CETP deficiency and concerns in CETP inhibitor development

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Abstract

Although some CETP-lowering SNPs in the CETP gene are variably associated with improved clinical outcomes in patients with coronary artery disease (CAD), three low molecular weight oral CETP inhibitors (torcetrapib, dalcetrapib, and evacetrapib) have not shown any clinical benefits despite remarkable increases in HDL-cholesterol levels ranging 31-138%. Potential causes for that failure are discussed in terms of off-target effects of some inhibitors, potential misleading interpretation of genetic epidemiological surveys in CETP gene, and inadequate alteration in HDL function induced by the inhibitors. Possible modalities of CETP inhibition such as antisense nucleotides and monoclonal antibodies are discussed.

Key words
Age-associated macular degeneration (AMD)
Cholesteryl ester transfer protein (CETP)
Coronary artery disease (CAD)
D442G Polymorphism
Hyperalphalipoproteinemia
Lipoprotein(a) [Lp(a)]
Proprotein convertase subtilisin/kexin 9 (PCSK9)
Pharmacogenetics
Small dense LDL (sdLDL)
Single nucleotide polymorphism (SNP)

1. Introduction

Since the first failure to develop a CETP inhibitor, subsequent clinical trials of CETP inhibitors have been discontinued unexpectedly; torcetrapib was terminated in December 2006, and dalcetrapib was also terminated in May 2012 prematurely. Recently the phase 3 trial of evacetrapib in patients with high-risk atherosclerotic cardiovascular disease (ACCELERATE) was terminated in October 2015 due to insufficient efficacy. One remaining phase 3 trial of anacetrapib is still in progress. The effects on lipoproteins of anacetrapib appeared to be similar to those of evacetrapib (1).

These trials have demonstrated the difficulty in the development of CETP inhibitors in relying on the changes in plasma lipids and the classical theory of low LDL-cholesterol (LDL-C) and high HDL-cholesterol (HDL-C) by CETP deficiency. In support of these results, a meta-regression analysis of 51 trials in secondary prevention of coronary artery disease (CAD) showed no significant association between changes in HDL-C and risk ratios of clinical endpoints (2). Thus, simple measurement of HDL-C itself is not a good biomarker for morbidity and mortality due to CAD and strokes in clinical trials of CETP inhibitors. Potential role of HDL function assay will be discussed in this chapter.

2. New findings in Function and Regulation of CETP

CETP could promote reverse cholesterol transport as long as the LDL receptor and other receptors are up regulated as shown in transgenic mice. Thus, in circumstances leading to lower LDL-C levels, CETP activators may be beneficial for atherosclerosis prevention. Liver X receptor (LXR) is known as a strong nuclear factor inducing CETP gene expression. Etoposide, a DNA topoisomerase II inhibitor as used in cancer chemotherapy, is an inducer of CETP via LXRα (3). In addition, farnesoid X receptor (FXR) is also an activator for CETP gene expression (4).

In addition to plasma lipid transfer/exchange activity, CETP may have an

intracellular function of inter-organelle cytosolic lipid transfer activity. A reduction of TG storage was shown in CETP-over-expressing SW872 adipocytes, which is compatible with a small, active adipocyte phenotype (5). As discussed later, the high-CETP adipocyte phenotype may be advantageous for weight reduction on a low carbohydrate diet.

3. Coronary artery disease (CAD) and age-associated macular degeneration (AMD) disease risk in association with CETP gene (Table 1)

In the large prospective cohort studies, protective effects on CAD were expected in the lower CETP and higher HDL alleles of TaqIB2 (intron 1, +279G>A rs708272) and -629C>A (rs1800775) including the Women's Genome Health Study and the Copenhagen City Heart Study (6,7).

The effect of the TaqIB allele was recently evaluated with a meta-analysis using a Mendelian randomization approach. However, the association was between low CETP- high HDL and a reduction in CAD risk was marginally significant (8). It appears that the CAD risk reduction due to TaqIB polymorphism is strengthened in populations having low smoking rates or low HDL-C levels. In such a meta-analysis, ethnical differences and population characteristics (whether it is community or hospital based) are important, since a hospital-based population tends to be enriched for previous vascular disease and statin use.

In a genome-wide approach several potentially important SNPs were identified (9). Using the upstream promoter SNPs such as -2568 C>A or G>T (rs3764261) and -2708G>A (rs12149545) as well as TaqIB, it was not confirmed that low CETP allele was protective against CAD in the secondary prevention setting (10). Moreover, reduced protection against MI by statin was associated with in the low CETP allele (11). Thus, in these circumstances, selection bias of excess inclusion of cases with high CETP genotype in the CAD category is possible. Also, a positive pharmacological interaction between statin use and a high CETP genotype needs to be considered, since patients with TaqIB2, a low CETP genotype, is resistant to statin therapy as shown in the REGRESS cohort (12).

