

EDITORIAL COMMENT

## Will Cholesteryl Ester Transfer Protein Inhibition Succeed Primarily by Lowering Low-Density Lipoprotein Cholesterol?

### Insights From Human Genetics and Clinical Trials\*

Sekar Kathiresan, MD

*Boston and Cambridge, Massachusetts*

Developing new medicines to treat atherosclerotic cardiovascular disease (ASCVD), the leading cause of death in the United States and in Europe, has never been more challenging (1). The number of new drug approvals is down, whereas the cost of developing a new medicine is increasing. Many new drugs fail in the clinic because of efficacy, that is, the drug does not reduce risk for ASCVD when tested in humans.

See page 2041

To overcome these challenges, the pharmaceutical industry is urgently searching for 2 solutions: “validated” gene targets and biomarkers that predict cardiovascular outcomes in a clinical trial (1,2). The term “validated” generally refers to increased confidence that targeting the gene will reduce risk of disease in humans. In addition to a validated gene target, the drug development process requires a biomarker that can help assess drug efficacy, dose-find in early clinical development, and predict disease risk.

Over the last 3 decades, the biomedical research community has intensively studied a specific gene target—*CETP*—and a related biomarker—high-density lipoprotein (HDL)—for their potential in altering risk for ASCVD (3,4). Studies in cellular models, animal models, and humans have led to 2 hypotheses. The first, the cholesteryl ester transfer protein

(*CETP*) hypothesis, is focused on *CETP* as a gene target and suggests that: 1) increased enzymatic activity of *CETP* promotes atherosclerosis; and 2) inhibition of *CETP* activity will reduce risk for ASCVD (3). The second, the HDL hypothesis, is focused on HDL as a biomarker and suggests that: 1) lower levels of HDL (as measured by the cholesterol content in HDL) increase risk for ASCVD; and 2) therapies that raise HDL will lower risk for ASCVD (4). Despite considerable research, the answers to both hypotheses remain unsettled, and in the last few years, several results have challenged prior assumptions.

Against this background, in this issue of the *Journal*, Johannsen et al. (5) use naturally occurring human genetic variation to address these research questions. They test the hypothesis that common genetic variation at the *CETP* gene relates to plasma lipids and risk for incident ASCVD. In 10,261 participants from the prospective Copenhagen City Heart Study, they genotype 2 common single nucleotide polymorphisms (*CETP* -629C>A or rs1800775 and *Taq1BG*>A or rs708272) that have previously been associated with decreased *CETP* mass and decreased *CETP* activity (6,7). They find that the alleles associated with lower *CETP* activity are associated with a range of lipid biomarkers including: 1) higher apolipoprotein A-I containing lipoproteins (as measured by HDL cholesterol); 2) lower apolipoprotein B-containing lipoproteins (as measured by low-density lipoprotein [LDL] cholesterol and triglycerides); and 3) lower plasma lipoprotein(a) (Table 1). They also test whether these polymorphisms associate with traits relevant to the side effects of pharmacological *CETP* inhibition and find no such associations.

Beyond enzymatic activity and biomarkers, genetics has a unique potential to offer insights into human disease endpoints (8). As such, the investigators’ primary question was whether these *CETP* polymorphisms associate with incident ASCVD and mortality. They find that the *CETP* alleles associated with intermediate endpoints (lower *CETP* activity, higher HDL cholesterol, lower LDL cholesterol, lower triglycerides, and lower lipoprotein(a)) are indeed associated with decreased risk for ASCVD and mortality. Overall, these robust findings confirm the observations reported in a meta-analysis from 2008 and 2 additional independent studies since then (Table 1) (7,9,10).

What are the implications of this study for the *CETP* hypothesis and the HDL hypothesis? With regard to the *CETP* hypothesis, these genetic association results seem to “validate” *CETP* as a gene target and suggest that drugs that inhibit *CETP* activity (and produce a similar lipid profile as the gene variants) are likely to reduce risk for ASCVD in clinical trials.

If so, how do we make sense of the failure of 2 different *CETP* inhibitors—torcetrapib and dalcetrapib—to reduce risk for ASCVD in large randomized controlled trials? For torcetrapib, a leading possibility is off-target side effects. In

\*Editorials published in the *Journal of the American College of Cardiology* reflect the views of the authors and do not necessarily represent the views of *JACC* or the American College of Cardiology.

From the Center for Human Genetic Research and Cardiovascular Research Center, Massachusetts General Hospital, Boston, Massachusetts; Department of Medicine, Harvard Medical School, Boston, Massachusetts; and the Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge, Massachusetts. Dr. Kathiresan is a consultant to Merck, Pfizer, Celera, and Alnylam.

