $\pm$ 0.5**	0.0 $\pm$ 0.0**	2.0 $\pm$ 0.6**	1.2 $\pm$ 0.3**	0.6 $\pm$ 0.1**	2.4 $\pm$ 0.6
CETP activity, %	61 $\pm$ 20**	2 $\pm$ 3**	86 $\pm$ 19**	61 $\pm$ 8**	29 $\pm$ 3**	100 $\pm$ 19

HE indicates heterozygote, HO: homozygote, Int14 + 1 G $\rightarrow$ A HE/D442G HE, compound heterozygote for Int14 + 1 G $\rightarrow$ A and D442G. Values are expressed as mean $\pm$ SD. * $p < 0.01$, ** $p < 0.001$ compared with control subjects.

heterozygous Int14 + 1 G → A mutation had about half the CETP mass of the normal controls. On the other hand, the CETP concentration in the subjects with the homozygous D442G mutation was 0.72 ± 0.16 µg/ml and 1.23 ± 0.27 µg/ml, for the males and females, respectively, but in the heterozygous subjects was 1.56 ± 0.42 µg/ml and 2.09 ± 0.56 µg/ml, for the males and females, respectively, showing only a mild change (a 17% decrease). The specific activity of plasma CETP in the subjects with the D442G mutation was similar to that in the normolipidemic controls without CETP gene mutations. In each genotype with the common CETP mutations except for the homozygous Int14 + 1 G → A mutation, the mean plasma CETP levels in the male subjects were significantly lower than those of the female subjects.

Among the completely CETP-deficient subjects, the homozygous subjects with the Int14 + 1 G → A mutation had markedly elevated levels of HDL cholesterol (3–6-fold increase), while the heterozygous subjects had moderately increased HDL cholesterol levels (12, 13, 15, 16, 18, 20, 21, 35). The TG levels of the subjects were slightly increased compared with those of the controls. The concentrations of apo A-I, C-II, C-III and E in CETP deficiency were also markedly increased, while the apo B level was normal or slightly decreased. These increases in apo C-III and E are derived from the increment of HDL particles containing apo C-III and E. In subjects with other genotypes of common CETP gene mutations, the plasma HDL cholesterol levels and the concentrations of apo A-I, A-II, C-II, C-III and E were significantly increased, although the difference was not so marked compared with that for the homozygous Int14 + 1 G → A mutation. Among the subjects with two common CETP gene mutations, CETP activity and mass concentration were negatively correlated with plasma HDL cholesterol, apo A-I, A-II, C-II, C-III and E, and positively with plasma LDL cholesterol.

Effect of CETP gene polymorphisms on plasma CETP mass and HDL cholesterol in the heterozygous subjects with common CETP gene mutations

Several common polymorphisms have been reported in the human CETP gene including – 629 A/C in promoter region (36), *TaqIB* polymorphism in intron 1 (37), *MspI* in intron 8 (37) and I405V in exon 14 (11). Among these polymorphisms, *TaqIB* polymorphism has been intensely studied, although the polymorphism is not expected to directly influence CETP transcriptional regulation or RNA splicing but rather to affect CETP gene expression, due to its location in intron 1 (+ 279 G/A). In normolipidemic subjects, the B2 allele (absence of the *TaqIB* restriction site) at this polymorphism site has been associated with decreased CETP levels and increased HDL cholesterol levels (38–42), thus resembling a mild phenotype of CETP

deficiency. Moreover, it has been shown that the *TaqIB*-B2 allele had a dose-dependent association with CHD risk in many studies including the Framingham Offspring Study (43), although this association may be population-specific and highly influenced by environmental factors, such as alcohol consumption and smoking.

Our results suggested that the *TaqIB*-B2 allele is associated with both lower plasma CETP concentrations and higher HDL cholesterol levels in subjects not only without CETP gene mutations, but also with heterozygous CETP deficiency; this association was also observed in the heterozygous subjects with the Int14 + 1 G → A mutation, but not significantly (Table 3). The linkage disequilibrium that we have observed among 4 different polymorphisms and 2 common mutations in the Japanese, the CETP gene splitting into a 5' haplotype block and a 3' haplotype block, is similar to that observed previously in Caucasians (44, 45). Table 4 summarizes the haplotype based on 4 polymorphisms in homozygous subjects with Int14 + 1 G → A or D442G mutations. The 4 polymorphisms generated 8 main haplotypes in normolipidemic subjects without CETP gene mutations. On the other hand, in the homozygous subjects with the Int14 + 1 G → A mutation, all had 1 haplotype, and the homozygous subjects for D442G had 2 main haplotypes. These results seem to be supported by studies investigating the association between common CETP gene mutations and polymorphism(s) (15, 47, 48). Thus, we determined the haplotypes of the normal allele in the heterozygous subjects with common CETP gene mutations when haplotype analysis was performed. Moreover, there are some haplotypes in the D442G mutation of the CETP gene, and this mutation is frequent in West Asian populations. It is suggested that the D442G mutation originated earlier than the Int14 + 1 G → A mutation.

