Published in final edited form as:

Clin Lipidol. 2013 December ; 8(6): 635–648. doi:10.2217/clp.13.73.

Monogenic causes of elevated HDL cholesterol and implications for development of new therapeutics

Daniel B Larach¹, Marina Cuchel¹, and Daniel J Rader^{*,1,2}

¹Division of Translational Medicine & Human Genetics, Department of Medicine, Perelman School of Medicine at the University of Pennsylvania, PA, USA

²11–125 Smilow Center for Translational Research, 3400 Civic Center Boulevard, Building 421, PA 19104–5158, USA

Abstract

Identification of the *CETP*, *LIPG* (encoding endothelial lipase) and *APOC3* genes, and analysis of rare genetic variants in them, have allowed researchers to increase understanding of HDL metabolism significantly. However, development of cardiovascular risk-reducing therapeutics targeting the proteins encoded by these genes has been less straightforward. The failure of two CETP inhibitors is complex but illustrates a possible over-reliance on HDL cholesterol as a marker of therapeutic efficacy. The case of endothelial lipase exemplifies the importance of utilizing population-wide genetic studies of rare variants in potential therapeutic targets to gain information on cardiovascular disease end points. Similar population-wide studies of cardiovascular end points make apoC-III a potentially attractive target for lipid-related drug discovery. These three cases illustrate the positives and negatives of single-gene studies relating to HDL-related cardiovascular drug discovery; such studies should focus not only on HDL cholesterol and other components of the lipid profile, but also on the effect genetic variants have on cardiovascular end points.

Keywords

anacetrapib; apoC-III; CETP; cholesterol; dalcetrapib; endothelial lipase; evacetrapib; genetics; HDL; ISI-APOCIII_{RX}; torcetrapib

Over the past several decades, the widespread use of statins to lower LDL cholesterol (LDL-C) levels has contributed to a substantial decrease in the incidence of coronary heart disease (CHD) [1]. Despite this, considerable residual CHD risk remains, even in patients with optimal LDL-C [2,3]. Consequently, substantial research has been directed toward identifying additional lipid metabolism targets that have the potential to further decrease cardiovascular risk. Studies of human genetics have featured prominently in these

© 2013 Future Medicine Ltd

*Author for correspondence: Tel.: +1 215 573 4176 Fax: +1 215 573 8606 rader@mail.med.upenn.edu.

Financial & competing interests disclosure

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

endeavors, revealing previously unknown biology that has led, in turn, to the development of new therapeutic targets. For example, linkage analysis in families with autosomal dominant hypercholesterolemia of unknown etiology led to the identification of gain-of-function mutations in *PCSK9* [4]; subsequent targeted sequencing of *PCSK9* in individuals with extremely low LDL-C identified loss-of-function mutations [5] that were found to be protective against CHD [6]. Based on the strength of this human genetic data, *PCSK9* is a major target for pharmacologic inhibition as a means of lowering LDL-C, with promising early-phase results [7].

In addition to LDL-C, HDL cholesterol (HDL-C) has been an attractive focus of research since it was identified as having an inverse relationship with CHD risk in epidemiological studies [8]. However, despite a great deal of research and significant financial investment into pharmaceutical development, the feasibility of preventing cardiovascular disease through strategies that increase HDL-C remains unclear [9]. Despite the fact that the high HDL-C phenotype is often of polygenic origin [10], research into single-gene conditions that increase HDL-C could lead to new therapeutic targets. This review examines three known monogenic causes of elevated HDL-C: loss-of-function mutations in CETP (encoded by *CETP*), endothelial lipase (EL; encoded by *LIPG*) and apoC-III (encoded by *APOC3*). CETP transfers cholesteryl esters from mature HDL particles to atherogenic apoB-containing lipoproteins, such as LDL and VLDL, in exchange for triglycerides (TGs) [11]. EL hydrolyzes HDL phospholipids, thus destabilizing the HDL particle, leading to increased catabolism [12]. ApoC-III inhibits lipoprotein lipase (LPL), leading to decreased hydrolysis of TG-laden cholesterol particles and a reciprocal decrease in HDL-C. Examination of these three conditions elucidates the critical role studies of genetic variants in single genes can play in improving our understanding of HDL metabolism. They also emphasize the essential nature of such genetic studies in identifying drug targets; indeed, the recognition that loss-of-function mutations in these three genes lead to increased HDL-C levels placed all three genes on a short-list of potential therapeutic targets for raising HDL-C. However, these examples also emphasize the fact that translational research on newly identified HDL-related genes must focus not only on HDL-C concentration, but also on HDL function, measures of atherosclerosis and cardiovascular disease end points.