Table 1 Genetic Variation at the CETP Gene, Plasma Lipids, and Risk for Atherosclerotic Cardiovascular Disease

First Author (Ref. #)	Year	Study Design	n	Variant	Minor Allele Frequency	CETP Activity	HDL Cholesterol	LDL Cholesterol	Triglycerides	Lp(a)	CVD
Thompson et al. (7)*	2008	Meta-analysis of prospective cohort and case-control studies	27,196 coronary disease cases; 55,538 control subjects	Taq1B (rs708272) -629C>A (rs1800775)	42%	-8.6%	+2.3 mg/dl	-1.2 mg/dl	-2.6 mg/dl	Not reported	0.95 (0.92–0.99) 0.95 (0.91–1.00)
Ridker et al. (9)*	2009	Prospective cohort	18,245 women; 198 incident MI events	Taq1B (rs708272)	43%	Not studied	+3.1 mg/dl	-1.5 mg/dl	-4.5 mg/dl	Not reported	0.76 (0.62–0.94)
Voiight et al. (10)*	2012	Meta-analysis of case-control studies	16,503 MI cases; 46,576 MI-free control subjects	rs3764261†	32%	Not studied	+3.4 mg/dl	-1.4 mg/dl	-2.1 mg/dl	Not reported	0.96 (0.93–1.00)
Johanssen et al. (5)‡	2012	Prospective cohort	10,261 individuals; 2,087 incident ischemic heart disease events	Taq1B (rs708272) -629C>A (rs1800775)	44%	Not studied	+7.7 mg/dl	-3.9 mg/dl	-8.9 mg/dl	39% lower in Taq1B minor allele homozygotes§	0.74 (0.65–0.85)

\*CETP activity and lipid value changes are mean difference per copy of allele. †Lipid differences as presented in Teslovich et al. (26). ‡Lipid differences and CVD risk comparisons are between carriers of 4 versus 0 HDL cholesterol-increasing alleles at the 2 polymorphisms. §On background of -629 CC wild type. Taq1B genotype was associated with stepwise reductions in Lp(a) ( $p = 0.02$ ).

CETP = cholesteryl ester transfer protein; CVD = cerebrovascular disease; HDL = high-density lipoprotein; LDL = low-density lipoprotein; Lp(a) = lipoprotein(a); MI = myocardial infarction.

the ILLUMINATE (Investigation of Lipid Level Management to Understand Its Impact in Atherosclerotic Events) randomized controlled trial involving 15,067 participants, torcetrapib increased HDL cholesterol by 72%, decreased LDL cholesterol by 25%, and decreased triglycerides by 9% (11). However, torcetrapib also increased blood pressure and aldosterone level. On net, when compared with placebo, torcetrapib treatment increased risk for ASCVD by 25%.

If torcetrapib's failure is potentially due to off-target effects, how does one understand the recent failure of dalcetrapib, a compound that did not have effects on blood pressure or aldosterone levels (12)? The dal-OUTCOMES trial randomized >15,000 participants to test the hypothesis that CETP inhibition with dalcetrapib will reduce cardiovascular morbidity and mortality in patients with recent ACS. In May 2012, the data safety and monitoring board stopped the trial at a second interim analysis due to "lack of clinically meaningful efficacy" (13). Dalcetrapib may have failed due to issues related to clinical trial design such as insufficient statistical power, insufficient duration of follow-up, and wrong study population. As the study findings remain to be published in a peer-reviewed journal, it is at present difficult to evaluate these considerations.

Beyond study design, dalcetrapib's failure may lie with something more fundamental, the fact that dalcetrapib raised HDL cholesterol in isolation. In contrast to other CETP inhibitors, dalcetrapib only alters 1 lipid fraction—HDL cholesterol (12). In Phase II clinical trials, dalcetrapib raised HDL cholesterol by about 25% to 30% without significant effects on LDL cholesterol, triglycerides, or blood pressure (14). The dal-OUTCOMES study investigators expected each 1 mg/dl increase in HDL cholesterol to lead to a 1.5% relative risk reduction in the primary ASCVD endpoint (14). The average participant treated with dalcetrapib was expected to have HDL cholesterol increase from 40 to 51 mg/dl, and this increase was projected to correspond to a 15% reduction in risk for the primary ASCVD endpoint. Critically, these calculations rest on the validity of the HDL hypothesis—that higher HDL causally protects from risk for ASCVD.

