Expression of simian CETP in normolipidemic Fisher rats has a profound effect on large sized apoE-containing HDL

Zoulika Zak,* Laurent Lagrost,[1,†] Thomas Gautier,† David Masson,† Valérie Deckert,† Linda Duverneuil,† Jean-Paul Pais de Barros,† Naig Le Guern,† Laure Dumont,† Martina Schneider,† Valérie Risson,* Philippe Moulin,[§] Delphine Autran, Gillian Brooker,[††] Jean Sassard,** and Alain Bataillard***

Département de Physiologie et de Pharmacologie Clinique,* CNRS UMR 5014, IFR 39, Université Claude Bernard Lyon I, 69008 Lyon, France; Laboratoire de Biochimie des Lipoprotéines,† INSERM U498, Faculté de Médecine, BP87900, 21079 Dijon Cedex, France; Service d'Endocrinologie-U11,[§] 69500 Bron, France; Laboratoire de Métabolisme des Lipides,** Hôpital de l'Antiquaille, Lyon, France; and University of Edinburgh Medical School,[††] Edinburgh, Scotland, UK

Abstract **In order to investigate the direct effect of cholesteryl ester transfer protein (CETP) on the structure and composition of HDL in vivo, simian CETP was expressed in Fisher rat that spontaneously displays high plasma levels of HDL1. In the new CETPTg rat line, the production of active CETP by the liver induced a significant 48% decrease in plasma HDL cholesterol, resulting in a 34% decrease in total cholesterol level ($P < 0.01$ in both cases). Among the various plasma HDL subpopulations, the largest HDL were those mostly affected by CETP, with a 74% decrease in HDL1 versus a significantly weaker 38% decrease in smaller HDL2 ($P < 0.0001$). Apolipoprotein E (apoE)-containing HDL1 were selectively affected by CETP expression, whereas apoA content of HDL remained unmodified. The reduction in the apoE content of serum HDL observed in CETPTg rats compared to controls (53%, $P < 0.02$) suggests that apoE in HDL may constitute in vivo a major determinant of their ability to interact with CETP. These results bring new insight into the lack of HDL1 in plasma from CETP-deficient heterozygotes despite their substantial 50% decrease in CETP activity. In addition, they indicate that HDL1 constitute reliable and practicable sensors of very low plasma CETP activity in vivo.**—Zak, Z., L. Lagrost, T. Gautier, D. Masson, V. Deckert, L. Duverneuil, J-P. Pais de Barros, N. Le Guern, L. Dumont, M. Schneider, V. Risson, P. Moulin, D. Autran, G. Brooker, J. Sassard, and A. Bataillard. **Expression of simian CETP in normolipidemic Fisher rats has a profound effect on large-sized apoE-containing HDLs.** ***J. Lipid Res.*** **2002.** 43: **2164–2171.**

Supplementary key words CETP deficiency • transgenesis • apolipoprotein E

Cholesteryl ester transfer protein (CETP) is a plasma glycoprotein that catalyzes an hetero-exchange of cholesteryl esters (CE) and triglycerides (TG) between HDL and apolipoprotein B (apoB) containing lipoproteins (1, 2). Over the last decade, considerable speculations have been emitted on the pro- or anti-atherogenic properties of CETP. Recently, the experimental blockade of CETP in cholesterol-fed rabbits, an animal species with elevated CETP activity and high atherosclerosis susceptibility, by the use of either antisense oligonucleotides (3, 4), chemical inhibitors (5), or vaccination (6) led to a significant decrease in the extent of atherosclerotic lesions. In the mouse, a CETP-deficient species (7), the pro- or antiatherogenic effects of the expression of high levels of human or simian CETP were dependent on the concomitant overexpression of other genes involved in plasma lipoprotein metabolism (8, 9, 10, 11). In the Dahl hyperlipidemic and hypertensive rat, another CETP deficient species, CETP expression produced a significant rise in atherogenic lesions (12). Finally, studies in CETP-deficient patients did not clarify the point as to whether CETP is a pro- or antiatherogenic factor, depending on the population and the metabolic context studied (13).

Besides its long-term effect on atherogenesis, CETP also exerts a strong and direct effect on HDL structure and composition. In this context, and from a metabolic point of view, the mouse and rabbit present some limitations, with large HDL1 particles representing only minor com-

Manuscript received 2 July 2002 and in revised form 8 August 2002.

Published, JLR Papers in Press, September 1, 2002.
DOI 10.1194/jlr.M200253-JLR200

Abbreviations: CETP, cholesteryl ester transfer protein; TC, total cholesterol; TG, triglycerides.

[1] To whom correspondence should be addressed.
e-mail: laurent.lagrost@u-bourgogne.fr

ponents in these species. Thus, at least for HDL purposes, the rat seems to constitute one of the most relevant models of human CETP deficiency. Indeed, and as observed in wild-type rats, plasma from patients with homozygous CETP deficiency contains large-sized, apoE-containing, and cholesteryl ester-rich HDL1 (14–16). Interestingly, HDL1, unlike smaller HDL2 and HDL3, are no more detectable in CETP-deficient heterozygotes with 50% decrease in CETP activity compared to control subjects (16), suggesting an early and selective action of CETP on HDL1.

Although infusion of partially purified human CETP in rats allowed us to document its role in the metabolism of HDL, abnormally high doses were injected, and the overexpression was only transient, not exceeding a few hours (17, 18). Thus, the latter experimental protocol was not appropriate to the determination of the physiological impact of a controlled and persistent CETP expression as it actually occurs in humans, rabbits, or CETP-transgenic lines. To date, only one study addressed the effect of CETP expression in the rat and the consequence of elevated CETP expression was studied in combination with polygenic hypertension as an additional risk factor in order to accelerate the effect of the transgene on atherogenesis (12). As a consequence, non-transgenic hypertensive rats already displayed an abnormal rise in the total cholesterol (TC) to HDL cholesterol (HDL-C) ratio as compared to other normolipidemic and normotensive genetic backgrounds (12, 19). Most importantly, the Dahl salt-sensitive hypertensive rats with low HDL levels did not mimic the situation in homozygous CETP-deficient patients who display marked hyperalphalipoproteinemia, with cholesterol being mainly transported in large size HDL1 particles (14).

