The role of plasma lipid transfer proteins in lipoprotein metabolism and atherogenesis

David Masson\textsuperscript{a,c,d}, Xian-Cheng Jiang\textsuperscript{b}, Laurent Lagrost\textsuperscript{c}, Alan R. Tall\textsuperscript{a}.

a: Division of Molecular Medicine, Department of Medicine, Columbia University, New York, NY 10032, USA.
b: Department of Anatomy and Cell Biology, State University of New York Downstate Medical Center, Brooklyn, NY 11203, USA.
c: INSERM, Centre de Recherche-UMR866, Faculté de Médecine, Institut Fédératif de Recherche Santé-STIC, Université de Bourgogne, 21079 Dijon, France.
d: to whom correspondence should be addressed: 630 West 168th Street, P&S 8-401, New York NY 10032 United States, e-mail: dm2561@columbia.edu or david.masson@chu-dijon.fr, Tel: 1 212 305 5789
Abstract

The plasma lipid transfer proteins promote the exchange of neutral lipids and phospholipids between the plasma lipoproteins. Cholesteryl ester transfer protein (CETP) facilitates the removal of Cholesteryl esters (CE) from HDL and thus reduces HDL levels, while phospholipid transfer protein (PLTP) promotes the transfer of phospholipids from triglyceride-rich lipoproteins into HDL and increases HDL levels. Studies in transgenic mouse models as well as in humans with rare genetic deficiencies (CETP) or common genetic variants (CETP, PLTP) highlight the central role of these molecules in regulating HDL levels. Human CETP deficiency is associated with dramatic elevations of HDL cholesterol and apoA-1 levels, while PLTP variants with increased expression are associated with higher HDL levels. A recent meta-analysis suggests that common CETP alleles causing reduced CETP and increased HDL levels are associated with reduced Coronary Heart Disease (CHD). The failure of a clinical trial with the CETP inhibitor torcetrapib may have been related in part to off-target toxicity. Ongoing phase 3 clinical trials with other CETP inhibitors may help to clarify if this strategy can ultimately be successful in the treatment of atherosclerosis.
**CHOLESTERYL ESTER TRANSFER PROTEIN**

**Discovery and characterization of CETP:** More than 40 years ago in this journal, Nichols and Smith described a factor in human plasma that was able to stimulate the reciprocal exchange of triglycerides and cholesteryl esters between lipoprotein subclasses (1). The first biochemical characterization of a plasma lipid transfer protein came 13 years later (2). Complete purification of CETP (3) and the subsequent cloning of the CETP cDNA (4) were achieved in 1987. These studies indicated that mature CETP is a 476 amino acid protein (74Kda) with a highly hydrophobic aminoacid content and four N-linked glycosylation sites. Neutralizing CETP monoclonal antibodies (mAbs) showed that this molecule was responsible for all CE and triglyceride (TG) transfer activity in human plasma and that inhibition of activity in rabbits resulted in increased HDL levels and a reduced content of CE in VLDL (5). Shortly after, the elucidation of human genetic deficiency of CETP established the key role of this molecule in human lipoprotein metabolism (6). Recently, the crystal structure of CETP revealed an elongated boomerang shaped molecule with the curvature of the concave surface likely fitting to the convex curvature of the lipoprotein surface (7) (Figure 1). A unique 60-Å-long hydrophobic tunnel with two distinct openings traverses the core of the protein. This tunnel can be filled with two cholesteryl ester molecules and plugged by two phospholipid molecules at each end. It has been proposed that upon CETP binding at the lipoprotein surface, phospholipids bound at the mouth of the tunnel merge into the phospholipid monolayer and allow neutral lipids to enter and exit the tunnel. A C-terminal amphipathic helix (Fig 1, black arrow) recognized by the neutralizing CETP mAb (5) is situated at the mouth of the N-terminal entrance to the lipid binding tunnel and may play a role in facilitating entry of neutral lipid into CETP. The CETP structure is consistent with the earlier findings that CETP binds neutral lipids and shuttles them between plasma lipoproteins in a carrier-mediated mechanism (8).