However, several lines of human genetic evidence now suggest that the epidemiologic association of higher HDL cholesterol with lower risk for ASCVD may not reflect a causal relationship. First, lifelong low HDL cholesterol due to Mendelian mutations in 3 genes—*ABCA1*, *APOA1*, or *LCAT*—is not consistently associated with increased risk for ASCVD (reviewed in Vergeer et al. [4]). Second, in the Copenhagen City Heart Study, carriers of loss-of-function mutations in *ABCA1* had ~17 mg/dl lower HDL cholesterol but were not at increased risk for ASCVD (15). Third, we recently reported that ~2.6% of individuals carry an HDL cholesterol-boosting variant in the endothelial lipase gene and despite having

higher HDL cholesterol, these individuals did not have lower risk for myocardial infarction (10). Finally, we evaluated 14 common variants that affected HDL cholesterol in isolation (without affecting other lipid fractions) and found that a genotype score crafted from these variants did not relate to risk for myocardial infarction (10). So, dalcetrapib may have failed because it altered only a noncausal biomarker.

When combined, the dalcetrapib clinical trial results and the human genetic findings summarized here cast doubt on the notion that raising HDL cholesterol in isolation will reduce risk for ASCVD. For several decades, the biomedical research community has assumed that if an intervention raises HDL cholesterol, then that intervention will reduce risk for ASCVD. Now, it seems prudent to rethink this assumption and re-evaluate the use of HDL cholesterol as a biomarker predictive of ASCVD in intervention studies.

In contrast with the HDL cholesterol biomarker, human genetic studies strongly suggest that both LDL cholesterol and plasma lipoprotein(a) cause ASCVD. Rare mutations that lead to extremely high LDL cholesterol consistently increase risk for ASCVD (16,17). About 3% of individuals carry an LDL cholesterol-lowering variant in the proprotein convertase subtilisin/kexin type 9 gene, and these individuals are at lower risk for myocardial infarction (18–20). A genotype score crafted from 13 variants that affected LDL cholesterol in isolation was strongly associated with myocardial infarction risk (10). In addition, polymorphisms that increase plasma lipoprotein(a) consistently confer increased risk for ASCVD (21–23).

What are the implications of these data for the 2 CETP inhibitors—anacetrapib and evacetrapib—that remain in clinical development (24,25)? These CETP inhibitors do not seem to have off-target effects on blood pressure or aldosterone. The pattern of lipid effects for anacetrapib and evacetrapib (higher HDL cholesterol, lower LDL cholesterol, lower triglycerides, and lower lipoprotein(a)) mirrors that seen by Johannsen et al. for the 2 *CETP* variants that are associated with lower CETP activity and lower risk for ASCVD. As such, the work of Johannsen et al. increases confidence that anacetrapib and evacetrapib will successfully reduce risk for ASCVD.

By providing direct evidence in humans prior to a clinical trial, studies of genetic variation such as that of Johannsen et al. represent powerful approaches to validate gene targets and pinpoint causal biomarkers. CETP inhibition by anacetrapib and evacetrapib is likely to succeed. However, we have learned that any potential benefit clearly cannot be ascribed to the HDL biomarker per se. Insights from human genetics and the completed dalcetrapib trial suggest that the success of CETP inhibitors may be more related to their effect on LDL cholesterol and lipoprotein(a) rather than through HDL elevation.

**Reprint requests and correspondence:** Dr. Sekar Kathiresan, Sekar Kathiresan, Massachusetts General Hospital, Center for Human Genetic Research, 185 Cambridge Street, CPZN 5.252, Boston, Massachusetts 02114. E-mail: skathiresan@partners.org.