Similarly, low CETP levels are pro-atherogenic in several studies of secondary prevention and/or statin treatment.

Although the Framingham Heart Study showed that low CETP-high HDL due to TaqIB2 was protective against CAD, lower CETP activity was associated with increased CAD risk in a subsequent study (13). Thus, it is important to define causes of lower CETP activity, which is by genetic or environmental. Smoking, infection, and diabetes are potential causes lowering CETP activity. In the Asian Indian Diabetic Cohort, -2568C>A was associated with increased HDL levels as well as hypertension. Also, low CETP activity was associated with increased risk of CAD in this cohort. Additionally, the Suita Study reported that rs3764261 manifests increased HDL-C levels but increased CAD risk in the Japanese (14). In that study, the minor allele of A was related with increased HDL-cholesterol levels, but it was more prevalent in cases with MI (OR=1.13, p=0.02). However, such an association was not replicated in another study (15).

Exon 9 is known as the alternative cassette exon, so mRNA splicing out of the sequence encoded by the exon 9 would result in inactive protein formation. Papp et al demonstrated that two SNPs (rs9930761 and rs5883) in intron 8 and exon 9 were determinants for efficacy of alternative splicing in the human liver, the former SNP is located in the branch site of intron 8, and the latter SNP altering the exonic splicing enhancer sequence (16)(Table 1). The rare combination of SNPs (rs5883T/rs9930761C) was associated with increased HDL-C levels, but also with increased incidence of MI, stroke, and all-cause mortality in males in the secondary prevention cohort of INVEST (see discussion of the TaqIB study). The rs5883T SNP increased the Δexon 9 transcript, resulting in lower CETP activity, as shown in transfection studies using the exons 8-10 mini-gene construct in HepG2 and HEK293 cells (17).

The D442G allele (exon 15 A>G, rs2303790) was a risk factor for AMD in East Asians (18), but it appeared to be protective for CAD (19). D442G showed the strongest association with HDL-C, while D allele showed a tendency to associate with CAD (OR=1.20, p=0.02) (15). It is likely that the apoE2 isoform

works as a risk for AMD, but it is protective factor for CAD. On the contrary, apoE4 is a protective factor for AMD, but it is a risk factor for CAD. Although retinal drusen and arterial plaques are both contain lipoproteins, the orientation from vascular lumen was opposite; drusen progression towards the retinal pigment epithelium is away from the choriocapillary vessels, but plaque progression was towards the vascular lumen in atherosclerosis (20). We observed a female in the seventies with complete CETP deficiency and no AMD (unpublished observation). Thus, any relationship between AMD and CETP deficiency needs to be established.

In the Omagari study, where the Japanese type mutation of intron14 G (+1)>A was enriched, a case-control study was examined in a voluntary subset of population with hyperalphalipoproteinemia with HDL-C > 100 mg/dL (21). In that study, hyperalphalipoproteinemia with lower CETP mass (1.7 mg/L vs. non-CETP deficiency 2.8 mg/L) was associated with cardiovascular disease and ischemic strokes, particularly in females. Since the CETP-deficient hyperalphalipoproteinemic group included 2 homozygotes and 69 heterozygotes, that finding would reflect clinical characteristics of heterozygous CETP deficiency caused by intron14 G(+1)>A mutation. This is contrast with the previous findings in Honolulu Heart Study in which D442G heterozygotes were predominant (22).

4. Role of CETP activity in adipocyte, metabolic syndrome, and diet therapy for weight reduction

A diet-lipid interaction was found in between the -2568CC genotype and a low carbohydrate, high fat weight-loss diet (23). In that study, the CC genotype, associated with higher CETP activity, had lower baseline HDL-C levels and increased HDL-C response and decreased TG.

These data suggested that a high CETP genotype might improve the plasma lipoprotein profile during body weight reduction on a low carbohydrate diet. In

contrary, the low CETP genotype of I405V was associated with visceral fat accumulation in response to overfeeding (24). CETP may promote intracellular TG transport, because cytosolic lipid transfer activity has been proposed as a CETP function.

5. HDL function and anti-inflammatory effects in CETP deficiency and CETP inhibitor (Figure 1)

The preβ1HDL level was increased in the anacetrapib treatment of dyslipidemic hamsters (25), which is compatible with findings in human with homozygous CETP deficiency but not in heterozygotes. Evacetrapib increased cholesterol efflux capacity and preβ1HDL levels in dyslipidemic patients (26). However, a recent simulation study of HDL remodeling demonstrated that a CETP inhibitor of RG7232 could not increase preβ1HDL levels (27).