In the present studies, the role of CETP in the metabolism of lipoproteins was addressed by creating a new line of CETP transgenic rats in the normolipidemic Fisher background with elevated plasma levels of large HDL1. This rat model allowed us to address the consequences in terms of lipoprotein structure, and lipid and apolipoprotein composition of the sustained expression of moderate CETP levels in a range that was compatible with that classically measured in normolipidemic and hyperlipidemic patients.

MATERIALS AND METHODS

Transgene design and development

The transgene was a 3.6 kb pair construct assembled by fusing the *cynomolgus* monkey CETP cDNA to the mouse metallothionein promoter (a generous gift from Dr. R.T. Marotti). To generate transgenic rats, the CETP transgene was microinjected into the pronucleus of superovulated Fisher females. Injected embryos were reimplanted into pseudo-pregnant female of the same homogenous genetic background. DNA from tails of 3-week-old animals were used for the identification of transgenic rats by polymerase chain reaction (PCR) amplification. The primers were directed to amplify a 603-bp fragment of the CETP cDNA (CETP forward, 5′ CTTGTCCATCGCCACCAGCC 3′; CETP reverse, 5′ AGGGAGTGGAAGACTTGCTCGGA 3′). The PCR was confirmed by Southern blot using a probe including the last 514 bp of the mouse MT-1 promoter and the first 66 bp of the CETP cDNA. Among the progeny, three male founders carried the transgene. They were bred successfully, and two of them (#8102 and #8103) transmitted the transgene to their progeny. Heterozygous male rats from the F2 generation were tested for serum CETP activity (see below), and the present studies were performed using the line with the highest expression level, i.e., line #8103.

Fluorescence in situ hybridization

Fluorescence in situ hybridization was performed by Genaxis (France). Metaphase chromosomes were prepared from fibroblast culture of transgenic rat tendon. The transgene was nick-translated in digoxygenin and used as a probe. The signal was visualized by epifluorescence microscopy.

Animals

Phenotypic characterizations were performed with wild-type and heterozygous CETP transgenic (CETPTg) rats on an homogenous Fisher background. All animals were maintained in controlled conditions of temperature (21 ± 1°C), humidity (60 ± 10%), and lighting (12 h cycle, 8 AM–8 PM). The protocols were in accordance with our institutional guidelines for animal care. Animals (16 to 24 weeks old) were fed a regular rodent chow diet (A03, UAR France). Blood samples were collected into plain glass tubes by jugular vein puncture from 1% isoflurane-anesthetized fasted rats.

RNA analysis

Total RNA was extracted from frozen liver, muscle, heart, and adipose (fat pad) tissue of control and CETPTg rats by the method of Chomczynski and Sacchi (20). To eliminate residual genomic DNA from RNA samples, a DNAse treatment was performed. Two micrograms of DNAse-treated RNA were subjected to RT-PCR using CETP-specific primers (see above). Glyceraldehyde-3-phosphate deshydrogenase (GAPDH) was used in each reaction for semi-quantitative analysis (GAPDH forward, 5′ ACCACAGTCCATGCCATCAC 3′; GAPDH reverse, 5′ TCCACCACCCTGTTGCTGTA 3′). Samples were separated by electrophoresis in 2% agarose gels containing 0.5 mg/ml ethidium bromide. CETP- and GAPDH-specific primers gave 603 bp and 452 bp PCR products, respectively. Signal intensity was visualised under UV light, and PCR conditions were determined as to be in the exponential phase of amplification for the two genes. Data were normalized using GADPH as an internal standard, and results were expressed as CETP to GAPDH mRNA ratio.

Measurement of CETP

CETP activity was measured as the capacity of the serum sample to promote the transfer of radiolabeled cholesteryl esters from a tracer dose of radiolabeled endogenous HDL3 toward apoB containing lipoproteins (21). Briefly, each incubation mixture contained 25 μl of rat plasma, radiolabeled human HDL3 (2.5 nmol of cholesteryl ester), and iodoacetate (final concentration 1.5 mM) in a final volume of 50 μl. Incubations were performed in triplicate for 3 h at 37°C. At the end of the incubation, the $d < 1.068$ and the $d > 1.068$ g/ml fractions were separated by ultracentrifugation, and they were transferred into counting vials containing 2 ml of scintillation fluid. The radioactivity was assayed for 2 min in a Wallac 1410 liquid scintillation counter (Amersham Pharmacia Biotech). Results from triplicate determination were expressed as the percentage of total radioactivity transferred from the lipoprotein tracer to the $d < 1.068$ g/ml fraction after deduction of blank values from the non-incubated control mixtures maintained at 4°C.

CETP enzyme-linked immunosorbent assay

CETP mass concentration in rat serum was measured by using a competitive ELISA adapted to a Biomek 2000 laboratory Automation Workstation (Beckman), as previously described (22). Anti-CETP TP2 antibodies were purchased from the Ottawa Heart Institute (Ottawa, Canada).

Fractionation of serum lipoproteins

Serum lipoproteins were fractionated by fast protein liquid chromatography (FPLC) on a Superose 6HR 10/30 gel filtration column (Amersham Pharmacia Biotech). Lipoproteins were eluted at a constant flow rate of 0.3 ml/min with TSE buffer (50 mM Tris, 0.15 M NaCl, 1 mM EDTA, and 0.02% NaN_3; pH 7.4). For each serum sample, up to 44 distinct fractions were collected, and TC and TG levels were determined. The gel filtration column was calibrated with globular protein standards of known Stokes' diameter (thyroglobulin, 17.0 nm; ferritin, 12.2 nm; aldolase, 8.2 nm, albumin, 7.2 nm) (Gel Filtration Calibration Kit, Pharmacia). Fractions 4 to 11 contained VLDL, fractions 12 to 17 contained LDL, fractions 18 to 24 contained HDL1, fractions 25 to 32 contained HDL2, and fractions 33 to 44 contained HDL3.