CETP

CETP deficiency, observational epidemiology & genetic variation

CETP was first purified and characterized in 1987 [13]. Three years later, two Japanese siblings with extremely high HDL-C levels and increased HDL size were found to lack CETP due to loss-of-function mutations [14]. A larger investigation identified the same mutations causing CETP deficiency in four out of 11 families with high HDL-C levels [15]. Subjects homozygous for the mutation had markedly increased HDL-C and apoA-I levels, together with decreased LDL-C and apoB levels, while heterozygotes showed modestly elevated HDL-C levels. These studies solidified CETP as a protein of interest for lipoprotein metabolism and identified it as a therapeutic target for inhibition as a strategy to raise HDL-C.

Many subsequent investigations of CETP's function focused on its association with both pro- and anti-atherogenic cardiovascular markers. CETP activity has been shown to correlate directly with LDL-C and non-HDL-C, and inversely with HDL-C, in patients with hyper-cholesterolemia and combined hyperlipidemia [16], and with serum LDL-C (but not HDL-C) in healthy Japanese patients [17]. Multiple studies have also identified a correlation between increased CETP concentrations and various dyslipidemic states, such as dysbetalipoproteinemia, severe chylomicronemia, nephrotic syndrome and familial hypercholesterolemia [18–20], as has been reviewed elsewhere [11].

Evaluation of CETP's association with atherosclerosis and CHD is less straightforward. It has been posited that CETP may in fact be antiatherogenic and that dyslipidemia causes elevated CETP, rather than the converse [21]; some preclinical data substantiates the hypothesis that CETP plays a role in reverse cholesterol transport [22,23]. There have been several observational studies of the association of plasma CETP mass and/or activity with CHD. A nested case–control study of 111 male CHD cases and 116 male CHD-free controls found that while plasma mass CETP levels were not significantly different between the two groups overall, a subgroup analysis of subjects with low TGs (below the population mean) found that CETP levels were lower in cases than in controls [24]. A similar case–control study of 114 CHD cases and 105 controls drawn from the same cohort again showed no difference in CETP levels between the two groups, but found that CETP activity was significantly higher in cases than in controls [25]. A prospective study of CETP activity in 2679 subjects found that low levels of activity were associated with increased CHD risk in men, but not in women [26]. Finally, a prospective study of 3256 patients undergoing coronary angiography found that patients in the lowest quartile of plasma CETP mass level had an increased mortality risk when compared with patients in the highest quartile [27].

Studies of the association of genetic variation in *CETP* with CHD may be more likely to reveal potential causal associations. In early studies, sequencing of 201 Japanese patients with HDL-C ≥ 100 mg/dl found that 67% carried deleterious mutations in *CETP* [28]. However, ten of the 12 subjects with atherosclerotic cardiovascular disease were heterozygous for *CETP* mutations, implying that while CETP deficiency may lead to high HDL-C, it may not be cardioprotective. A later study of 104,505 Japanese subjects by the same group found that those with genetic CETP deficiency due to a splice site mutation and markedly elevated HDL-C did not live longer than those without the mutation, and that the frequency of the mutation was higher in patients with coronary artery disease than in healthy controls [29]. In a smaller population of 3469 Japanese–American men, heterozygotes for CETP deficiency had modestly elevated HDL-C overall, but those who had HDL-C between 41–60 mg/dl had an increased prevalence of CHD [30]. However, a follow-up study showed a lack of a significant association of *CETP* mutations with incident CHD [31]. The I405V polymorphism in *CETP* has been associated with low CETP activity and increased HDL-C in a study of 576 men, but also with possibly increased risk of CHD in those with hypertriglyceridemia [32], greater CHD risk in Caucasian women [33] and increased carotid intima–media thickness (CIMT) in men [34].