CETP deficiency produces large HDL2 particles, but no significant effects on HDL3. HDL2 could be good acceptor of cholesterol efflux capacity via SR-BI in Fu5AH cells (28), whereas HDL3 is an acceptor of cholesterol efflux via ABCA1 in LXR agonist treated J774 cells. HDL-TG levels and the HDL-TG/PL ratio were negatively correlated with cholesterol efflux activity in Fu5AH cells (29). Thus, low HDL-TG seen in CETP deficiency or patients with CETP inhibitors may be beneficial for cholesterol efflux activity via SR-BI and ABCG1 receptors.

Anti-oxidative activity was maintained in HDL of heterozygous CETP deficiency (30). HDL3-S1P level had been reduced, and eNOS activation was impaired in CETP deficiency. However, HDL2 from CETP deficient patients were more effective than control HDL2 in inhibiting VCAM-1 expression (31). It is shown that HDL inhibited TNF α -induced MCP-1 secretion, monocyte adhesion and NF- κ B activation in endothelial cells in the anacetrapib treated hamsters (32).

Overall, CETP inhibitors did not reduce CRP levels despite significant reduction of LDL and Lp(a) levels. Similarly, serum SAA levels were increased by the treatment of anacetrapib and atorvastatin in APOE*3Leiden.CETP mice (33). It

is important to know whether the inflammation is correlated with any of HDL function and what is the mechanism inhibiting antioxidant activity of HDL when CETP inhibitors are used. However, it is possible that HDL lost its antioxidant activity as a result when HDL protected LDL from its oxidation.

In a post hoc analysis of the MEGA study of low-dose pravastatin in the Japanese, mild hyperalphalipoproteinemia (HDL-C 60 - 89 mg/dL), was a protective factor against CAD (Hazard ratio 0.48) as compared with HDL-C <60 mg/dL, but conclusive evidence for a reduction of CAD was not obtained in marked HALP in which HDL-C levels are greater than 90 mg/dL (34). Two cases with vasospastic angina were found in the marked hyperalphalipoproteinemic group (n=239). The role of this condition and unidentified other confounding factors must be considered in the HDL-epidemiological survey.

6. Small and dense LDL, Lp(a) and proprotein convertase subtilisin/kexin 9 (PCSK9) levels in CETP deficiency and CETP inhibitors (Figure 2)

CETP inhibition increases HDL levels but also decreases LDL levels. One of the potential causes of lowering LDL is reduction of PCSK9 levels as shown in the studies of anacetrapib and a new small CETP inhibitor (K-312) (35, 36). Anacetrapib reduced plasma PCSK9 levels by -19% (37). It appears to be mediated by reduced levels of mature form of SREBP2 (38).

Since low CETP activity decreased hetero-exchange of CE and TG between HDL and LDL, leading to less TG in LDL, therefore small and dense LDL (sdLDL) was predicted to be an unlikely product after the TG lipolysis by hepatic lipase activity. On the contrary, high CETP activity produces more sdLDL. However, a recent study has suggested that while the anacetrapib decreased plasma levels of VLDL-IDL-LDL, but the very small and dense LDL4b fraction was rather increased (39). The mechanism for the discrepancy is not known, however, the smallest LDL4b fraction may be remained in the plasma because of lack of CE addition from HDL.

Anacetrapib reduced Lp(a) levels by ~50% but not CRP levels (40). In vitro studies, Lp(a) catabolism can be regulated by PCSK9 through the LDL receptor (41). However, Lp(a) levels were not significantly decreased in CETP-lowering SNPs (7). It has been suggested that plasma Lp(a) levels are low in homozygous CETP deficiency. However, Lp(a) lowering effect has not been clearly demonstrated in heterozygous CETP deficiency yet.

The relationship between CETP, HDL, and LDL suggested that CETP might be a modifier gene for atherogenecity via lipoprotein levels and their functions. Large LDL and HDL seen in CETP deficiency are anti-atherogenic in the primary prevention in almost all studies, however, such an association was less clear in the secondary prevention or in statin users as discussed above. Mixed results in the secondary prevention offer more extensive studies involved functional assays of HDL and CETP genotyping in the near future.

7. Effects on reverse cholesterol transport of CETP inhibitors

Although anacetrapib promotes reverse cholesterol transport and bulk cholesterol excretion in hamsters, some studies suggested that CETP inhibition alone dose not stimulate reverse cholesterol transport (42,43). As suggested, additional effects mediated by the LDL-lowering drug berberine are required (44). Berberine induced LDL receptor production mediated by inhibition of PCSK9 transcription, which was made thorough ubiquitin-proteasome degradation of hepatocyte nuclear factor 1α (45).