Native polyacrylamide gradient gel electrophoresis

The $d < 1.21$ g/ml lipoprotein fraction from individual rat sera (50 μl) was ultracentrifugally isolated at 100,000 rpm in a TLA-100 rotor in a Beckman Optima TLX ultracentrifuge. The size distribution of HDL was determined by electrophoresis of total lipoproteins on Spiragel 1.5–25% (Spiral-Couternon, France), according to the general procedure recommended by the manufacturer. At the end of the electrophoresis, the gels were stained with the Coomassie Brillant Blue G, and HDL distribution profiles were obtained by analysis of polyacrylamide gradient gels on a Bio-Rad GS-670 imaging densitometer. The size of the HDL subfractions was determined by comparison with globular protein standards (HMW protein calibration kit, Pharmacia) that were submitted to electrophoresis together with the samples. The relative abundance of HDL1 (12.9–20.0 nm) and HDL2 (8.7–12.9 nm) was quantitated as the corresponding area under the scan curve, and results were expressed in AUC units.

SDS-polyacrylamide gel electrophoresis of rat HDL apolipoproteins

The $1.02 < d < 1.21$ g/ml HDL fraction was ultracentrifugally isolated from rat sera at 100,000 rpm in a TL-100 rotor on a Beckman Optima TLX ultracentrifuge. Isolated HDL (protein, 0.5 g/l) were incubated for 15 min at 80°C in the presence of SDS (25 g/l) and dithiotreitol (33 g/l) in TBS buffer (Tris, 10 mmol/l; NaCl, 150 mmol/l; NaN_3, 3 mmol/l; pH 7.4). Samples were then applied on a SDS polyacrylamide gradient gel (Phastgel 8/25, Amersham Pharmacia Biotech), and migration was conducted as recommended by the manufacturer. Apolipoproteins were stained by Coomassie Brilliant Blue G, and apparent molecular weights of individual bands were determined by comparison with protein standards (High Molecular Weight calibration kit, Pharmacia) that were submitted to electrophoresis together with the samples.

Lipid and lipoprotein analysis

Serum TC and TG were measured by enzymatic methods using Boehringer Mannheim reagents, and assays were performed on a Cobas-Fara centrifuge analyser (Hoffman Laroche).

Statistical analysis

Results are expressed as mean ± SE. Student's *t*-test or nonparametric Mann-Whitney U-test were used to compare differences between data means, as appropriate.

RESULTS

A transgenic line was established from a male founder, and in the established CETP transgenic line the gene of the simian protein was transmitted to the progeny in a mendelian fashion. As determined by RT-PCR, CETP mRNA levels were expressed in the liver, with no detectable mRNA levels in other tissues, including adipose tissue, muscle, and heart (**Fig. 1**). No CETP mRNA was detected in tissues from control rats (Fig. 1). In CETPTg rats, a unique site of insertion was localized on chromosome 3q23-q24 as determined by fluorescence in situ hybridization. Southern blot analysis of hemizygous males indicated that approximately 10 copies of the CETP transgene were incorporated in the genome of CETPTg rats (data not shown).

To determine if CETPTg rats actually expressed CETP, CETP mRNA and activity levels were determined in total plasma samples from heterozygous males. As shown in **Fig. 2**, the expression of both CETP mRNA in the liver and the level of serum cholesteryl ester transfer activity were quite variable from one animal to another, with a close correlation between the two parameters ($r = 0.79$, $P < 0.01$). These observations came in support of the liver as the major contributor to the serum CETP pool (Fig. 1), and the level of cholesteryl ester transfer activity in transgenic animals was mainly a function of the circulating level of the protein (Fig. 2). Interestingly, a broad variety of CETP gene/activity expression was observed from one animal to another, and we took advantage of this fact to select a population of CETPTg males with mean serum CETP level of 2.4 mg/l (i.e., similar to the mean CETP concentration reported in normolipidemic subjects) (22). Individual CETP mass concentrations ranged from very low levels (i.e., down to the non-detectable levels measured in control rats or homozygous CETP deficient patients) to high levels (i.e., up to 4–5 mg/l concentrations similar to those reported in dyslipidemic populations) (**Fig. 3**).

On a standard chow diet, serum TG levels did not differ significantly between control and CETPTg rats (**Table 1**). In contrast, the expression of the CETP transgene pro-

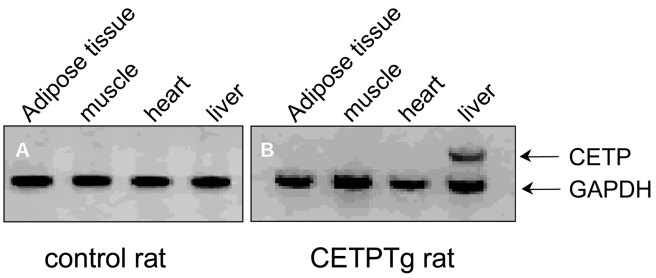

Fig. 1. Sites of expression of the cholesteryl ester transfer protein (CETP) transgene. CETP transgene mRNA levels were determined in adipose tissue, muscle, heart, and liver of control (A) and CETPTg (B) rats, as described in Materials and Methods. Glyceraldehyde-3-phosphate deshydrogenase (GAPDH) was used as an internal standard.

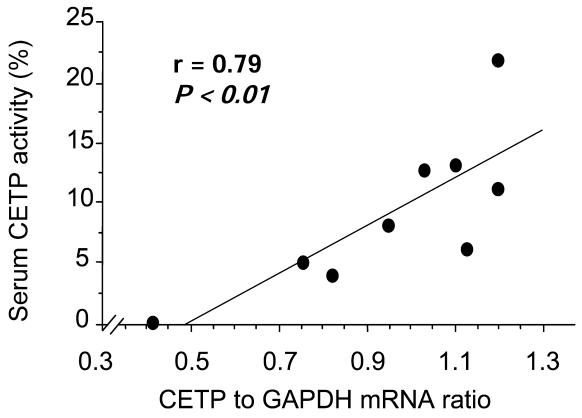

Fig. 2. Correlation between serum CETP activity and hepatic CETP mRNA levels. Cholesteryl ester transfer activity and hepatic mRNAs were quantitated in nine distinct CETPTg rats as described in Materials and Methods.