Despite this, evidence drawn from much larger samples focused on common variants at the *CETP* locus suggests that genetic reduction in CETP activity not only increases HDL-C and

decreases LDL-C, but is also associated with a modest reduction in CHD risk. A meta-analysis incorporating 196,367 subjects found that the I405V mutation, a -629C>A polymorphism, and the intronic *CETP* variant Taq1B were all associated with lower CETP mass and activity, increased HDL-C, increased apoA-I, decreased LDL-C, and a small reduction in CHD risk [35]. However, a pooled analysis of 10,526 undifferentiated subjects and 10,947 subjects at high CHD risk found that carriage of the Taq1B variant was associated with increased CHD risk in the former group, but with decreased risk in the latter group [36]. Another meta-analysis of 12,482 myocardial infarction (MI) cases and 41,331 controls found that a 2490C>A polymorphism at the *CETP* locus was associated with increased HDL-C, reduced LDL-C and reduced risk of MI [37]. While one prospective study of 6780 subjects found that neither Taq1B nor -629C>A status affected the direct relationship between the apoB/ apoA-I and total cholesterol:HDL-C ratios, and CHD risk [38], another prospective cohort study of 18,245 healthy women found decreased MI risk and increased HDL-C in carriers of the Taq1B, 852 + 783T>C, and 179 + 3052A>G polymorphisms in or near *CETP* [39]. A third prospective cohort study of 10,261 individuals found that the Taq1B and -629C>A polymorphisms were associated with lower CETP activity, increased HDL-C, decreased LDL-C and a significantly lower risk of ischemic cardio- and cerebro-vascular disease [40].

From the evidence discussed above, the observational epidemiology regarding the association of CETP mass and activity with CHD events is internally inconsistent and conflicts with the genetic data. A potential explanation is that the association of decreased CETP mass with increased CHD events may be confounded – a hypothesis borne out by Mendelian randomization analyses – suggesting that genetic variants at the *CETP* locus that reduce CETP mass and/or activity appear to modestly reduce cardiovascular risk [35].

CETP inhibition

Given CETP deficiency's association with increased HDL-C, pharmacologic inhibition of CETP has been of considerable interest for some time. However, the question of whether such inhibition leads to improved cardiovascular outcomes has not been conclusively answered. Although several preclinical studies detailing the use of antisense oligodeoxynucleotides [41] and a vaccine against CETP [42,43] have been published, pharmacological attention has focused primarily on small-molecule CETP inhibitors.

The first widely studied inhibitor, torcetrapib, was shown to reduce aortic atherosclerosis in rabbits fed an atherogenic diet for 16 weeks [44]. At the study's end, aortic atherosclerosis and aortic cholesterol content were 60% lower in 24 torcetrapib-treated animals than in 23 controls. Early clinical studies focused on torcetrapib's effects on lipoprotein profiles. For example, a Phase I dose-finding study of 40 healthy individuals noted elevations in HDL-C, apoA-I, apoE and TGs along with reduced non-HDL-C, apoB and cholesteryl ester content [45]. Another study in low HDL-C subjects found that torcetrapib administration, both with and without accompanying statin therapy, increased HDL-C levels and particle size significantly, while decreasing LDL-C [46], as did larger randomized controlled trials of low HDL-C subjects [47,48]. However, Phase III evaluations of torcetrapib's effects, not only on lipid levels, but also on atherosclerosis, did not show benefit and, in fact, demonstrated