8. Off-target effects and vascular effects of CETP inhibitors

Torcetrapib increased aldosterone levels via CYP11B2 (46). However, endothelin-1 was also increased by torcetrapib. Endothelin-1 could be another cause of excess aldosterone, because endothelin-1 is a potent stimulate of aldosterone secretion from adrenal glands (47). However, despite no obvious off-target effects in dalcetrapib and evacetrapib, recent failures of clinical trials would suggest that an unknown problem of CETP inhibiton itself (48). Indeed,

dalcetrapib did not reduce the risk of recurrent cardiovascular events despite moderately increased HDL levels, but adverse findings of increased blood pressure and CRP levels were simultaneously found (49). As described, the failure of evacetrapib was not explained by the current HDL hypothesis, because the inhibitor indeed increased cholesterol efflux capacity and preβ1-HDL levels (26).

The failure of dalcetrapib was subsequently evaluated by genome-wide approach. Interestingly, the cardiovascular effect was varied by SNPs in the ADCY9 gene, an adenylate cyclase gene located in chromosome 16 (50). The AA genotype was associated with decreased of cardiovascular events, but the GG was on the contrary increased in the dalcetrapib treatment group. ADCY9 may determine cAMP levels through activating β-adrenergic receptors, possibly involving ABCA1 efflux activity via cAMP.

9. New therapy inhibiting CETP activity by other than low molecular weight compounds and selective TG transfer inhibition

Current CETP inhibitors may have a common disadvantage in increasing CETP mass in HDL. In contrast, a naturally CETP deficient phenotype is expected in the antisense oligonucleotide. Antisense oligonucleotide inhibited CETP mRNA could enhance macrophage RCT, resulting in increase in fecal cholesterol excretion and decreased TG secretion in liver of hyperlipidemic, CETP transgenic, LDLR KO mice (51).

A more interesting approach might be manipulating substrate preference of CETP. Neutral lipid preference of CETP is varied among animal species and in human missense SNPs. D442G, the most common SNP in the Asian population, has lower preference for TG relative to CE as a substrate for lipid transfer, resulting in less TG gain from VLDL and more CE loss from HDL. Therefore, the mild CETP deficient phenotype tends to produce higher VLDL and lower HDL than that expected (52).

Indeed, the human TP2 monoclonal antibody (mAb) and the rabbit 14-8F mAb against the C-terminus of CETP inhibited TG transfer greater than CE transfer (53). The mAb therapy would be expected to prefer CE transfer relative to TG. Indeed, vaccine therapy against CETP may be promising because it appears to be selective TG transfer inhibitor and beneficial effects of that inhibition were expected from the D442G SNP studies and the rabbit vaccine study (54).

Increased apoA-I was expected to result from delayed catabolism of apoA-I by CETP inhibitors. However, other mechanisms for increasing apoA-I via apoA-I production have been described. RVX-208, a selective binder to the BET domain protein produces its effect by an epigenetic pathway, and perhexiline produces its effect by modulation of Krüppel-like factor 14 (55). With these mechanisms, HDL size would be predicted to be smaller than that seen in CETP inhibitors.

10. Conclusion

Torcetrapib appeared to be failed due to off-target effects on blood pressure by excess aldosterone despite increased HDL levels. Since dalcetrapib is a weaker inhibitor, therefore no effect was expected on LDL and Lp(a). It appeared that beneficial effects by HDL were offset by some detrimental factor in dalcetrapib. Again, evacetrapib is a potent inhibitor producing the expected lipoprotein phenotype, but the recent failure of its phase 3 clinical trial suggested using increased HDL and decreased LDL levels are not useful as predictors of clinical efficacy. The REVEAL, clinical trial of anacetrapib is still in progress, and lipoprotein changes are expected to be similar to those by evacetrapib. If anacetrapib is clinically ineffective, genome-wide pharmacogenetic approach would be important to determine the cause of the failure in clinical trials.

Accumulating evidence suggested that the current approach of CETP inhibition is pessimistic, but several directions remain to be explored for altering the target, including TG specific transfer inhibition or inhibitors affecting CETP production and translation by antisense oligonucleotide or mAb. These possibilities definitely need more investigation.

Confounding results have been seen in epidemiological survey of low CETP activity, which may be attributable to variable causes of low CETP activity, namely genetic or environmental. Indeed, inflammatory cytokines would decrease CETP expression levels as well as apoA-I. Therefore, low CETP activity does not always result in high HDL levels in some studies. More research is needed to disclose what the phenotype of low CETP but normal HDL levels means.

Importantly, CETP genetic epidemiological study has provided variable associations with clinical endpoints so far, perhaps depending in part on the setting of primary or secondary prevention of CAD. Additional data and careful analyses are still needed to clarify possible population-specific bias. Such research might require subclass analysis of LDL and HDL and assessment of

the HDL function as well as information of the statin use.