Recently, Lu *et al.* investigated in detail the association between promoter polymorphisms and CETP/HDL cholesterol in a sample of 357 elderly Japanese men (49). Their haplotype analyses indicated that the – 2,505 C/A polymorphism (50) and 1,946 VNTR [gaaa]_n (51) might explain the variations in the CETP concentrations, and may independently determine the variations in HDL cholesterol levels, whereas the – 629 A/C and *TaqIB* polymorphisms were not instrumental in determining CETP concentrations or HDL cholesterol levels. The importance of 1,946 VNTR for plasma HDL cholesterol was also described by Thompson *et al.* (45). 1,946 VNTR seems to be significantly associated with CETP/HDL cholesterol, even in heterozygous subjects with common CETP gene mutations.

Lipoprotein abnormalities in homozygous CETP deficiency

In 1984, we described two patients with marked HALP who suffered from angina pectoris and premature cor-

Table 3. Relationship of the CETP *TaqI*B polymorphism to plasma CETP mass and HDL cholesterol levels in Japanese subjects who had the common CETP mutations.

(A) Normolipidemic subjects without CETP gene mutations						
	Male			Female		
	<i>n</i>	CETP mass (μg/ml)	HDL-C (mmol/l)	<i>n</i>	CETP mass (μg/ml)	HDL-C (mmol/l)
B1B1	42	2.05 ± 0.50	1.06 ± 0.28	25	2.61 ± 0.61	1.26 ± 0.36
B1B2	77	1.95 ± 0.49	1.19 ± 0.30	26	2.30 ± 0.58	1.29 ± 0.32
B2B2	28	1.72 ± 0.45	1.23 ± 0.35	13	1.95 ± 0.72	1.52 ± 0.45
<i>p</i>		0.023	0.035		0.013	0.133

(B) Heterozygous subjects with Int14 +1 G → A mutation						
	Male			Female		
	<i>n</i>	CETP mass (μg/ml)	HDL-C (mmol/l)	<i>n</i>	CETP mass (μg/ml)	HDL-C (mmol/l)
B1B1	13	1.22 ± 0.46	2.20 ± 0.70	17	1.66 ± 0.41	2.64 ± 0.65
B1B2	13	1.03 ± 0.30	2.48 ± 0.83	22	1.24 ± 0.27	2.61 ± 0.41
B2B2	0	–	–	0	–	–
<i>p</i>		0.159	0.360		0.003	0.893

(C) Heterozygous subjects with D442G mutation						
	Male			Female		
	<i>n</i>	CETP mass (μg/ml)	HDL-C (mmol/l)	<i>n</i>	CETP mass (μg/ml)	HDL-C (mmol/l)
B1B1	23	1.85 ± 0.47	1.53 ± 0.52	36	2.08 ± 0.45	1.91 ± 0.49
B1B2	35	1.59 ± 0.44	1.71 ± 0.65	48	2.06 ± 0.65	2.02 ± 0.54
B2B2	13	1.36 ± 0.37	2.09 ± 0.41	20	1.66 ± 0.50	2.40 ± 0.54
<i>p</i>		0.037	0.024		0.044	0.004

Table 4. Reconstructed haplotype frequencies for – 629 A/C, *TaqI*B, Int7 + 8 C/T and I405V polymorphisms of the CETP gene.

	Haplotype				Frequency		
	– 629 A/C	<i>TaqI</i> B	Int7 + 8 C/T	I405V (A/G)	Int14 + 1 G → A HO (<i>n</i> = 38)	D442G HO (<i>n</i> = 17)	Controls (<i>n</i> = 227)
1	C	B1	C	A	0.000	0.349	0.315
2	A	B2	T	G	0.000	0.000	0.290
3	C	B1	C	G	0.000	0.000	0.130
4	A	B2	C	G	0.000	0.000	0.096
5	A	B1	C	A	1.000	0.033	0.064
6	A	B1	C	G	0.000	0.000	0.049
7	A	B2	T	A	0.000	0.616	0.026
8	A	B2	C	A	0.000	0.000	0.024
9	C	B1	T	A	0.000	0.000	0.003
10	C	B2	C	A	0.000	0.000	0.002
11	C	B2	T	A	0.000	0.001	0.000