harm. The ILLUSTRATE trial of 1188 patients with pre-existing coronary artery disease on statin therapy randomized to torcetrapib or placebo found a significant increase in HDL-C and a decrease in LDL-C in the torcetrapib-treated group, but no significant difference in the progression of coronary atherosclerosis, as measured by intravascular ultrasound [49]. The RADIANCE 1 trial of 850 patients with heterozygous familial hypercholesterolemia showed a similarly beneficial effect on torcetrapib-treated subjects' lipid profiles, but no significant difference in maximum CIMT, and a significant increase in annualized change in mean CIMT compared with the statin-only group [50]. RADIANCE 2, an evaluation of 752 mixed dyslipidemia patients randomized to torcetrapib plus statin or statin alone, also found increased HDL-C and decreased LDL-C in the torcetrapib group, but no significant change in maximum CIMT per year [51]. Torcetrapib development ended following the early cessation of the ILLUMINATE trial of 15,067 patients at high risk for CHD due to an increased risk of cardiovascular events and all-cause mortality in the torcetrapib-treated group [52]. Torcetrapib is now known to have off-target effects, such as stimulation of aldosterone secretion and increased blood pressure, which may have contributed to its adverse effects [53].

The second widely studied CETP inhibitor was the less potent compound dalcetrapib. A study in rabbits found a 40–45% HDL-C increase and a 15–20% LDL-C decrease, as well as a 70% decrease in aortic atheroma lesion area (compared with an 80% decrease in a statin-treated group) [54]. Another rabbit study noted a significant HDL-C increase compared with control; however, no difference in aortic atheroma area was seen, and a correlation analysis found no relationship between atherosclerosis development and either CETP activity or HDL-C level [55]. A Phase II clinical trial of mildly dyslipidemic human subjects showed that increased HDL-C and slightly decreased LDL-C accompanied a dose-dependent decrease in CETP activity [56]. Although the dal-PLAQUE Phase IIb trial found a decrease in MRI-assessed carotid total vessel area in dalcetrapib-treated patients compared with placebo, it showed no difference between groups in other coprimary end points, such as PET:computed tomography most-diseased-segment target:background ratio, MRI-assessed carotid wall area and MRI-assessed normalized wall index [57]. Dalcetrapib development was halted following the early cessation for futility of the Phase III dal-OUTCOMES mortality and morbidity study [58]. In dal-OUTCOMES, 15,871 patients with recent acute coronary syndrome were randomized to dalcetrapib or placebo in a 1:1 ratio and followed for the composite end point of CHD death, non-fatal MI, ischemic stroke, unstable angina or cardiac arrest with resuscitation. The study was halted after interim analysis found that while dalcetrapib increased HDL-C by 31–40% in the treatment group (compared with 4–11% in the placebo group), it did not reduce LDL-C, and, most importantly, did not reduce the risk of recurrent cardiovascular events (hazard ratio for dalcetrapib vs placebo: 1.04; 95% CI: 0.93–1.16; $p = 0.52$) [58]. Potential explanations for this result include that increased HDL-C through CETP inhibition is not cardio protective, that inhibition of CETP does not promote (and may even impair) reverse cholesterol transport, that the increase in HDL-C was not adequate, that there was no accompanying reduction in LDL-C and that dalcetrapib had off-target effects that offset any protection conferred by the drug.

Despite the lack of promising results for torcetrapib and dalcetrapib, the hypothesis that CETP inhibition is atheroprotective has not been definitively discarded. As noted above,

torcetrapib's failure has been attributed to off-target effects considered unique to that drug, while dalcetrapib's lack of efficacy might be attributed to its lack of potency – perhaps it does not raise HDL-C enough to confer clinical benefit. A CETP inhibitor that raises HDL-C to a greater degree without causing harmful off-target effects might yet prove therapeutically useful. Anacetrapib and evacetrapib are CETP inhibitors still in clinical development that might meet these criteria.