References

1. Mabuchi H, Nohara A, Inazu A. Cholesteryl ester transfer protein (CETP) deficiency and CETP inhibitors.
Molecules and Cells, 37(11):777-84, 2014

2. Hourcade-Potelleret F, Laporte S, Lehnert V, Delmar P, Benghozi R, Torriani U, Koch R, Mismetti P. Clinical benefit from pharmacological elevation of high-density lipoprotein cholesterol: meta-regression analysis. Heart, 101:847-853, 2015

3. Liu M, Chen Y, Zhang L et al. Regulation of hepatic cholesteryl ester transfer protein expression and reverse cholesterol transport by inhibition of DNA topoisomerase II.
J Biol Chem, 290:14418-14429, 2015

4. Gautier T, de Haan W, Grober J et al. Farnesoid X receptor activation increases cholesteryl ester transfer protein expression in humans and transgenic mice.
J Lipid Res, 54:2195-2205, 2013

5. Izem L, Greene DJ, Bialkowska K, Morton RE. Overexpression of full-length cholesteryl ester transfer protein in SW872 cells reduces lipid accumulation.
J Lipid Res, 56:515-525, 2015

6. Ridker PM, Pare G, Parker AN, Zee RYL, Miletich JP, Chasman DI. Polymorphism in the CETP gene region, HDL cholesterol, and risk of future myocardial infarction. Genomewide analysis among 18245 initially healthy women from the Women's Genome Health Study.
Circ Cardiovasc Genet, 2:26-33, 2009

7. Johannsen TH, Frikke-Schmidt R, Schou J, Nordestgaard BG, Tybjaerg-Hansen A. Genetic inhibition of CETP, ischemic vascular disease and

mortality, and possible adverse effects.
Journal of the American College of Cardiology, 60:2041-8, 2012

8. Wu Z, Lou Y, Qiu X, Liu Y, Lu L, Chen Q, Jin W. Association of cholesteryl ester transfer protein (CETP) gene polymorphism, high density lipoprotein cholesterol and risk of coronary artery disease: a meta-analysis using a Mendelian randomization approach.
BMC Medical Genetics, 15:118, 2014

9. Willer CJ, Sanna S, Jackson AU et al. Newly identified loci that influence lipid concentrations and risk of coronary artery disease.
Nature Genet, 40:161-169, 2008

10. Corsetti JP, Ryan D, Rainwater DL, Moss AJ, Zareba W, Sparks CE. Cholesteryl ester transfer protein polymorphism (TaqIB) associates with risk in postinfarction patients with high C-reactive protein and high-density lipoprotein cholesterol levels.
Arterioscler Thromb Vasc Biol, 30:1657-1664, 2010

11. Leusink M, Onland-Moret NC, Asselbergs FW et al. Cholesteryl ester transfer protein polymorphisms, statin use, and their impact on cholesterol levels and cardiovascular events.
Clin Pharmacol Thera, 95:314-320, 2014

12. Regieli JJ, Jukema JW, Grobbee DE et al. CETP genotype predicts increased mortality in statin-treated men with proven cardiovascular disease: an adverse pharmacogenetic interaction.
Eur Heart J, 29:2792-2799, 2008

13. Vasan RS, Pencina MJ, Robins SJ, Zachariah JP, Kaur G, D'Agostino RB, Ordovas JM. Association of circulating cholesteryl ester transfer protein activity with incidence of cardiovascular disease in the community.
Circulation, 120:2414-2420, 2009

14. Hiura Y, Shen C-S, Kokubo Y et al. Identification of genetic markers associated with high-density lipoprotein-cholesterol by genome-wide screening in a Japanese population. The Suita Study.
Circ J, 73:1119-1126, 2009

15. Takeuchi F, Isono M, Katsuya T et al. Association of genetic variants influencing lipid levels with coronary artery disease in Japanese individuals.
PLoS ONE, 7(9):e46385, 2012

16. Papp AC, Pinsonneault JK, Wang D et al. Cholesteryl ester transfer protein (CETP) polymorphisms affect mRNA splicing, HDL levels, and sex-dependent cardiovascular risk.
PLoS ONE 7(3):e31930, 2012

17. Suhy A, Hartmann K, Newman L et al. Genetic variants affecting alternative splicing of human cholesteryl ester transfer protein.
Biochem Biophys Res Commu, 443:1270-1274, 2014

18. Thompson A, Di Angelantonio E, Sarwar N et al. Association of cholesteryl ester transfer protein genotypes with CETP mass and activity, lipid levels, and coronary risk.
JAMA, 299:2777-2788, 2008