**Role of CETP in lipoprotein metabolism:** In humans and monkeys, CETP is expressed in liver (parenchymal and nonparenchymal cells), small intestine (enterocytes), adipose tissue and spleen (4). There is also significant expression of CETP in macrophages and bone marrow transplantation experiments using as donors mice expressing the human CETP transgene under the control of its natural promoter suggest that macrophages make a significant contribution to plasma CETP levels (9). CETP gene expression is stimulated by
dietary cholesterol and endogenous hypercholesterolemia as a result of activation of liver X receptor (LXR)/Retinoid X receptor (RXR) transcription factors bound to the proximal promoter of the human CETP gene (10). In human plasma, CETP is present at concentrations of about 2 µg/ml, with moderately higher levels in dyslipidemic subjects, and is mainly associated with high density lipoproteins (5). Plasma cholesteryl ester transfer activity is dependent on both CETP concentration and the ability of CETP to interact with lipoproteins. This interaction can be stimulated by free fatty acids generated during hydrolysis of dietary TGs (5) or inhibited by specific apolipoproteins such as apo C-I or apo F (11, 12). Due to the mechanism of lipid transfer, CETP can only promote the net mass transfer of lipids between lipoprotein subclasses that have different CE/TG ratios. Therefore CETP facilitates the transfer of cholesteryl esters from CE-rich LDL and HDL towards VLDL. CETP promotes the reciprocal enrichment of LDL and HDL with TGs derived from VLDL (5). Unlike CEs, TGs can be hydrolysed in the plasma compartment through the action of lipases. Subsequent hydrolysis of TG in LDL promotes their remodelling with the generation of small dense LDL with a smaller neutral lipid core. A similar mechanism also occurs in HDL, and hydrolysis of large-triglyceride-enriched HDL generates smaller HDL3 together with the release of lipid poor apolipoprotein A-I (5).

Role of CETP in atherosclerosis

Predictions from lipoprotein physiology: By transferring CEs from HDL toward apo B containing lipoproteins, CETP decreases the concentration of HDL cholesterol and apoA-1 and increases the concentration of CE in VLDL and remnants. In addition, CETP activity raises levels of LDL cholesterol and apo B, most likely due to down-regulation of hepatic LDL receptors (5). Small, dense LDLs generated by CETP and lipases may be particularly atherogenic because of increased affinity for artery wall proteoglycans and increased susceptibility to oxidation. In contrast to these pro-atherogenic effects, the remodelling of HDL particles by CETP is accompanied by the release of lipid-poor apoA-1 which is the preferential acceptor for ABCA1 mediated cholesterol efflux from macrophage foam cells. However, this effect may be offset by a decrease in large HDL particles that promote cholesterol efflux in part via the ABCG1 pathway (13). In the steady state, CETP activity appears not to change the overall efficiency of reverse cholesterol transport, though the lipoproteins mediating reverse cholesterol transport are likely different i.e. primarily remnants and LDL in the presence of CETP activity, and HDL in CETP deficiency. Any beneficial
effect of CETP inhibition likely accrues from decreased cholesterol uptake and increased cholesterol efflux in macrophage foam cells and in vascular cells of atherosclerotic plaques (14).

**Animals studies:** Interestingly, CETP is not present in all animal species. Introduction of the human CETP gene in transgenic mouse models has led to varied effects on atherosclerosis. CETP expression increased atherosclerosis in hypercholesterolemic mouse models such as apo E and LDL-Receptor deficient mice (15, 16), and in a mouse model of mixed hyperlipidemia expressing apoE(Leiden) (17). In contrast, in hypertriglyceridemic apo CIII Tg mice expression of CETP can be either non- or anti-atherogenic (18). In mice CETP activity appears to be pro-atherogenic when it causes both reduced HDL levels and increased levels of CE in remnants and LDL, while it is non-atherogenic in a setting of hypertriglyceridemia and prominent accumulation of small lipid-poor, apoA-1 rich HDL particles.