Anacetrapib binds CETP significantly more tightly than dalcetrapib in a fashion similar to torcetrapib [59]. Unlike torcetrapib, however, multiple trials have so far found no increased incidence of hypertension or adverse cardiovascular effects in anacetrapib-treated patients. Phase I studies demonstrated increased HDL-C and apoA-I, and decreased LDL-C, without increases in blood pressure [60]. A larger study in patients with primary hypercholesterolemia or mixed dyslipidemia randomized to combination statin and anacetrapib, anacetrapib alone, statin and placebo, or placebo alone found significantly increased HDL-C and decreased LDL-C and lipoprotein(a) (Lp(a)) for all anacetrapib-treated groups compared with placebo, without significant effect on subjects' blood pressure levels [61]. At the highest doses of anacetrapib, increases in HDL-C of up to 139% were seen, as well as decreases in LDL-C and Lp(a) of up to 40 and 50%, respectively. Similarly, the DEFINE trial of 1623 CHD patients on statin therapy randomized to anacetrapib or placebo showed a significant increase in HDL-C (138%), a significant decrease in LDL-C (40%) and Lp(a) (36%), no changes in blood pressure, aldosterone, or electrolyte levels, and no increased incidence of adverse cardiovascular events in the treatment group when compared with placebo [62]. The 30,000-subject REVEAL trial of anacetrapib in patients with pre-existing cardiovascular disease is scheduled to be completed at the beginning of 2017 [201]. Of note, anacetrapib's effects on LDL-C – nearly comparable to those of statin monotherapy – and on Lp(a) provide potential mechanisms beyond HDL-C raising by which anacetrapib might be cardio-protective [61]. Dalcetrapib, by contrast, did not have an appreciable effect on LDL-C levels [58].

Evacetrapib was the fourth CETP inhibitor to enter Phase III clinical development. A Phase II study of high LDL-C or low HDL-C patients randomized to evacetrapib at a range of doses, statin alone, evacetrapib/statin combination therapy or placebo found that evacetrapib alone increased HDL-C by 54–129% and decreased LDL-C by 14–36% compared with placebo. Evacetrapib given in combination with a statin increased HDL-C by 79–89% and decreased LDL-C by 11–14% compared with statin monotherapy [63]. Evidence that this improved lipid profile is in fact atheroprotective requires completion of the 11,000-subject ACCELERATE Phase III trial, scheduled for completion in September 2015 [202].

Despite the allocation of considerable resources, the question of whether or not CETP inhibition is a viable strategy to decrease cardiovascular risk has yet to be answered conclusively. As noted above, genetic variants in CETP that reduce its activity not only increase HDL-C but also reduce LDL-C. Similarly, anacetrapib and evacetrapib significantly decrease LDL-C in addition to raising HDL-C. Both drugs also lower Lp(a) through mechanisms that are unclear at present. These effects on LDL-C and Lp(a) may make it difficult to apportion mechanistic responsibility for any cardiovascular benefit from CETP inhibition that may be proven in the future. Should either remaining CETP inhibitor

be shown to decrease cardiovascular risk, it will be unclear whether cardioprotection is due to increased HDL-C, decreased LDL-C, decreased Lp(a) or some combination of all three factors. Cumulatively, these issues highlight the risk inherent in using genetic studies to identify HDL-related therapeutic targets without concurrent examination of atherosclerotic burden and cardiovascular risk, as well as the difficulty of specifically proving whether raising HDL-C has a beneficial effect on cardiovascular risk at all.