HO indicates homozygote. Control subjects had no known mutations of the CETP gene. Haplotypes were reconstructed using the PHASE program (version 1.0) (Ref. 46).

neal opacity. We hypothesized that marked HALP is not always beneficial, but can be atherogenic (52). We extensively analyzed lipoproteins from CETP-deficient subjects, and demonstrated that LDL and HDL were abnormal biochemically and biologically. Fig. 2 summarizes the characteristics of plasma lipids and lipoproteins in CETP-deficient subjects homozygous for the Int14 + 1 G → A mutation. The subjects showed extremely high HDL cholesterol and relatively low LDL cholesterol levels. The increase in HDL cholesterol was attributed to the increase in HDL₂ cholesterol, while HDL₃ cholesterol was not increased. Large and apolipoprotein E-rich HDL particles accumulated in the plasma of the CETP-deficient subjects (53). HDL particles in CETP deficiency, which are more enriched with CE but poorer in TG than control HDL, had a reduced capacity for inhibiting acetyl LDL-induced accumulation of CE in mouse peritoneal macrophages, and less capacity than normal HDL₂ for cholesterol efflux from macrophages preloaded with CE by acetylated LDL (54). This suggests that large CE-rich HDL₂ particles from CETP-deficient subjects do not have an anti-atherogenic function. Two types of HDL particles exist in human plasma: HDL particles containing only apo A-I (LpA-I) and those containing both apo A-I and A-II (LpA-I/A-II). The LpA-I level is low in subjects with CETP deficiency, resulting in a significant decrease in the efflux and LCAT-mediated esterification of cholesterol (55). Furthermore, Oliveira *et al.* demonstrated that the esterification of cholesterol was reduced in subjects with both heterozygous and homozygous CETP deficiency (56).

A stable isotope study demonstrated that the fractional catabolic rates (FCRs) of apo A-I and A-II were significantly reduced in CETP-deficient subjects, whereas the rates of apo A-I and apo A-II production were normal (57). Thus, homozygous CETP deficiency causes a marked delay in the catabolism of apo A-I and A-II. In contrast, the FCRs of apo B were significantly increased in the subjects, while the production rate of apo B was slightly decreased (58). However, the LDL particles from CETP-deficient subjects are small and very heterogeneous (polydisperse LDL) and apo B-rich (59, 60). These particles have a reduced affinity to the LDL receptor of fibroblasts (61), which may be susceptible to *in vivo* oxidation (Fig. 2). In fact, plasma oxidized-LDL is increased in CETP-deficient subjects (62). Taken together, homozygous CETP deficiency causes functional abnormalities as well as compositional changes in both HDL and LDL. Further analysis is essential to investigate whether heterozygous CETP-deficient subjects have lipoprotein abnormalities similar to those of homozygous CETP-deficient subjects, and to determine the other HDL functions, such as anti-oxidant, anti-thrombotic and anti-inflammatory properties.

Relationship between CETP deficiency and atherosclerosis

Several epidemiological studies in the Japanese have investigated the atherogenicity of CETP deficiency. We found a unique area in the northern part of Japan called Omagari (Akita Prefecture), where a null type mutation, Int14 + 1 G → A, of the CETP gene was present in high frequency (63). The prevalence of marked HALP and the Int14 + 1 G → A mutation were 10-fold and 20-fold higher in this area than in other areas of Japan, respectively. This discovery provided us with an opportunity to ascertain whether or not marked HALP caused by CETP gene mutation is associated with longevity. The prevalence of both the Int14 + 1 G → A mutation and marked HALP declined with aging. Furthermore, we examined the relationship between HDL cholesterol levels and ischemic ECG changes, showing that the negative correlations which have been demonstrated by numerous epidemiologic studies were observed only in the range of HDL

Fig. 2. Lipoprotein abnormalities in CETP deficient subjects homozygous for Int14 + 1 G → A, null mutation. (A) Biochemical characteristics of lipoproteins in CETP deficiency. Left panel: Plasma lipoproteins ($d < 1.210$ g/ml) were run on 4–30% of nondenaturing polyacrylamide gradient gel electrophoresis. Right panels: Summary of biochemical characteristics. (B) Atherogenic properties of LDL (Left panel) as well as HDL (Right panel) in CETP deficiency. Left panel: The affinity of LDL for LDL receptor was examined. LDL from CETP deficiency had reduced binding ability to LDL receptor on human skin fibroblasts. Right panel: Preventive effect of HDL on the accumulation of cholesterol in mouse peritoneal macrophages was examined. HDL from CETP deficiency had no preventive effect.