TABLE 1. Lipid and lipoprotein concentrations

Lipids	Rats	
	Control	CETPTg
Total cholesterol (g/l)	0.76 ± 0.07	0.50 ± 0.04[a]
	n = 7	n = 14
Triglycerides (g/l)	2.29 ± 0.32	2.18 ± 0.13
	n = 7	n = 14
Total cholesterol (μg/ml) in lipoprotein fractions		
VLDL-C	90.4 ± 8.1	102.2 ± 8.6
LDL-C	27.0 ± 2.2	28.3 ± 5.1
HDL-C	690.1 ± 64.7	355.6 ± 60.7[a]
HDL1-C	232.0 ± 34.0	61.0 ± 16.5[a]
HDL2-C	433.3 ± 27.4	268.1 ± 43.4[a]
HDL3-C	24.9 ± 5.8	26.5 ± 4.6

Lipoprotein preparation and lipid analysis were determined as described in Materials and Methods. Values shown are mean ± SE (n = 7 control and n = 14 CETPTg rats for total cholesterol and triglycerides; n = 5 control and n = 8 CETPTg rats for cholesterol in lipoprotein fractions).

[a] Significantly different from control rats. $P < 0.01$.

duced a 34% decrease in the mean concentration of serum TC. A selective 48% decrease in the HDL-C levels accounted for the change in plasma cholesterol, with no significant alterations in the cholesterol content of the VLDL and LDL fractions (Table 1). As shown on FPLC gel filtration profiles (**Fig. 4**), the significant decrease in cholesterol levels concerned mainly large HDL particles (fractions 18 to 24) with no change in VLDL (fractions 4 to 11), and with no detectable levels of LDL (fractions 12 to 17). In CETPTg rats (Fig. 4A), the specific loss of cholesterol in the large HDL1 gave rise to an HDL peak with a shape that was quite similar to those observed in humans and control C57BL/6 mice, i.e., two species with high and low levels of plasma LDL, respectively, and with virtually no plasma HDL1 (Fig. 4B). A more detailed analysis of the rat HDL profile by polyacrylamide gradient gel electrophoresis indicated that HDL1 (diameter, 12.9–20.0 nm) were mostly affected by CETP expression, leading to a significant decrease in the mean apparent diameter of the serum HDL population (12.16 ± 0.06 nm in controls vs. 11.88 ± 0.21 nm in CETPTg rats; $P < 0.05$) (**Fig. 5**). CETP expression produced a more profound 74% drop in the cholesterol content of large size HDL1 as compared to the limited 38% reduction in HDL2, with no significant effect on the discrete HDL3 fraction (Table 1). Consistent conclusions could be drawn from the semi-quantitative analysis of native polyacrylamide gels, with a 64% decrease in the population of HDL1 size in CETPTg rats, but only a 34% decrease in the population of HDL2 size (**Fig. 6A**).

In support of distinct behaviours of HDL1 and HDL2 as lipoprotein substrates in the CETP-mediated lipid transfer process, a marked decrease in HDL1 was observed with any level of transgene expression and it was in contrast with the gradual, dose-dependent decrease in HDL2 over the CETP activity range studied (Fig. 6B, C). Finally, and in order to bring some molecular insights into the preferential reduction of large HDL1 among all the CETPTg rat sera, the apolipoprotein content of isolated HDL was analyzed by denaturing polyacrylamide gradient gel electrophoresis. As shown in **Fig. 7**, the CETP transgene promoted a significant and selective reduction in the apoE content of serum HDL in CETPTg rats compared to control rats (−53%, $P < 0.02$) with no significant changes in apoA-I, apoA-II, and apoA-IV (i.e., the other major components of serum HDL).

DISCUSSION

A new line of CETPTg rats was created in order to assess in vivo the role of CETP on the structure and composition

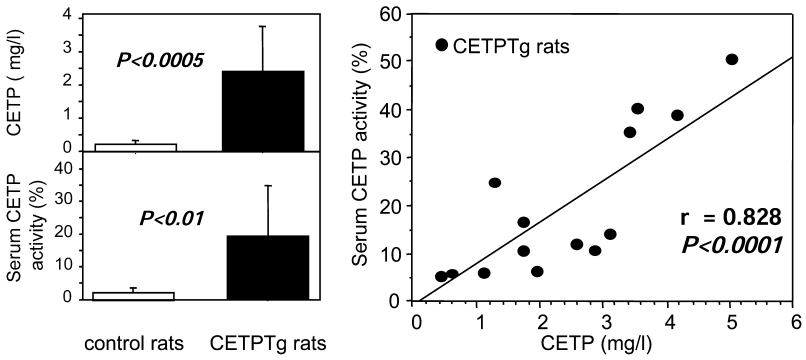

Fig. 3. CETP mass and activity in serum from control and CETPTg rats. Cholesteryl ester transfer activity and mass levels were determined in total plasma from control (n = 7) and CETPTg rats (n = 14), as described in Materials and Methods.

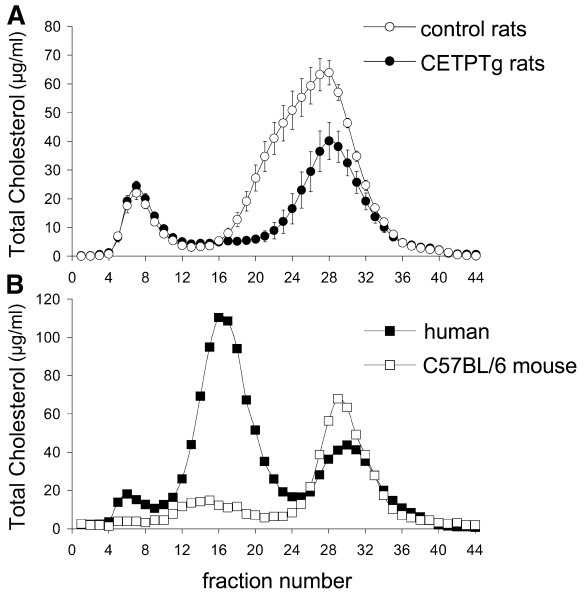

Fig. 4. Gel filtration chromatography of control rat, CETPTg rat, human, and C57BL/6 mouse sera. Sera were passed through a Superose 6-HR column on an FPLC system, and the cholesterol content of individual fractions was determined as described in Materials and Methods. For control (opened circles) and CETPTg (closed circles) rat profiles (A), each point is the mean ± SEM of n = 5 and n = 8 animals, respectively. B: Typical profiles of human (closed squares) and C57BL/6 control mouse (open squares).

of plasma lipoproteins in the Fisher genetic background. In contrast to what has been previously reported in other animal models used for CETP transgenesis, including C57BL/6 mice (8, 23) and Dahl rats (12), Fisher rats show high plasma levels of large size HDL1 that are known to