19. Cheng C-Y, Yamashiro K, Chen LJ et al. New loci and coding variants confer risk for age-related macular degeneration in East Asians.
Nature Commu, 6:6063, 2015

20. Curcio CA, Johnson M, Huang J-D, Rudolf M. Aging, age-related macular degeneration, and the response-to-retention of apolipoprotein B-containing lipoproteins.
Progress in Retinal and Eye Research, 28:393-422, 2009

21. Hirano K-i, Nagasaka H, Kobayashi K, Yamaguchi S, Suzuki A, Toda T, Doyu M.
Disease-associated marked hyperalphalipoproteinemia.
Molecular Genet Metabolism Reports, 1:264-268, 2014

22. Curb JD, Abbott RD, Rodriguez BL et al. A prospective study of HDL-C and cholesteryl ester transfer protein gene mutations and the risk of coronary heart disease in the elderly.
J Lipid Res, 45:948-953, 2004

23. Qi Q, Durst R, Schwarzfuchs D et al. CETP genotype and changes in lipid levels in response to weight-loss diet intervention in the POUNDS LOST and DIRECT randomized trials.
J Lipid Res, 56:713-721, 2015

24. Teran-Garcia M, Despres J-P, Tremblay A, Bouchard C. Effects of cholesterol ester transfer protein (CETP) gene on adiposity in response to long-term overfeeding.
Atherosclerosis, 196:455-460, 2008

25. Wang S-P, Daniels E, Chen Y et al. In vivo effects of anacetrapib on preβHDL: improvement of HDL remodeling without effects on cholesterol absorption.
J Lipid Res, 54:2858-2865, 2013

26. Nicholls SJ, Ruotolo G, Brewer HB et al. Cholesterol efflux capacity and pre-beta-1 HDL concentrations are increased in dyslipidemic patients treated with evacetrapib.
J Am Coll Cardiol, 66:2201-10, 2015

27. Lu J, Cleary Y, Maugeais C, Kiu Weber C, Mazer NA. Analysis of “On/Off” kinetics of a CETP inhibitor using a mechanistic model of lipoprotein metaboilsm and kinetics.

CPT Pharmacometrics Syst. Pharmacol, 4:465-473, 2015

28. Miwa K, Inazu A, Kawashiri M et al. Cholesterol efflux from J774 macrophages and Fu5AH hepatoma cells to serum is preserved in CETP-deficient patients.
Clin Chim Acta, 402:19-24, 2009

29. Autran D, Attia N, Dedecjus M, Durlach V, Girard-Globa A. Postprandial reverse cholesterol transport in type 2 diabetic patients: effect of a lipid lowering treatment.
Atherosclerosis, 153:453-460, 2000

30. Chantepie S, Bochem AE, Chapman MJ, Hovingh GK, Kontush A. High-density lipoprotein (HDL) particle subpopulations in heterozygous cholesteryl ester transfer protein (CETP) deficiency: maintenance of antioxidative activity.
PLoS ONE, 7(11):e49336

31. Gomaraschi M, Ossoli A, Pozzi S et al. eNOS activation by HDL is impaired in genetic CETP deficiency.
PLoS ONE, 9:e95925, 2014

32. Han S, LeVoci L, Fisher P et al. Inhibition of cholesteryl ester transfer protein by anacetrapib does not impair the anti-inflammatory properties of high density lipoprotein.
Biochim Biophys Acta, 1831:825-833, 2013

33. Kühnast S, van der Tuin SJL, van der Hoorn JWA et al. Anacetrapib reduces progression of atherosclerosis, mainly by reducing non-HDL-cholesteol, improves lesion stability and adds to the beneficial effects of atorvastatin.
Eur Heart J, 36:39-48, 2015

34. Nakamura H, Mizuno K for the MEGA Study Group. Cardiovascular and

cancer events in hyper-high-density lipoprotein cholesterolemic patients: a post hoc analysis of the MEGA study.
Lipids in Health and Disease, 13:133, 2014

35. Van der Tuin SJL, Kuhnast S, Berbee JFP et al. Anacetrapib reduces (V)LDL cholesterol by inhibition of CETP activity and reduction of plasma PCSK9.
J Lipid Red, 56:2085-2093, 2015

36. Miyosawa K, Watanabe Y, Murakami K et al. New CETP inhibitor K-312 reduces PCSK9 expression: a potential effect on LDL cholesterol metabolism.
Am J Physiol Endocrinol Metab, 309:E177-E190, 2015

37. Millar JS, Reyes-Soffer G, Jumes P et al. Anacetrapib lowers LDL by increasing apoB clearance in mildly hypercholesteolemic subjects.
J Clin Invest, 125:2510-2522, 2015