cholesterol < 1.81 mmol/l = 70 mg/dl. In the range of HDL cholesterol ≥ 1.81 mmol/l, the incidence of ischemia was, rather, increased (Fig. 3). These results show that CETP deficiency is not associated with longevity, but instead may be atherogenic. Recently, we performed the quantification of atherosclerotic areas in CETP deficiency with HDL cholesterol ≥ 2.59 mmol/l. Subjects with any known major risk factors including hypertension, diabetes mellitus, high LDL cholesterol, smoking and obesity were excluded from this study. Parameters tested were plaque score (PS) in the carotid artery and pulse wave velocity (PWV) from the aortic root to the femoral artery. Both PS and PWV were higher in subjects with CETP deficiency, compared with those of age- and sex-matched controls, suggesting that CETP deficiency is substantially atherogenic.

Two other epidemiological studies were reported in populations with the D442G missense mutation. One of them was performed in Japanese-American men living in Hawaii (Honolulu Heart Program) (64), the other in the Japanese population living in Kochi Prefecture, Japan (65). In 3,496 Japanese-American men living in Hawaii, the incidence of CHD was higher in subjects with the D442G mutation than in those without mutations, although the difference was significant only in subjects whose HDL cholesterol level was between 1.06 and 1.55 mmol/l (40–60 mg/dl). However, men with increased HDL levels (>1.55 mmol/l) in this population had a low risk of CHD, irrespective of the presence or absence of the CETP

gene mutation, appearing to be protected against atherosclerosis. The other study in the Kochi Prefecture demonstrated results similar to those in the Japanese-American men living in Hawaii.

The above data show that CETP deficiency is atherogenic, but that atherogenicity in CETP deficiency is not always correlated with the level of plasma HDL cholesterol. Thus, further analysis is essential for the complete understanding of the atherogenicity of CETP deficiency.

Conclusion

CETP deficiency is the most important and common cause of HALP in the Japanese. Further analysis is essential for the complete understanding of the atherogenicity of human CETP deficiency, especially the heterozygous deficiency.

Acknowledgements: We thank Masatoshi Kondo for his useful discussion. The skillful technical assistance of Mrs. Satomi Ishihara is gratefully acknowledged.

References

- (1) Gordon T, Castelli WP, Hjortland MC, Kannel KB, and Dawber TR: High density lipoproteins as a protective factor against coronary heart disease. *Am J Med*, 62: 707–714, 1977
- (2) Breslow JL: Familial disorders of high-density lipoprotein metabolism. In: *The Metabolic and Molecular Bases of Inherited Disease*. 7th Ed, ed by Scriver CR, Beaudet AL, Sly WS, and Valle V, pp 2031–2052, McGraw-Hill, New York, 1995
- (3) Tall AR: An overview of reverse cholesterol transport. *Eur Heart J, SupplA*: A31–A35, 1998
- (4) Bruce C, Chouinard RA, and Tall AR: Plasma lipid transfer protein, high-density lipoproteins, and reverse cholesterol transport. *Annu Rev Nutr*, 18: 297–330, 1998
- (5) Tall AR: Plasma lipid transfer protein. *Annu Rev Biochem*, 64: 235–257, 1995
- (6) Yamashita S, Sakai N, Hirano K, Arai T, Ishigami M, Maruyama T, and Matsuzawa Y: Molecular genetics of plasma cholesteryl ester transfer protein. *Curr Opin Lipidol*, 8: 101–110, 1997
- (7) Yamashita S, Hirano K, Sakai N, and Matsuzawa Y: Molecular biology and pathophysiological aspects of plasma cholesteryl ester transfer protein. *Biochim Biophys Acta*, 1529: 257–275, 2000
- (8) Acton S, Rigotti A, Landschulz KT, Xu S, Hobbs HH, and Krieger M: Identified of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science*, 271: 518–520, 1996
- (9) Drayna D, Jarnagin AS, McLean J, Henzel W, Kohr W, Fielding CJ, and Lawn R: Cloning and sequenc-

Fig. 3. Relationship between plasma high density lipoprotein (HDL) cholesterol levels and the incidence of ischemic ECG changes in Omagari (modified from Ref. 61). •, denotes the incidence of an ST-segment depression of > 1 mm in the subjects in each HDL cholesterol range.