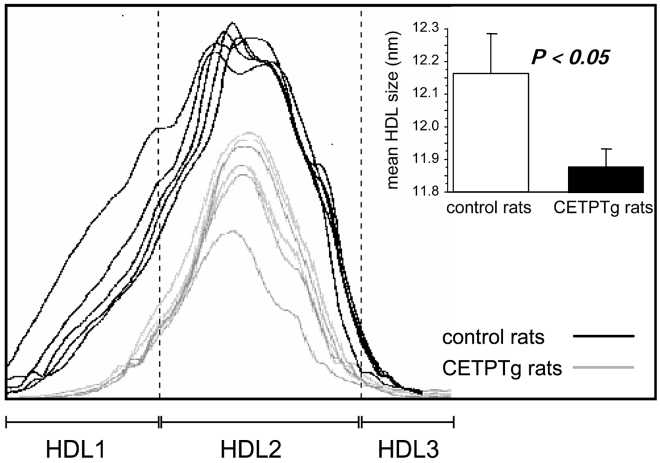

Fig. 5. Native polyacrylamide gradient gel electrophoresis of serum HDL from control and CETPTg rats. Total serum lipoproteins from control and CETPTg rats were submitted to electrophoresis on 15–250 g/l polyacrylamide gradient gels that were subsequently stained for proteins as described in Materials and Methods. HDL profiles (insert) were obtained by image analysis, and mean apparent diameters (insert) were calculated as compared to protein standards. Vertical bars are mean ± SEM. Statistical significance by Student's *t*-test.

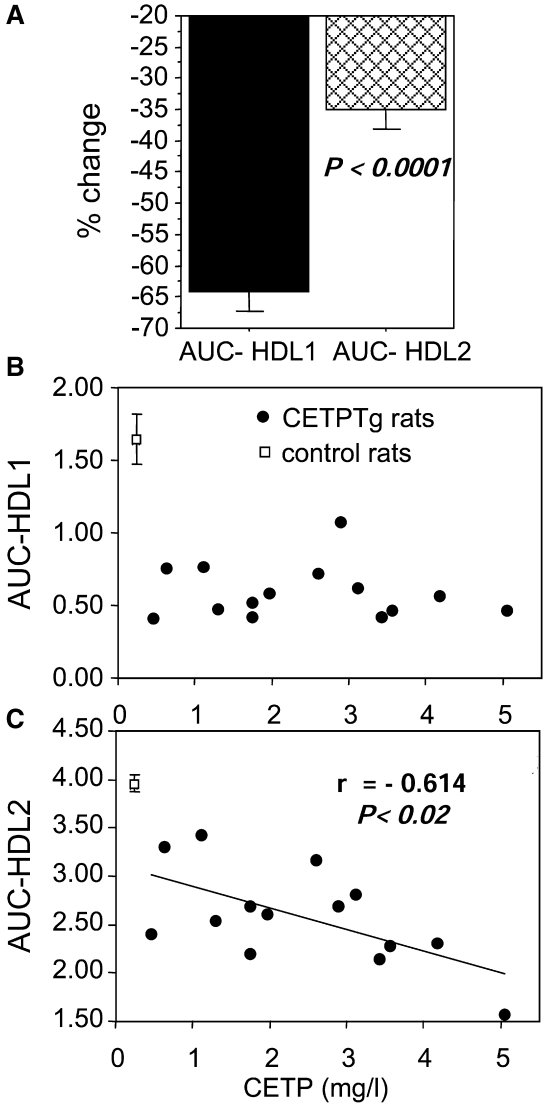

Fig. 6. Effect of CETP expression on the abundance of HDL1 and HDL2 in CETPTg rats compared with control rats. Total serum lipoproteins were separated by native polyacrylamide gradient gel electrophoresis (see Fig. 5), HDL profiles were obtained by image analysis, and the relative abundance of HDL1 and HDL2 was quantitated as the relative area under the scan curve (AUC). CETP mass concentration in total sera was determined by ELISA. A: Vertical bars show the percent change in the abundance of HDL1 and HDL2 in CETPTg rats versus controls; statistical significance by Student's *t*-test. B and C: Correlations between the relative abundance of HDL1 and HDL2, and CETP mass concentration among control (opened squares) and CETPTg (closed circles) rats; linear regression analysis.

be markedly increased in human patients with CETP deficiency (24). Thus, in the present studies the different levels of persistent CETP expression in the high-HDL1 Fisher rat allowed us to monitor in a comprehensive and physiological manner the effect of CETP on HDL. Controlled expression of CETP levels in the Fisher rats was shown to exert a profound effect on plasma HDL, that was characterized mainly by a selective but pronounced reduction in the relative abundance of large, apoE-containing HDL1

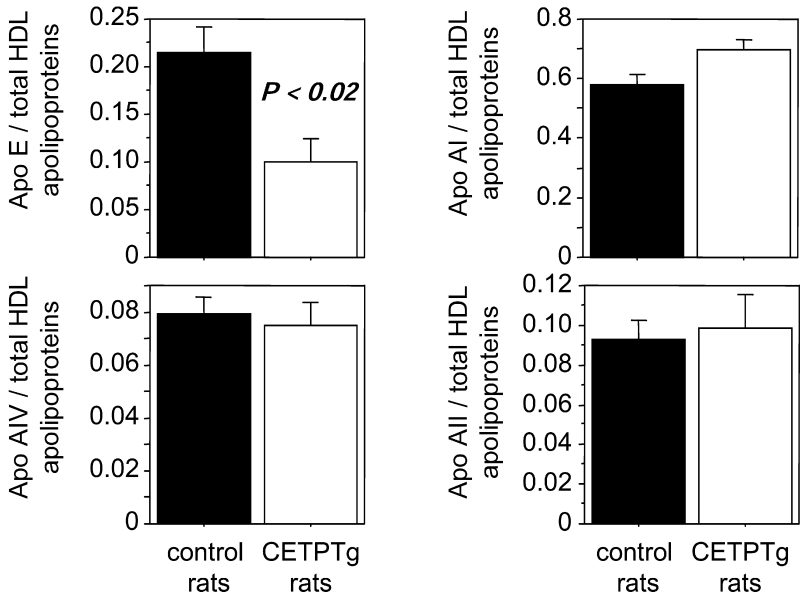

Fig. 7. Effect of CETP expression on HDL apolipoproteins in control and CETPTg rats. HDL from control (n = 6) and CETPTg (n = 7) rats were isolated by ultracentrifugation, and apolipoproteins were separated by SDS-electrophoresis in 8–25% polyacrylamide gradient gel (see Materials and Methods). Protein bands were vizualized after Coomassie staining, and the molecular weights were determined by comparison with protein standards. The apolipoprotein profiles were obtained by image analysis, the corresponding area under the scan curve was determined for each band, and the abundance of apoA-I, apoA-IV, apoA-II, and apoE was calculated and expressed as apolipoprotein to total HDL apolipoprotein ratio.

indicating a peculiar sensitivity of these particles to CETP action.