Endothelial lipase

Analysis of the protracted course of CETP investigation raises an important issue regarding single-gene studies in cardiovascular disease. Such studies have played and will continue to play a critical role in increasing our knowledge of lipid metabolism and identifying targets for drug development. However, cases of major genetic effects in these genes are often too rare to perform rigorous studies of cardiovascular end points. The progression of inquiry into EL provides an alternative model for single-gene investigation in which the decision to pursue drug development is guided by population-level genetic studies of low-frequency variants of large effect. Large-scale genetic research can provide data on the effect single genes have on overall cardiovascular risk prior to the initiation of costly and lengthy clinical trials.

EL (gene name *LIPG*) was first cloned and characterized in 1999 [64,65]; mice overexpressing EL were found to have decreased HDL-C and apoA-I levels [64]. EL destabilizes the HDL particle by hydrolyzing HDL phospholipids. The small, remodeled particle releases lipid-poor apoA-I; both apolipoprotein and HDL particles are subsequently catabolized at an increased rate, resulting in decreased HDL-C and apoA-I [12]. Similar decreases in HDL-C size and particle number, as well as apoA-I levels, have been found in other overexpression studies in mice [66–68]. The inverse relationship between EL activity and HDL-C levels has been reinforced by loss-of-function studies in mice. Inhibition of EL with an antibody significantly increased HDL-C levels in mice [69]. Another study found increased HDL-C in both *Lipg*^{-/-} and *Lipg*^{+/-} mice due to an increase in the absolute number of HDL particles [68]. Another noted that *Lipg*^{-/-} mice have elevated HDL-C, apoA-I and apoE levels, with an increased number of large HDL particles and decreased HDL clearance [70].

Measures of cholesterol efflux and overall reverse cholesterol transport in cell lines and animals are also affected by overexpression and knockout of EL, but not always in the manner one might hypothesize. For example, over-expression of EL in a mouse model led to greatly diminished HDL-C, apoA-I and scavenger receptor-BI-mediated cholesterol bidirectional flux, but also to increased ABCA1-mediated cholesterol efflux, despite the lower amount of apoA-I present; this was attributed to ABCA1's affinity for small, immature HDL particles of the type formed through EL's action [66]. In another study, EL overexpression was noted to cause cholesterol efflux from macrophages by promoting apoA-I binding to ABCA1, while EL suppression led to decreased cholesterol efflux [71]. However, a mouse study has found that while EL overexpression led to increased hepatic cholesterol content, it did not increase the excretion of cholesterol into feces [72]. Finally, EL/hepatic lipase (HL) double-knockout mice have been shown to have increased HDL-C

and cholesterol efflux capacity as compared with single-knockout mice, as expected, but did not demonstrate an increased rate of *in vivo* macrophage reverse cholesterol transport [73].

A number of analyses have examined the effect of *LIPG* common variants and rare mutations on lipid levels in human subjects. In a 2002 study, the *EL* gene was sequenced in 20 people with high HDL-C; six coding variants were identified [74]. One variant, T111I, was associated with increased HDL-C in some studies [70,75] but not others [76–79]. The T111I *EL* variant was shown to have normal lipolytic function *in vitro* [80] and was subsequently found to be in linkage disequilibrium with a promoter single nucleotide polymorphism (SNP) that affects *EL* transcription [81]. Thus, its association with HDL-C, if any, is likely to be due to its strong linkage disequilibrium with a causal SNP that influences transcription.

A second variant, N396S, was subsequently found to be highly significantly associated with increased HDL-C and apoA-I [81]. It was also shown to reduce lipolytic function *in vitro* and *in vivo*. The N396S variant was confirmed to be significantly associated with increased HDL-C in another large-scale study of more than 100,000 subjects that will be discussed below [37]. Despite some technical difficulties in measuring *EL* activity in human plasma, a number of assays have been developed, and data derived from them are consistent with the concept that reduced *EL* activity is associated with increased HDL-C levels [82,83].