- ing of the human cholesteryl ester transfer protein cDNA. *Nature*, 327: 632–634, 1987
- (10) Lagrost L: Regulation of cholesteryl ester transfer protein (CETP) activity; review of in vitro and in vivo studies. *Biochim Biophys Acta*, 1215: 209–236, 1994
 - (11) Agellon L, Quinet E, Gillette T, Drayna D, Brown M, and Tall AR: Organization of the human cholesteryl ester transfer protein gene. *Biochemistry*, 29: 1372–1376, 1990
 - (12) Kurasawa T, Yokoyama S, Miyake Y, Yamamura T, and Yamamoto A: Deficiency of serum cholesteryl ester transfer between high and low density lipoproteins in human serum and a case with decreased transfer rate in association with hyperalphalipoproteinemia. *J Biochem*, 98: 1499–1508, 1985
 - (13) Koizumi J, Mabuchi H, Yoshimura A, Michishita I, Takeda M, Itoh H, Sakai Y, Sakai T, Ueda K, and Takeda R: Deficiency of serum cholesteryl-ester transfer activity in patients with familial hyperalphalipoproteinemia. *Atherosclerosis*, 58: 175–186, 1985
 - (14) Brown ML, Inazu A, Hesler CB, Agellon LB, Mann C, Whitlock ME, Marcel YL, Milne RW, Koizumi J, Mabuchi H, Takeda R, and Tall AR: Molecular basis of lipid transfer protein deficiency in a family with increased high-density lipoproteins. *Nature*, 342: 448–451, 1989
 - (15) Inazu A, Brown ML, Hesler CB, Agellon LB, Koizumi J, Takata K, Maruhamma Y, Mabuchi H, and Tall AR: Increased high-density lipoprotein levels caused by a common cholesteryl-ester transfer protein gene mutation. *N Engl J Med*, 323: 1234–1238, 1990
 - (16) Yamashita S, Hui DY, Sprecher DL, Matsuzawa Y, Sakai N, Tarui S, Kaplan D, Wetterau JR, and Harmony JA: Total deficiency of plasma cholesteryl ester transfer protein in subjects homozygous and heterozygous for the intron 14 splicing defect. *Biochem Biophys Res Commun*, 170: 1346–1351, 1990
 - (17) Takahashi K, Jiang XC, Sakai N, Yamashita S, Hirano K, Bujo H, Yamazaki H, Kusunoki J, Miura T, Kussie P, Matsuzawa Y, Saito Y, and Tall AR: A missense mutation in the cholesteryl ester transfer protein gene with possible dominant effects on plasma high density lipoproteins. *J Clin Invest*, 92: 2060–2064, 1993
 - (18) Inazu A, Jiang XC, Haraki T, Yagi K, Kamon N, Koizumi J, Mabuchi H, Takeda R, Takata K, Moriyama Y, Doi M, and Tall AR: 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*, 94: 1872–1882, 1994
 - (19) Sakai N, Yamashita S, Hirano K, Menju M, Arai T, Kobayashi K, Ishigami M, Yoshida Y, Hoshino T, Nakajima N, Kameda-Takemura K, and Matsuzawa Y: Frequency of exon 15 missense mutation (442D:G) in cholesteryl ester transfer protein gene in hyperalphalipoproteinemic Japanese subjects. *Atherosclerosis*, 114: 139–145, 1995
 - (20) Nagano M, Yamashita S, Hirano K, Kujiraoka T, Ito M, Sagehashi Y, Hattori H, Nakajima N, Maruyama T, Sakai N, Egashira T, and Matsuzawa Y: Point mutation (– 69 G → A) in the promoter region of cholesteryl ester transfer protein gene in Japanese hyperalphalipoproteinemic subjects. *Arterioscler Thromb Vasc Biol*, 21: 985–990, 2001
 - (21) Nagano M, Yamashita S, Hirano K, Ito M, Maruyama T, Ishihara M, Sagehashi Y, Oka T, Kujiraoka T, Hattori H, Nakajima N, Egashira T, Kondo M, Sakai N, and Matsuzawa Y: Two novel missense mutations in the CETP gene in Japanese hyper-alpha-lipoproteinemic subjects: high-throughput assay by Invader[®] assay. *J Lipid Res*, 43: 1011–1018, 2002
 - (22) Arai T, Yamashita S, Sakai N, Hirano K, Okada S, Ishigami M, Maruyama T, Yamane M, Kobayashi H, Nozaki S, Funahashi T, Kameda-Takemura K, Nakajima N, and Matsuzawa Y: A novel nonsense mutation (G181X) in the human cholesteryl ester transfer protein gene in Japanese hyperalphalipoproteinemic subjects. *J Lipid Res*, 37: 2145–2154, 1996
 - (23) Takata K, Mishima Y, Shirahata H, Umemoto S, Toyota Y, and Hiraoka A: A novel nonsense mutation of cholesteryl ester transfer protein gene and the clinical characterization of Japanese primary hyperalphalipoproteinemias (Abstract). *Atherosclerosis*, 4: 301, 2003
 - (24) Gotoda T, Kinoshita M, Shimano H, Harada K, Shimada M, Ohsuga J, Teramoto T, Yazaki Y, and Yamada N: Cholesteryl ester transfer protein deficiency caused by a nonsense mutation detected in the patient's macrophage mRNA. *Biochem Biophys Res Commun*, 194: 519–524, 1993
 - (25) Sakai N, Santamarina-Fojo S, Yamashita S, Matsuzawa Y, and Brewer HB Jr: Exon skipping caused by intron 10 splicing donor site mutation in cholesteryl ester transfer protein gene results in abnormal downstream splice site selection. *J Lipid Res*, 37: 2065–2073, 1996
 - (26) Hirano K, Yamashita S, Funahashi T, Sakai N, Menju M, Ishigami M, Hiraoka H, Kameda-Takemura K, Tokunaga K, Hoshino T, Kumasaka K, and Matsuzawa Y: Frequency of intron 14 splicing defect of cholesteryl ester transfer protein gene in the Japanese general population; relation between the mutation and hyperalphalipoproteinemia. *Atherosclerosis*, 100: 85–90, 1993