In the present studies, the expression of the CETP transgene was shown to vary widely between individuals of the same CETPTg line. Although no clear rational for the variability of the transgene expression in the established CETPTg line can be brought, it is worthy to note that, a similar variegated expression of a human apoA-I transgene was reported within one rat transgenic line (25). In the present studies, the variegated pattern of expression was unlikely to have resulted from factors intrinsic to the transgene construct since it has been previously used in mice to establish four transgenic lines with reproducible levels of expression (26). Moreover, the copy number within the line appeared to be stable and there was no evidence of transgene rearrangement. As it has been reported in other cases of variegation (27), the tight correlation between CETP mRNA expression and plasma CETP activity rather suggests that the unstable transgene expression could relate to a variable at the transcriptional level. In the present studies, we took advantage of the variable level of CETP expression in the CETP transgenic rat line that was established from a male founder. This led us to select transgenic rats with different expressions of CETP activity, i.e., with low, intermediate, and high levels similar to those reported in CETP-deficient patients, normolipidemic subjects, and dyslipidemic patients, respectively.

As expected from the known metabolic function of CETP, its expression in transgenic rats provoked significant alterations in plasma lipid parameters. They were characterized mainly by significant reductions in total and HDL-C levels, with no significant variations of the plasma TG levels or cholesterol content of apoB-containing lipoproteins. While detailed analysis of the lipid profile revealed a selective impact of a moderate, but sustained CETP expression on the large, apoE-rich HDL1 in vivo, it confirmed no significant alterations in the VLDL and LDL fractions upon CETP gene expression. In fact, the rat exhibits an extensive hepatic uptake of triglyceride-rich lipoprotein remnants that interact very efficiently with specific hepatic receptors (28). It results that the VLDL-IDL-LDL transformation is considerably reduced in the rat as compared to humans because of a rapid clearance of the apoE-containing VLDL particles in the former case. This situation may not be modified by CETP gene expression with virtually no detectable levels of LDL in both control and CETPTg rats (Fig. 4A). Data of the present study indicate further that the earlier evidence of a direct effect of a massive and single injection of CETP on HDL structure and composition (17, 29, 30) is of metabolic relevance. In addition, they extent previous in vivo observations in the monkey, demonstrating that the significant, inverse correlation previously observed between plasma CETP activity and large, apoE-containing HDL actually relies on a direct interconnection of the two parameters (31). Given the unique metabolic properties of large, apoE-containing HDL1, including in particular its ability to bind to the cellular LDL receptor (14), the selective effect of CETP on this peculiar lipoprotein subpopulation might well constitute a major clue to the enigma of the role of CETP in atherogenesis. Interestingly, HDL1-like particles are only found in some of the animal species with very low CETP activity, and it has longer been recognized that animals with high plasma levels of large HDL display concomitantly low levels of CETP (32). With regard to human studies, it is worthy to note that in the presence of abundant apoE-rich large HDL1 is a characteristic of hyperalphalipoproteinemia with complete CETP deficiency, with no detectable amounts of these particles in normo- or hyperalphalipoproteinemic subjects with normal or half normal CETP activity (14, 16). Unlike rat, wild-type mouse, at least of the C57BL/6 genetic background, does not naturally exhibit elevated amounts of large, apoE-rich HDL1 (8, 33) (Fig. 4B). This point might well account for the relatively weak changes, if any in the structure and composition of HDL in transgenic mice expressing the

single CETP gene (8, 26, 34). In fact, the effect of CETP on HDL in mouse became mostly apparent in animals expressing human CETP either in an hyperlipidemic compound line also overexpressing human apoC-III (10) or in a normolipidemic compound line also overexpressing human apoA-I (9). It must be emphasized that in the apoA-ITg/CETPTg line no HDL1-sized particles were detected despite high plasma HDL levels, and HDL size changes were mainly confined to the HDL2 fraction upon the coexpression of the CETP transgene (9). Overall, mouse HDL are not so large as those in plasma of human CETP-deficient subjects (33), neither are HDL in plasma from Dahl hyperlipidemic/hypertensive rats (12). In this context, the CETPTg rat line characterized in this study is a unique model that brings new support to a direct link between the presence of a unique apoE-rich HDL1 subpopulation and defective neutral lipid transfers between triglyceride-rich VLDL and cholesteryl ester-rich HDL, as observed in CETP-deficient patients (14). It results that a sudden rise in the relative amount of triglyceride-rich acceptors in CETPTg rat plasma is likely to accelerate the disappearance of large, apoE-rich HDL1 by stimulating the replacement of cholesteryl esters by the hydrolyzable TG in HDL: one hypothesis that deserves further attention by the mean of dietary manipulation.

Structurally, the CETP-sensitive HDL1 differ from the two main other plasma HDL subclasses, i.e., HDL2 and HDL3, by at least two distinct aspects: *1*) their extralarge size, with a mean diameter ranging from 12.9 up to 20.0 nm, and *2*) their apolipoprotein composition, with a specific apoE-enrichment. When the molecular basis for the preferential disappearance of HDL1 from plasma of CETPTg rats is analyzed in the light of previous observations, the mean size per se, i.e., an estimate of the lipoprotein surface curvature, is unlikely to account for the observed changes, with somewhat more efficient interaction of CETP with small HDL than with large HDL (35). Conversely, with regard to the apolipoprotein composition, the apoE enrichment of large HDL1 might well play a significant role. Indeed, previous in vitro studies reported the existence of a preferential interaction of CETP with apoE-containing lipoproteins, suggesting that apoE might constitute a specific activator of the CETP-mediated lipid transfers (36). The results of the present in vivo study come in support of this view, and suggest further that the apoE content of HDL may constitute in vivo a major determinant of their ability to act as a lipoprotein substrate in the CETP-mediated lipid transfer process. In this context, the striking rise in apoE-rich HDL levels would be a direct consequence of the lack of CETP-mediated HDL remodelling in both Int14A homozygotes and Int14A/D442G compound heterozygotes with complete CETP deficiency (37).