38. Dong B, Singh AB, Fung C, Kan K, Liu J. CETP inhibitors downregulate hepatic LDL receptor and PCSK9 expression in vitro and in vivo through a SREBP2 dependent mechanism.
Atherosclerosis, 235:449-462, 2014

39. Krauss RM, Wojnooski K, Orr J et al. Changes in lipoprotein subfraction concentration and composition in healthy individuals treated with the CETP inhibitor anacetrapib.
J Lipid Res, 53:540-547, 2012

40. Bloomfield D, Carlson GL, Sapre A et al. Efficacy and safety of the cholesteryl ester transfer protein inhibitor anacetrapib as monotherapy and coadministered with atorvastatin in dyslipidemic patients.
Am Heart J, 157:352-60, 2009

41. Romagnuolo R, Scipione CA, Boffa MB, Marcovina SM, Seidah NG, Koschinsky ML. Lipoprotein (a) catabolism is regulated by proprotein convertase

subtilisin/kexin type 9 through the low density lipoprotein receptor.
J Biol Chem, 290:11649-11662, 2015

42. Castro-Perez J, Briand F, Gagen K et al. Anacetrapib promotes reverse cholesterol and bulk cholesterol excretion in Syrian golden hamsters.
J Lipid Res, 52:1965-1973, 2011

43. Niesor EJ, Magg C, Ogawa N et al. Modulating cholesteryl ester transfer protein activity maintains efficient preβ1-HDL formation and increases reverse cholesterol transport.
J Lipid Res, 51:3443-54, 2010

44. Briand F, Thieblemont Q, Muzotte E, Sulpice T. Upregulating reverse cholesterol transport with cholesteryl ester transfer protein inhibition requires combination with the LDL-lowering drug berberine in dyslipidemic hamsters.
Arterioscler Thromb Vasc Biol, 33:13-23, 2013

45. Dong B, Li H, Singh AB, Cao A, Liu J. Inhibition of PCSK9 transcription by berberine involves down-regulation of hepatic HNF1 α protein expression through the ubiquitin-proteasome degradation pathway.
J Biol Chem, 290:4047-4058, 2015

46. Clerc RG, Stauffer A, Weibel F et al. Mechanisms underlying off-target effects of the cholesteryl ester transfer protein inhibitor torcetrapib involve L-type calcium channels.
J Hypertension, 28:1676-1686, 2010

47. Simic B, Hermann M, Shaw SG et al. Torcetrapib impairs endothelial function in hypertension.
Eur Heart J, 33:1615-1624, 2012

48. Cao G, Beyer TP, Zhang Y et al. Evacetrapib is a novel, potent, and selective inhibitor of cholesteryl ester transfer protein that elevates HDL

cholesterol without inducing aldosterone or increasing blood pressure.
J Lipid Res, 52:2169-2176, 2011

49. Schwartz GG, Olsson AG, Abt M et al. Effects of dalcetrapib in patients with a recent acute coronary syndrome.
N Engl J Med, 367:2089-99, 2012

50. Tardif J-C, Rheaume E, Perreault LPL et al. Pharmacogenetic determinants of the cardiovascular effects of dalcetrapib.
Circ Cardiovasc Genet, 8:372-382, 2015

51. Bell, TA III, Graham MJ, Lee RG, et al. Antisense oligonucleotide inhibition of cholesteryl ester transfer protein enhances RCT in hyperlipidemic, CETP transgenic, LDLr-/- mice.
J Lipid Res, 54:2647-2657, 2013

52. Morton RE, Izem L. Modification of CETP function by changing its substrate preference: a new paradigm for CETP drug design.
J Lipid Res, 56:612-619, 2015

53. Saito K, Kobori K, Hashimoto H, Ito S, Manabe M, Yokoyama S. Epitope mapping for the anti-rabbit cholesteryl ester transfer protein monoclonal antibody that selectively inhibits triglyceride transfer.
J Lipid Res, 40:2013-2021, 1999

54. Liaw Y-W, Lin C-Y, Lai Y-S et al. A vaccine targeted at CETP alleviates high fat and high cholesterol diet-induced atherosclerosis and non-alcoholic steatohepatitis in rabbit.
PLoS ONE, 9(12):e111529, 2014

55. Guo Y, Fan Y, Zhang J et al. Perhexiline activates KLF14 and reduces atherosclerosis by modulating apoA-I production.
J Clin Invest, 125:3819-3830, 2015

Table1. CETP gene SNPs, location and rs numbers

-2708G>A		Rs12149545
-2568C>A (G>T)		Rs3764261
-1337C>T		Rs17231506
-629C>A		Rs1800775