- (27) Inazu A, Koizumi J, Haraki T, Yagi K, Wakasugi T, Takegoshi T, Mabuchi H, and Takeda R: Rapid detection and prevalence of cholesteryl ester transfer protein deficiency caused by an intron 14 splicing defect in hyperalpha-lipoproteinemia. *Hum Genet*, 91: 13–16, 1993
- (28) Fors L, Lieder KW, Vavra SH, Kwiatkowski RW: Large-scale SNP scoring from unamplified genomic DNA. *Pharmacogenomics*, 1: 219–229, 2000
- (29) Gotoda T, Kinoshita M, Ishibashi S, Inaba T, Harada K, Shimada M, Osuga J, Teramoto T, Yazaki Y, and Yamada N: Skipping of exon 14 and possible instability of both the mRNA and the resultant truncated protein underlie a common cholesteryl ester transfer protein deficiency in Japan. *Arterioscler Thromb Vasc Biol* 17: 1376–1381, 1997
- (30) Beamer LJ, Carroll SF, and Eisenberg D: Crystal structure of human BPI and two bound phospholipids at 2.4 angstrom resolution. *Science*, 276: 1861–1864, 1997
- (31) Bruce C, Beamer LJ, and Tall AR: The implications of the structure of the bactericidal/permeability-increasing protein on the lipid-transfer function of the cholesteryl ester transfer protein. *Curr Opin Struct Biol*, 8: 426–434, 1998
- (32) Desrumaux C, Labeur C, Verhee A, Tavernier J, Vandekerckhove J, Rosseneu M, and Peelman F: A hydrophobic cluster at the surface of the human plasma phospholipids transfer protein is critical for activity on high density lipoproteins. *J Biol Chem*, 276: 5908–5915, 2000
- (33) Oliveira HCF, Chouinard RA, Agellon LB, Bruce C, Ma L, Walsh A, Breslow JL, and Tall AR: Human cholesteryl ester transfer protein gene proximal promoter contains dietary cholesterol positive responsive elements and mediates expression in small intestine and periphery while predominant liver and spleen expression is controlled by 5'-distal sequences. *J Biol Chem*, 271: 31831–31838, 1996
- (34) Gaudet F and Ginsburg GS: Transcriptional regulation of the cholesteryl ester transfer protein gene by the orphan nuclear hormone receptor apo-lipoprotein AI regulatory protein-1. *J Biol Chem*, 270: 29916–29922, 1995
- (35) Yamashita S, Hui DY, Wetterau JR, Sprecher DL, Harmony JA, Sakai N, Matsuzawa Y, and Tarui S: Characterization of plasma lipoproteins in patients heterozygous for human plasma cholesteryl ester transfer protein (CETP) deficiency: Plasma CETP regulates high-density lipoprotein concentration and composition. *Metabolism*, 40: 756–763, 1991
- (36) Dacet C, Poirier O, Cambien F, Chapman J, and Rouis M: New functional promoter polymorphism, CETP-629, in cholesteryl ester transfer protein (CETP) gene related to CETP mass and high density lipoprotein cholesterol levels; role of Sp1/Sp3 in transcriptional regulation. *Arterioscler Thromb Vasc Biol*, 20: 507–515, 2000
- (37) Drayna D and Lawn R: Multiple RFLPs at the human cholesteryl ester transfer protein (CETP) gene. *Nucleic Acids Res*, 15: 4698, 1987
- (38) Kondo I, Berg K, Drayna DT, and Lawn RM: DNA polymorphism at the locus for human cholesteryl ester transfer protein (CETP) is associated with high density lipoprotein cholesterol and apolipoprotein levels. *Clin Genet*, 35: 49–56, 1989
- (39) Freeman DJ, Griffin BA, Holmes AP, Lindsay GM, Gaffney D, Packard CJ, and Shepherd J: Regulation of plasma HDL cholesterol and subfraction distribution by genetic and environmental factors: associations between the *TaqIB* RFLP in the CETP gene and smoking and obesity. *Arterioscler Thromb*, 14: 336–344, 1994
- (40) Hannuksela ML, Liinamaa MJ, Kesaniemi YA, and Savolainen MJ: Relation of polymorphisms in the cholesteryl ester transfer protein gene to transfer protein activity and plasma lipoprotein levels in alcohol drinkers. *Atherosclerosis*, 110: 35–44, 1994
- (41) Kuivenhoven JA, de Knijff P, Boer JMA, Smalheer HA, Botma GJ, Seidell JC, Kastelein JJ, and Pritchard PH: Heterogeneity at the CETP gene locus: influence on plasma CETP concentrations and HDL cholesterol levels. *Arterioscler Thromb Vasc Biol*, 17: 560–568, 1997
- (42) Ikewaki K, Mabuchi H, Teramoto T, Yamada N, Oikawa S, Sasaki J, Takata K, and Saito Y; Japan CETP Study Group: Association of cholesteryl ester transfer protein activity and *TaqIB* polymorphism with lipoprotein variations in Japanese subjects. *Metabolism*, 52: 1564–1570, 2003
- (43) Ordovas JM, Cupples LA, Corella D, Otvos JD, Osgood D, Martinez A, Lahoz C, Coltell O, Wilson PW, and Schaefer EJ: 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*, 20: 1323–1329, 2000
- (44) Corbex M, Poirier O, Fumeron F, Betouille D, Evans A, Ruidavets JB, Arveiler D, Luc G, Tiret L, and Cambien F: Extensive association analysis between the CETP gene and coronary heart disease phenotypes reveals putative functional polymorphisms and gene-environment interaction. *Genet Epidemiol*, 19: 64–80, 2000
- (45) Thompson JF, Lira ME, Durham LK, Clark RW, Bamberger MJ, and Milos PM: Polymorphisms in the CETP gene and association with CETP mass and HDL levels. *Atherosclerosis*, 167: 195–204, 2003
- (46) Stephens M, Smith NJ, and Donnelly P: A new sta-