In conclusion, the new line of normolipidemic CETPTg rats in the Fisher genetic background allowed us to assess the physiological impact of a human-like CETP activity on the lipoprotein metabolism in one animal model with elevated circulating levels of large, apoE-rich HDL1. Since HDL1 arose as reliable and practicable sensors of very low CETP activity, they might well constitute a relevant index of the efficacy of putative pharmacological CETP inhibitors in future clinical practice. Finally, the wide range of plasma CETP concentrations that could be achieved in the present studies within a group of CETPTg rats indicates that this new transgenic line may constitute a useful tool for the modelisation of combined low CETP activity and high HDL levels that were proven to be suitable for atherogenesis prevention in humans (38).

The authors thank Pascale Fournier, Joëlle Sacquet, and John Mullins for their excellent technical assistance, and Anick Girard-Globa for helpful suggestions. This study was supported by the Institut National de la Santé et de la Recherche Médicale, the Conseil Régional Rhône-Alpes, the Conseil Régional de Bourgogne, the Fondation de France, and Merck.

REFERENCES

1. Bruce, C., R. A. Chouinard, Jr., and A. R. Tall. 1998. Plasma lipid transfer proteins, high-density lipoproteins, and reverse cholesterol transport. *Annu. Rev. Nutr.* **18:** 297–330.
2. Lagrost, L. 1994. Regulation of cholesteryl ester transfer protein (CETP) activity: review of in vitro and in vivo studies. *Biochim. Biophys. Acta.* **1215:** 209–236.
3. Sugano, M., and N. Makino. 1996. Changes in plasma lipoprotein cholesterol levels by antisense oligodeoxynucleotides against cholesteryl ester transfer protein in cholesterol-fed rabbits. *J. Biol. Chem.* **271:** 19080–19083.
4. Sugano, M., N. Makino, S. Sawada, S. Otsuka, M. Watanabe, H. Okamoto, M. Kamada, and A. Mizushima. 1998. Effect of antisense oligonucleotides against cholesteryl ester transfer protein on the development of atherosclerosis in cholesterol-fed rabbits. *J. Biol. Chem.* **273:** 5033–5036.
5. Okamoto, H., F. Yonemori, K. Wakitani, T. Minowa, K. Maeda, and H. Shinkai. 2000. A cholesteryl ester transfer protein inhibitor attenuates atherosclerosis in rabbits. *Nature.* **406:** 203–207.
6. Rittershaus, C. W., D. P. Miller, L. J. Thomas, M. D. Picard, C. M. Honan, C. D. Emmett, C. L. Pettey, H. Adari, R. A. Hammond, D. T. Beattie, A. D. Callow, H. C. Marsh, and U. S. Ryan. 2000. Vaccine-induced antibodies inhibit CETP activity in vivo and reduce aortic lesions in a rabbit model of atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **20:** 2106–2112.
7. Jiao, S., T. G. Cole, R. T. Kitchens, B. Pfleger, and G. Schonfeld. 1990. Genetic heterogeneity of lipoproteins in inbred strains of mice: analysis by gel-permeation chromatography. *Metabolism.* **39:** 155–160.
8. Agellon, L. B., A. Walsh, T. Hayek, P. Moulin, X. C. Jiang, S. A. Shelanski, J. L. Breslow, and A. R. Tall. 1991. Reduced high density lipoprotein cholesterol in human cholesteryl ester transfer protein transgenic mice. *J. Biol. Chem.* **266:** 10796–10801.
9. Hayek, T., T. Chajek-Shaul, A. Walsh, L. B. Agellon, P. Moulin, A. R. Tall, and J. L. Breslow. 1992. An interaction between the human cholesteryl ester transfer protein (CETP) and apolipoprotein A-I genes in transgenic mice results in a profound CETP-mediated depression of high density lipoprotein cholesterol levels. *J. Clin. Invest.* **90:** 505–510.
10. Hayek, T., L. Masucci-Magoulas, X. Jiang, A. Walsh, E. Rubin, J. L. Breslow, and A. R. Tall. 1995. Decreased early atherosclerotic lesions in hypertriglyceridemic mice expressing cholesteryl ester transfer protein transgene. *J. Clin. Invest.* **96:** 2071–2074.
11. Foger, B., M. Chase, M. J. Amar, B. L. Vaisman, R. D. Shamburek, B. Paigen, J. Fruchart-Najib, J. A. Paiz, C. A. Koch, R. F. Hoyt, H. B. Brewer, Jr., and S. Santamarina-Fojo. 1999. Cholesteryl ester transfer protein corrects dysfunctional high density lipoproteins and reduces aortic atherosclerosis in lecithin cholesterol acyltransferase transgenic mice. *J. Biol. Chem.* **274:** 36912–36920.
12. Herrera, V. L., S. C. Makrides, H. X. Xie, H. Adari, R. M. Krauss, U. S. Ryan, and N. Ruiz-Opazo. 1999. Spontaneous combined hy-