**Human studies :**

**Genetic CETP deficiency:** Genetic CETP deficiency was discovered in Japanese families with increased HDL levels (6, 19). Homozygous deficient subjects display dramatic increases in HDL-C (approx. + 100-200%) as well as decreases in LDL-C and apoB levels (approx. -40%). HDL from homozygous CETP deficient subject are very large and enriched in apo E and show an enhanced ability to promote cholesterol efflux from macrophages in part through the ABCG1 pathway (13). LDL particles from CETP deficient subjects are heterogeneous in size and display a reduced affinity for the LDL receptor. In a cross-sectional survey of Japanese/American men, heterozygotes for CETP gene defects had an increased risk for coronary heart disease (20). However, this finding was not confirmed in a subsequent prospective study in the same population, in which a trend to a lower incidence of stroke and CHD was apparent in men with heterozygous CETP deficiency (21).

**CETP polymorphisms :** Although many studies have demonstrated associations between CETP single-nucleotide polymorphisms in Caucasian populations and small changes in plasma CETP and HDL concentration, the relationship between these polymorphisms and susceptibility to atherosclerotic CVD has been inconsistent. A recent meta-analysis of three different snps in the CETP gene (two of them in linkage disequilibrium) in 113,833 subjects including 27,196 cases of CHD showed a significant or borderline significant reduction in CHD for the CETP alleles associated with lower CETP and higher HDL levels (22) (Fig 2A). The protective effect of HDL elevation associated with lower CETP levels was similar to that afforded by HDL elevation in prospective epidemiological studies. One caveat to the
conclusions of this meta-analysis is that findings may have been influenced by publication bias. Recently a genotype score of nine validated SNPs that are associated with modulation in levels of LDL or HDL cholesterol, including CETP TaqIB, was found to be an independent risk factor for incident cardiovascular disease and in this analysis HDL and LDL associated SNPs acted independently (23). Overall, the evidence linking HDL-associated SNPs with CHD is weaker than that for LDL, consistent with the idea that LDL is the cause of atherosclerosis while HDL is a modifying factor. Notably, these studies show either no effect of CETP genetic variants on CHD, or a protective effect, but there is no consistent relationship linking reduced CETP levels to increased CHD.

**CETP inhibitors:** All CETP inhibition strategies have been effective at increasing HDL levels and decreasing atherosclerosis in rabbits (24). Clinical trials in humans using CETP inhibitors such as torcetrapib have shown marked increases in HDL, and moderate reductions in LDL (25). Unfortunately, a large phase III clinical trial called ILLUMINATE involving torcetrapib, was stopped prematurely as a result of an excess of deaths and cardiovascular disease in the group receiving torcetrapib and atorvastatin compared to atorvastatin alone (26). The reasons for the adverse outcome are uncertain. Torcetrapib administration was associated with a number of undesirable off-target effects that could have contributed to increase mortality/morbidity (Fig 2B). They included increases in blood pressure, sodium, bicarbonate and aldosterone levels as well as a decrease in potassium levels (26). It seems that these adverse effects were molecule specific and not related to CETP inhibition (27). The mechanism of hypertension appears to result in part from an increased production of adrenal steroids including aldosterone and cortisol (27). Post hoc analysis of the ILLUSTRATE study, showed that while the majority of torcetrapib-treated patients demonstrated no regression of coronary atherosclerosis, a significant regression of coronary atherosclerosis was observed in patients in the highest HDL-C quartile. In this post hoc analysis there was a continuous inverse relationship between HDL levels and the percent atheroma volume (28). Moreover, this relationship was only clearly seen in the group receiving the CETP inhibitor, strongly suggesting that in patients achieving high HDL levels, HDL particles were functional in promoting regression of atherosclerosis.