- tistical method for haplotype reconstruction from population data. *Am J Hum Genet*, 68: 978–989, 2001
- (47) Bruce C, Sharp DS, and Tall AR: Relationship of HDL and coronary heart disease to a common amino acid polymorphism in the cholesteryl ester transfer protein in men with and without hypertriglyceridemia. *J Lipid Res*, 39: 1071–1078, 1998
 - (48) Hsu LA, Ko YL, Hsu KH, Ko YH, and Lee YS: Genetic variations in the cholesteryl ester transfer protein gene and high density lipoprotein cholesterol levels in Taiwanese Chinese. *Hum Genet*, 110: 57–63, 2002
 - (49) Lu H, Inazu A, Moriyama Y, Higashikata T, Kawashiri MA, Yu W, Huang Z, Okamura T, and Mabuchi H: Haplotype analyses of cholesteryl ester transfer protein gene promoter: a clue to an unsolved mystery of TaqIB polymorphism. *J Mol Med*, 81: 246–255, 2003
 - (50) Klerkx AH, Tanck MW, Kastelein JJ, Molhuizen HO, Jukema JW, Zwinderman AH, and Kuivenhoven JA: Haplotype analysis of the CETP gene: not TaqIB, but the closely linked – 629 C → A polymorphism and a novel promoter variant are independently associated with CETP concentration. *Hum Mol Genet*, 12: 111–123, 2003
 - (51) Williams S, Hayes L, Elsenboss L, Williams A, Andre C, Abramson R, Thompson JF, and Milos PM: Sequencing of the cholesteryl ester transfer protein 5' regulatory region using artificial transposons. *Gene*, 197: 101–107, 1997
 - (52) Matsuzawa Y, Yamashita S, Kameda K, Kubo M, Tarui S, and Hara I: Marked hyper-HDL2-cholesterolemia associated with premature corneal opacity; a case report. *Atherosclerosis*, 62: 117–121, 1984
 - (53) Yamashita S, Sprecher DL, Sakai N, Matsuzawa Y, Tarui S, and Hui DY: Accumulation of apolipoprotein E-rich high density lipoproteins in hyperalphalipoproteinemic human subjects with plasma cholesteryl ester transfer protein deficiency. *J Clin Invest*, 86: 688–695, 1990
 - (54) Ishigami M, Yamashita S, Sakai N, Arai T, Hirano K, Hiraoka H, Kameda-Takemura K, and Matsuzawa Y: Large and cholesteryl ester-rich high-density lipoproteins in cholesteryl ester transfer protein (CETP) deficiency can not protect macrophages from cholesterol accumulation induced by acetylated low-density lipoproteins. *J Biochem*, 116: 257–262, 1994
 - (55) Ohta T, Nakamura R, Takata K, Saito Y, Yamashita S, Horiuchi S, and Matsuda I: Structural and functional differences of subspecies of apoA-I-containing lipoprotein in patients with plasma cholesteryl ester transfer protein deficiency. *J Lipid Res*, 36: 696–704, 1995
 - (56) Oliveria HCF, Ma L, Milne R, Marcovina SM, Inazu A, Mabuchi H, and Tall AR: Cholesteryl ester transfer protein (CETP) activity enhances plasma cholesteryl ester formation: studies in CETP transgenic mice and human genetic CETP deficiency. *Arterioscler Thromb Vasc Biol*, 17: 1045–1052, 1997