perlipidemia, coronary heart disease and decreased survival in Dahl salt-sensitive hypertensive rats transgenic for human cholesteryl ester transfer protein. *Nat. Med.* **5:** 1383–1389.
13. Hirano, K., S. Yamashita, and Y. Matsuzawa. 2000. Pros and cons of inhibiting cholesteryl ester transfer protein. *Curr. Opin. Lipidol.* **11:** 589–596.
14. Yamashita, S., D. L. Sprecher, N. Sakai, Y. Matsuzawa, S. Tarui, and D. Y. Hui. 1990. Accumulation of apolipoproteinE-rich high density lipoproteins in hyperalphalipoproteinemic human subjects with plasma cholesteryl ester transfer protein deficiency. *J. Clin. Invest.* **86:** 688–695.
15. Bisgaier, C. L., M. V. Siebenkas, M. L. Brown, A. Inazu, J. Koizumi, H. Mabuchi, and A. R. Tall. 1991. Familial cholesteryl ester transfer protein deficiency is associated with triglyceride-rich low density lipoproteins containing cholesteryl esters of probable intracellular origin. *J. Lipid Res.* **32:** 21–33.
16. Arai, T., T. Tsukada, T. Murase, and K. Matsumoto. 2000. Particle size analysis of high density lipoproteins in patients with genetic cholesteryl ester transfer protein deficiency. *Clin. Chim. Acta.* **301:** 103–117.
17. Ha, Y. C., L. B. Chang, and P. J. Barter. 1985. Effects of injecting exogenous lipid transfer protein into rats. *Biochim. Biophys. Acta.* **833:** 203–210.
18. Quig, D. W., and D. B. Zilversmit. 1986. Disappearance and effects of exogenous lipid transfer activity in rats. *Biochim. Biophys. Acta.* **879:** 171–178.
19. Chapman, M. J. 1980. Animal lipoproteins: chemistry, structure, and comparative aspects. *J. Lipid Res.* **21:** 789–853.
20. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162:** 156–159.
21. Lagrost, L., H. Gandjini, A. Athias, V. Guyard-Dangremont, C. Lallemant, and P. Gambert. 1993. Influence of plasma cholesteryl ester transfer activity on the LDL and HDL distribution profiles in normolipidemic subjects. *Arterioscler. Thromb.* **13:** 815–825.
22. Guyard-Dangremont, V., L. Lagrost, P. Gambert, and C. Lallemant. 1994. Competitive enzyme-linked immunosorbent assay of the human cholesteryl ester transfer protein (CETP). *Clin. Chim. Acta.* **231:** 147–160.
23. Marotti, K. R., C. K. Castle, T. P. Boyle, A. H. Lin, R. W. Murray, and G. W. Melchior. 1993. Severe atherosclerosis in transgenic mice expressing simian cholesteryl ester transfer protein. *Nature.* **364:** 73–75.
24. Yamashita, S., T. Maruyama, K. I. Hirano, N. Sakai, N. Nakajima, and Y. Matsuzawa. 2000. Molecular mechanisms, lipoprotein abnormalities and atherogenicity of hyperalphalipoproteinemia. *Atherosclerosis.* **152:** 271–285.
25. Swanson, M. E., T. E. Hughes, I. S. Denny, D. S. France, J. R. Paterniti, Jr., C. Tapparelli, P. Gfeller, and K. Burki. 1992. High level expression of human apolipoprotein A-I in transgenic rats raises total serum high density lipoprotein cholesterol and lowers rat apolipoprotein A-I. *Transgenic Res.* **1:** 142–147.
26. Marotti, K. R., C. K. Castle, R. W. Murray, E. F. Rehberg, H. G. Polites, and G. W. Melchior. 1992. The role of cholesteryl ester transfer protein in primate apolipoprotein A-I metabolism. Insights from studies with transgenic mice. *Arterioscler. Thromb.* **12:** 736–744.
27. Birchler, J. A., M. P. Bhadra, and U. Bhadra. 2000. Making noise about silence: repression of repeated genes in animals. *Curr. Opin. Genet. Dev.* **10:** 211–216.
28. Arbeeny, C. M., and V. A. Rifici. 1984. The uptake of chylomicron remnants and very low density lipoprotein remnants by the perfused rat liver. *J. Biol. Chem.* **259:** 9662–9666.
29. Groener, J. E., T. van Gent, and A. van Tol. 1989. Effect of lipid transfer protein on plasma lipids, apolipoproteins and metabolism of high-density lipoprotein cholesteryl ester in the rat. *Biochim. Biophys. Acta.* **1002:** 93–100.
30. Gavish, D., Y. Oschry, and S. Eisenberg. 1987. In vivo conversion of human HDL3 to HDL2 and apoE-rich HDL1 in the rat: effects of lipid transfer protein. *J. Lipid Res.* **28:** 257–267.
31. Kushwaha, R. S., D. L. Rainwater, M. C. Williams, G. S. Getz, and H. C. McGill, Jr. 1990. Impaired plasma cholesteryl ester transfer with accumulation of larger high density lipoproteins in some families of baboons (Papio sp.). *J. Lipid Res.* **31:** 965–973.
32. Ha, Y. C., and P. J. Barter. 1982. Differences in plasma cholesteryl ester transfer activity in sixteen vertebrate species. *Comp. Biochem. Physiol. B.* **71:** 265–269.
33. Okumura-Noji, K., K. Sasai, R. Zhan, H. Kawaguchi, H. Maruyama, T. Tada, H. Takahashi, M. Okazaki, T. Miida, N. Sakuma, G. Kimura, N. Ohta, and S. Yokoyama. 2001. Cholesteryl ester transfer protein deficiency causes slow egg embryonation of Schistosoma japonicum. *Biochem. Biophys. Res. Commun.* **286:** 305–310.
34. Jiang, X. C., L. Masucci-Magoulas, J. Mar, M. Lin, A. Walsh, J. L. Breslow, and A. Tall. 1993. Down-regulation of mRNA for the low density lipoprotein receptor in transgenic mice containing the gene for human cholesteryl ester transfer protein. Mechanism to explain accumulation of lipoprotein B particles. *J. Biol. Chem.* **268:** 27406–27412.
35. Speijer, H., J. E. Groener, E. van Ramshorst, and A. van Tol. 1991. Different locations of cholesteryl ester transfer protein and phospholipid transfer protein activities in plasma. *Atherosclerosis.* **90:** 159–168.
36. Kinoshita, M., H. Arai, M. Fukasawa, T. Watanabe, K. Tsukamoto, Y. Hashimoto, K. Inoue, K. Kurokawa, and T. Teramoto. 1993. Apolipoprotein E enhances lipid exchange between lipoproteins mediated by cholesteryl ester transfer protein. *J. Lipid Res.* **34:** 261–268.
37. Chiba, H., H. Akita, K. Tsuchihashi, S. P. Hui, Y. Takahashi, H. Fuda, H. Suzuki, H. Shibuya, M. Tsuji, and K. Kobayashi. 1997. Quantitative and compositional changes in high density lipoprotein subclasses in patients with various genotypes of cholesteryl ester transfer protein deficiency. *J. Lipid Res.* **38:** 1204–1216.
38. Zhong, S., D. S. Sharp, J. S. Grove, C. Bruce, K. Yano, J. D. Curb, and A. R. Tall. 1996. 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.