Two other CETP inhibitors, anacetrapib (Merck) and dalcetrapib (Roche) are in advanced clinical studies (29, 30). Unlike torcetrapib these agents do not appear to cause hypertension. Dalcetrapib has a distinct mechanism of action compared to anacetrapib and torcetrapib and likely covalently modifies a Cys-H group in the N-terminal part of the lipid binding tunnel of CETP. Anacetrapib is a more potent CETP inhibitor than dalcetrapib and produces larger
effects on HDL and LDL levels. Ongoing phase 3 clinical studies with various CETP inhibitors may help to determine if the addition of CETP inhibitors to statins can lead to a reduction in atherosclerotic CVD.

PHOSPHOLIPID TRANSFER PROTEIN

**Discovery and characterization of PLTP:** Earlier biochemical studies showed that a second lipid transfer protein distinct from CETP was present in human plasma (31). This protein was unable to transfer neutral lipids between LDL and HDL, but unlike CETP promoted net mass transfer of phospholipids from phospholipid vesicles or lipolyzed VLDL particles into HDL (32). Cloning of the cDNA revealed that PLTP is a 476 amino acid protein (Mr 81 Kda) with six N-linked glycosylation sites (33). PLTP displays an approx. 25 % amino acid identity with CETP and with two other proteins, the lipopolysaccharide (LPS) binding protein (LBP) and the bactericidal permeability increasing protein (BPI), involved in the defense of the organism against LPS from gram negative bacteria. The four proteins comprise the lipid transfer/LPS binding family. They also share significant homology with the PLUNC proteins that could be involved in the local innate immune response against bacteria in oral, nasal and respiratory epithelia (34). Similar to CETP, PLTP probably acts as a carrier that shuttle phospholipids between lipoprotein particles. In addition to phospholipids, PLTP is able to transfer other amphipatic compounds such as free cholesterol, LPS and vitamin E (35, 36).

**Role of PLTP in lipoprotein metabolism:** In contrast to CETP, PLTP is widely expressed in organs and cells (33). High levels of PLTP mRNA are especially seen in the brain, the lung and the gonads suggesting specific functions of PLTP in these organs. PLTP gene expression is controlled by nuclear receptors such as Farnesoid X receptor (FXR) and LXR (37). In addition to promoting transfer of phospholipids from VLDL and chylomicrons into HDL (38), PLTP may contribute to the remodelling of HDL particles. In vitro studies suggest that when the lipid composition of a particle is altered by phospholipid transfer, its apolipoproteins are destabilized. This induces the fusion of two remnant particles and in the loss of an apo AI molecule (39). Thus PLTP activity may contribute to generation of large alpha HDL as well as pre beta HDL particles. Vitamin E transfer mediated by PTLP is also important in lipoprotein metabolism, since PLTP contributes to decrease the vitamin E content of circulating lipoproteins and to increase their oxidability (36). Alteration of vitamin E content
of liver may also contribute to regulate apo B lipoprotein production, perhaps by influencing ROS generation and efficiency of insulin signaling in hepatocytes (40). In addition, PLTP may also work within cells to add lipid to nascent apo B thus limiting apoB degradation and increasing VLDL production (41).

PLTP and atherosclerosis:

**Animals models:** In contrast to CETP, PLTP is present in all animal species and is expressed at high levels in the mouse. In human apoAI transgenic mice, PLTP overexpression resulted in moderate increase in HDL cholesterol and apo A-I as well as a more pronounced increase in pre beta-HDL (42). Gene knock-out of PLTP resulted in about 50% reductions of apo A-I and HDL cholesterol and phospholipids levels (38). Surprisingly, in apoB-transgenic and apoE-deficient backgrounds, PLTP deficiency resulted in markedly decreased atherosclerosis. This was explained by a decrease in the production and levels of apoB-containing lipoprotein, an increase in their vitamin E content and a decrease in their susceptibility to oxidation (36, 41). Reduction of cholesterol absorption may have also contributed to the protective effect of PLTP deficiency (43). In PLTP transgenic mouse models, PLTP overexpression increased atherosclerosis susceptibility (44). In fact, the effects of PLTP on atherosclerosis probably result from a balance between systemic deleterious effects and local protective effects. As underscored by recent bone marrow transplantation studies, local PLTP expression in macrophages could be protective as long as systemic PLTP levels are not markedly elevated (45, 46).