 - (57) Ikewaki K, Rader DJ, Sakamoto T, Nishiwaki M, Wakimoto N, Schaefer JR, Ishikawa T, Fairwell T, Zech LA, Nakamura H, and Brewer HB Jr: Delayed catabolism of high density lipoprotein apolipoproteins A-I and A-II in human cholesteryl ester transfer protein deficiency. *J Clin Invest*, 92: 1650–1658, 1993
 - (58) Ikewaki K, Nishiwaki M, Sakamoto T, Ishikawa T, Fairwell T, Zech LA, Nagano M, Nakamura H, Brewer HB Jr, and Rader DJ: Increased catabolic rate of low density lipoproteins in humans with cholesteryl ester transfer protein deficiency. *J Clin Invest*, 96: 1573–1581, 1995
 - (59) Yamashita S, Matsuzawa Y, Okazaki M, Kako H, Yasugi T, Akioka H, Hirano K, and Tarui S: Small polydisperse low density lipoproteins in familial hyperalphalipoproteinemia with complete deficiency of cholesteryl ester transfer activity. *Atherosclerosis*, 70: 7–12, 1988
 - (60) Sakai N, Matsuzawa Y, Hirano K, Yamashita S, Nozaki S, Ueyama Y, Kubo M, and Tarui S: Detection of two species of low density lipoprotein particles in cholesteryl ester transfer protein deficiency. *Arterioscler Thromb*, 11: 71–79, 1991
 - (61) Sakai N, Yamashita S, Hirano K, Ishigami M, Arai T, Kobayashi K, Funahashi T, and Matsuzawa Y: Decreased affinity of low density lipoprotein (LDL) particles for LDL receptors in patients with cholesteryl ester transfer protein deficiency. *Eur J Clin Invest*, 25: 332–339, 1995
 - (62) Chiba H, Akita H, Kotani K, Kanno T, and Manabe M: Complete cholesteryl ester transfer protein deficiency increases oxidized-LDL in plasma. *Jpn J Clin Pathol*, 45: 55–57, 1997 (in Japanese)
 - (63) Hirano K, Yamashita S, Nakajima N, Arai T, Maruyama T, Yoshida Y, Ishigami M, Sakai N, Kameda-Takemura K, and Matsuzawa Y: Genetic cholesteryl ester transfer protein deficiency is extremely frequent in the Omagari area of Japan. *Arterioscler Thromb Vasc Biol*, 17: 1053–1059, 1997
 - (64) Zhong S, Sharp DS, Grove JS, Bruce C, Yano K, Curb JD, and Tall AR: Increased coronary heart disease in Japanese-American men with mutation in the cholesteryl ester transfer protein gene despite increased HDL levels. *J Clin Invest*, 97: 2917–2923, 1996
 - (65) Moriyama Y, Okamura T, Inazu A, Doi M, Iso H,

Mouri Y, Ishikawa Y, Suzuki H, Iida M, Koizumi J, Mabuchi H, and Komachi Y: A low prevalence of coronary heart disease among subjects with in-

creased high-density lipoprotein cholesterol levels, including those with plasma cholesteryl ester transfer protein deficiency. *Prev Med*, 27: 659–667, 1998