Intron1 TaqIB	+279G>A (C>T)	Rs708272
Intron8	-40T>C	Rs9930761
Exon9	+121C>T	Rs5883
Intron9 BamHI	G>A (C>T)	Rs289714
Exon14 I405V	G>A	Rs5882
Intron14G>A	+1G>A	Mutation in Japanese
Exon15 D442G	A>G	Rs2303790

Figure 1. HDL subclasses and their roles in lipoprotein metabolism

Figure 2. A relationship between CETP and CAD in association studies via effects on HDL and/or LDL

Figure 1

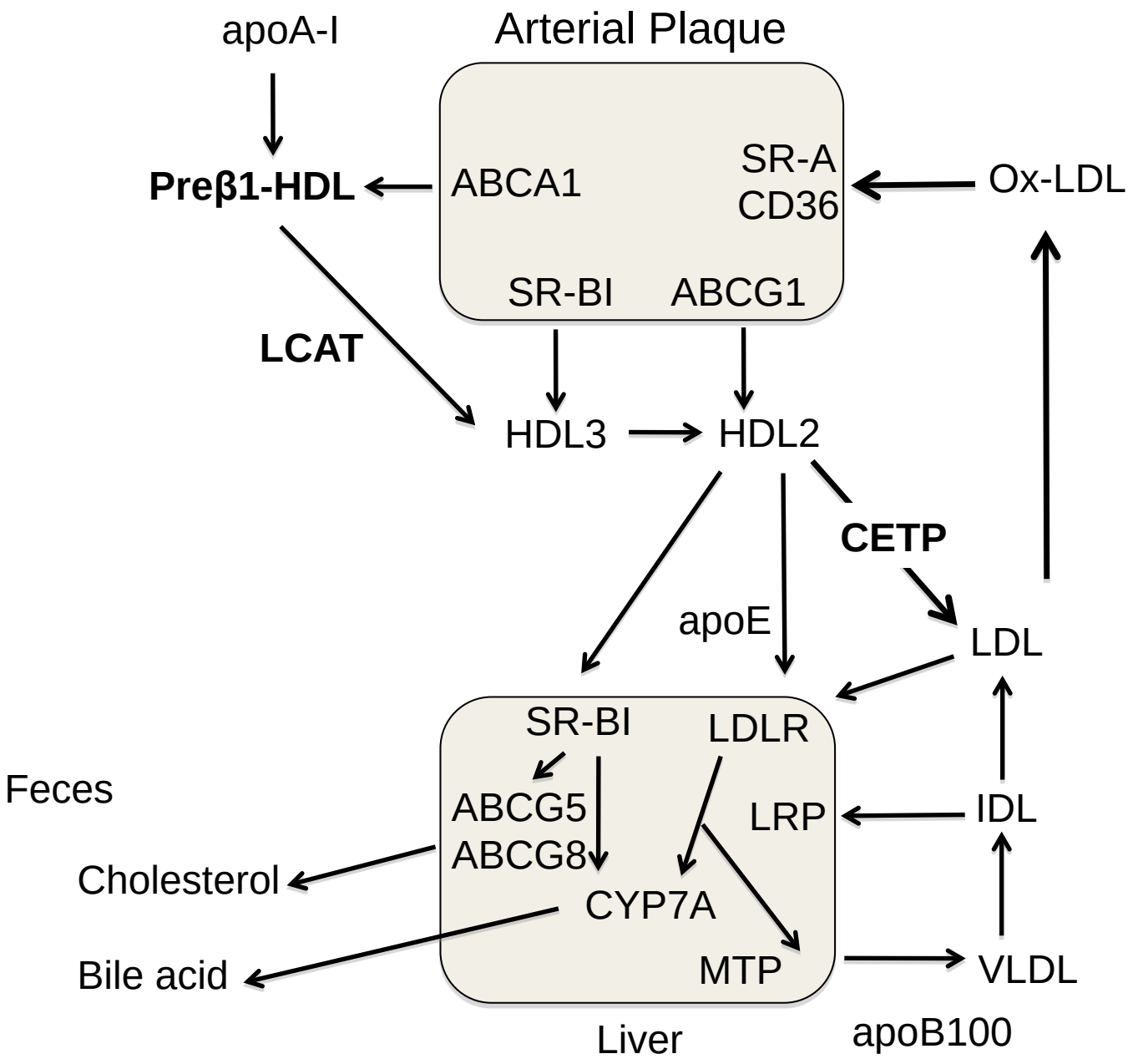
apoA-I
Arterial Plaque
Preβ1-HDL
ABCA1
SR-A
CD36
Ox-LDL
SR-BI
ABCG1
LCAT
HDL3
HDL2
CETP
apoE
LDL
SR-BI
LDLR
Feces
ABCG5
ABCG8
LRP
IDL
Cholesterol
CYP7A
Bile acid
MTP
VLDL
Liver
apoB100

Figure 2