**Human studies:** To date no genetic deficiency has been reported for PLTP. Importantly, two recent studies have identified snps near the PLTP gene that are associated with HDL (47) and also with TG levels for one variant (48). Interestingly, the variant associated with higher HDL and lower TGs also correlated with higher PLTP transcript levels in the liver (48). These are the first evidences for a direct link between PLTP and lipoprotein levels in humans. The effects on HDL levels are consistent with the transgenic mouse studies (38, 42) but the apparent lowering of TG levels in association with increased PLTP expression was not predicted.

**Future directions:** Clinical trials involving CETP inhibitors with minimized toxic off-target side-effects will likely show whether this strategy can be successfully used in the treatment of human atherosclerosis. However, major challenges remain concerning optimal dosage, selection of patient groups who may benefit from this therapy and the difficulty of showing
incremental effects of adding a new therapy to potent statins. While the recent GWA studies have indicated a role of PLTP in human lipoprotein metabolism, the elucidation of loss of function mutations would be a major step toward the elucidation of the role of this protein in human lipoprotein metabolism and atherosclerosis.

Acknowledgments: We are grateful to Dr. Xiayang Qiu, who provided the 3D CETP structure. D.M. is supported by a grant from the Philippe Foundation.
References


Figures legends

**Figure 1:** Structural model of human CETP (courtesy of Dr. Xiayang Qiu, Pfizer inc.). N-terminal domains are in green, C-terminal domains are in yellow, linker in red. The two cholesteryl ester molecules are in magenta and cyan and phospholipid are represented as black bonds. CETP displays an elongated boomerang shape with the curvature of the concave surface fitting to the convex curvature of the lipoprotein surface. A unique hydrophobic tunnel filled with two cholesteryl ester molecules traverses the core of the protein. This tunnel is plugged by two phospholipid molecules at each end. A C-terminal helix (black arrow) is situated at the mouth of the N-terminal entrance to the hydrophobic tunnel, and was previously identified as the target of the CETP neutralizing antibody TP-2.

**Figure 2:**

**A:** Observed Per-Allele Odds Ratios for Coronary Disease With CETP Variants vs Odds Ratios Derived From Prospective Studies of HDL-C Levels. A significant reduction in CHD is observed for the CETP alleles associated with lower CETP and higher HDL levels. Interestingly, the protective effect of HDL elevation associated with lower CETP levels is similar to that afforded by HDL elevation in prospective epidemiological studies.

**B:** ILLUMINATE study was stopped prematurely as a result of an excess of deaths and cardiovascular disease in the group receiving torcetrapib. Potential adverse effects of torcetrapib include off-target effects such as increases in blood pressure, sodium, bicarbonate and aldosterone levels as well as a decrease in potassium levels. However mechanism-related adverse effects cannot be ruled out. Beneficial effects would include increase in cholesterol efflux via ABCG1 resulting in decrease foam cell formation and a decrease coronary atherosclerosis. In the ILLUSTRATE study there was an inverse relationship between change in HDL and change in % atheroma volume in the group receiving torcetrapib.
Variant

\( TaqIB \) (rs708272)

\( I405V \) (rs5882)

\(-629C>A \) (rs1800775)

**Overall odds ratio (95% CI)**

\[ \text{Per-allele odds ratio for coronary disease associated with CETP variants in the current analysis} \]

\[ \text{Odds ratio for observed per-allele increase in HDL-C levels in prospective studies} \]

**Thompson, A. et al. JAMA 2008;299:2777-2788**

Copyright © (2008) American Medical Association. All rights reserved

**Figure 2**

**BENEFICIAL EFFECTS**

↑ Cholesterol efflux via ABCG1

↓ Macrophage foam cells

↓ Coronary Atherosclerosis

↑ Blood pressure (↑ Aldosterone, ↓ Potassium)

\{ Off Target \}

**MECHANISM-RELATED **

↑ Death from sepsis?

Other effects?

**ADVERSE EFFECTS**

TORCETRAPIB