## REFERENCES

1. Paul SM, Mytelka DS, Dunwiddie CT, et al. How to improve R&D productivity: the pharmaceutical industry's grand challenge. *Nat Rev Drug Discov* 2010;9:203–14.
2. Plump AS, Lum PY. Genomics and cardiovascular drug development. *J Am Coll Cardiol* 2009;53:1089–100.
3. Barter PJ, Rye KA. Cholesteryl ester transfer protein inhibition as a strategy to reduce cardiovascular risk. *J Lipid Res* 2012;53:1755–66.
4. Vergeer M, Holleboom AG, Kastelein JJ, Kuivenhoven JA. The HDL hypothesis: does high-density lipoprotein protect from atherosclerosis? *J Lipid Res* 2010;51:2058–73.
5. Johannsen TH, Frikke-Schmidt R, Schoou J, Nordestgaard BG, Tybjaerg-Hansen A. Genetic inhibition of *CETP*, ischemic vascular disease and mortality, and possible adverse effects. *J Am Coll Cardiol* 2012;60:2041–8.
6. Dacht C, Poirier O, Cambien F, Chapman J, Rouis M. New functional promoter polymorphism, *CETP*/–629, in cholesteryl ester transfer protein (*CETP*) gene related to *CETP* mass and high density lipoprotein cholesterol levels: role of Sp1/Sp3 in transcriptional regulation. *Arterioscler Thromb Vasc Biol* 2000;20:507–15.
7. 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* 2008;299:2777–88.
8. Kathiresan S, Srivastava D. Genetics of human cardiovascular disease. *Cell* 2012;148:1242–57.
9. Ridker PM, Paré G, Parker AN, Zee RY, Miletich JP, Chasman DI. Polymorphism in the *CETP* gene region, HDL cholesterol, and risk of future myocardial infarction: genomewide analysis among 18,245 initially healthy women from the Women's Genome Health Study. *Circ Cardiovasc Genet* 2009;2:26–33.
10. Voight BF, Peloso GM, Orho-Melander M, et al. Plasma HDL cholesterol and risk of myocardial infarction: a Mendelian randomization study. *Lancet* 2012;380:572–80.
11. Barter PJ, Caulfield M, Eriksson M, et al., for the ILLUMINATE Investigators. Effects of torcetrapib in patients at high risk for coronary events. *N Engl J Med* 2007;357:2109–22.
12. Stein EA, Stroes ES, Steiner G, et al. Safety and tolerability of dalcetrapib. *Am J Cardiol* 2009;104:82–91.
13. Roche. Roche Provides Update on Phase III Study of Dalcetrapib [media release]. May 7, 2012. Available at: [http://www.roche.com/media/media\\_releases/med-cor-2012-05-07.htm](http://www.roche.com/media/media_releases/med-cor-2012-05-07.htm). Accessed September 2012.
14. Schwartz GG, Olsson AG, Ballantyne CM, et al., for the dal-OUTCOMES Committees and Investigators. Rationale and design of the dal-OUTCOMES trial: efficacy and safety of dalcetrapib in patients with recent acute coronary syndrome. *Am Heart J* 2009;158:896–901.e3.
15. Frikke-Schmidt R, Nordestgaard BG, Stene MC, et al. Association of loss-of-function mutations in the *ABCA1* gene with high-density lipoprotein cholesterol levels and risk of ischemic heart disease. *JAMA* 2008;299:2524–32.
16. Rader DJ, Cohen J, Hobbs HH. Monogenic hypercholesterolemia: new insights in pathogenesis and treatment. *J Clin Invest* 2003;111:1795–803.
17. Abifadel M, Varret M, Rabes JP, et al. Mutations in *PCSK9* cause autosomal dominant hypercholesterolemia. *Nat Genet* 2003;34:154–6.
18. Cohen JC, Boerwinkle E, Mosley TH, Jr., Hobbs HH. Sequence variations in *PCSK9*, low LDL, and protection against coronary heart disease. *N Engl J Med* 2006;354:1264–72.
19. Kathiresan S. A *PCSK9* missense variant associated with a reduced risk of early-onset myocardial infarction. *N Engl J Med* 2008;358:2299–300.
20. Benn M, Nordestgaard BG, Grande P, Schnohr P, Tybjaerg-Hansen A. *PCSK9* R46L, low-density lipoprotein cholesterol levels, and risk of ischemic heart disease: 3 independent studies and meta-analyses. *J Am Coll Cardiol* 2010;55:2833–42.

21. Sandholzer C, Saha N, Kark JD, et al. Apo(a) isoforms predict risk for coronary heart disease: a study in six populations. *Arterioscler Thromb Vasc Biol* 1992;12:1214–26.
22. Kamstrup PR, Tybjaerg-Hansen A, Steffensen R, Nordestgaard BG. Genetically elevated lipoprotein(a) and increased risk of myocardial infarction. *JAMA* 2009;301:2331–9.
23. Clarke R, Peden JF, Hopewell JC, et al., for the PROCARDIS Consortium. Genetic variants associated with Lp(a) lipoprotein level and coronary disease. *N Engl J Med* 2009;361:2518–28.
24. Cannon CP, Shah S, Dansky HM, et al., for the Determining the Efficacy and Tolerability Investigators. Safety of anacetrapib in patients with or at high risk for coronary heart disease. *N Engl J Med* 2010;363:2406–15.
25. Nicholls SJ, Brewer HB, Kastelein JJ, et al. Effects of the CETP inhibitor evacetrapib administered as monotherapy or in combination with statins on HDL and LDL cholesterol: a randomized controlled trial. *JAMA* 2011;306:2099–109.
26. Teslovich TM, Musunuru K, Smith AV, et al. Biological, clinical and population relevance of 95 loci for blood lipids. *Nature* 2010;466:707–13.

---

**Key Words:** cardiovascular diseases ■ genetics ■ lipids ■ lipoproteins.