Cumulatively, evidence in cell lines, animals and humans – especially the human genetic data – makes a convincing case that an inverse relationship exists between *EL* activity and HDL-C. These data strongly indicate that pharmacological inhibition of *EL* would raise HDL-C – a hypothesis borne out by pre-clinical data in mice [70]. Based on the strong evidence base that reduced *EL* activity causes increased HDL-C, there has been substantial interest in pharmacological inhibition of *EL* as a therapeutic strategy to raise HDL-C.

However, the connection between *EL* activity and atherosclerosis or cardiovascular risk is more uncertain. In addition to its effect on HDL-C levels, *EL* has been shown to affect angiogenesis and vasorelaxation by affecting the binding of HDL-associated sphingosine-1-phosphate with endothelial cell receptors [84] and generating lysophosphatidylcholine species, respectively [85]. Atherosclerosis-prone *Lipg*^{-/-}/*ApoE*^{-/-} mice showed attenuated atherosclerosis when compared with mice in which apoE alone was deleted [86]. However, another group reported that similar *Lipg*^{-/-}/*ApoE*^{-/-} mice, as well as *Lipg*^{-/-}/*Ldlr*^{-/-} mice, had higher HDL-C but no difference in aortic atherosclerotic lesion areas compared with *Lipg*^{+/+}/*ApoE*^{-/-} and *Lipg*^{+/+}/*Ldlr*^{-/-} mice [87]. Thus, the effect of *EL* deficiency on atherosclerosis in mice is uncertain.

Consequently, there has been substantial interest in determining whether genetic variation in *EL* is associated with cardiovascular risk in humans. An association study of 17 common *EL* SNPs (not including N396S) in a population of 3916 subjects found no significant associations with MI after correction for multiple testing [88]. A case-control study (described above) of 3375 subjects found no association between five separate common *EL* SNPs (again not including N396S) and MI, but did note an association between three of them and deep vein thrombosis risk [75]. However, another study involving sequencing of

177 individuals with HDL-C levels at or above the 90th percentile identified six novel *LIPG* mutations, as well as the N396S variant and another known variant, R476W. The two known mutations were found to reduce *in vitro* EL activity by 50% (partial loss of function), while the newly identified rare variants showed complete loss of function, and significantly increased cholesterol efflux acceptor capacity. A meta-analysis of 15,496 subjects found a nonsignificant trend toward reduced coronary artery disease in carriers of the more frequent N396S and R476W variants [89].

The most convincing evidence to date against an association between EL and cardiovascular disease comes from a 2012 Mendelian randomization study that examined the frequency of the loss-of-function N396S variant in a collected population of 20,913 MI cases compared with 95,407 controls to determine whether partial genetic EL deficiency caused by this mutation was in fact cardioprotective [37]. As noted above, there was a highly significant increase in HDL-C in carriers of the N396S variant. Based on observational epidemiology, this increase in HDL-C, if causal, would be predicted to result in a concurrent 13% decrease in MI risk. However, the variant N396S allele was not associated with decreased risk of MI (odds ratio [OR]: 0.99; 95% CI: 0.88–1.11; $p = 0.85$). While the effect of heterozygous EL N396S on HDL-C levels is modest, thus perhaps making it harder to see clear benefit with regard to CAD, the number of subjects studied provided adequate power to detect even a small reduction in MI.

Cumulatively, these data call into question whether EL inhibition will decrease CHD risk, even though it would be fully expected to increase HDL-C levels. Although a series of molecular inhibitors of EL have been reported [90,91], none have been advanced into the clinic. Additional data, such as pharmacologic inhibition demonstrating reduced atherosclerosis in preclinical models and/or additional human genetic data with genetic instruments of greater effect, are necessary to give sufficient confidence in this mechanism as a therapeutic target.

ApoC-III

The cases of CETP and EL provide two lessons for the future of single-gene studies in HDL-C research. First, they emphasize that raising HDL-C levels alone does not necessarily confer reduced cardiovascular risk. Second, well-designed genetic studies can provide researchers with a great deal of information regarding the advisability of targeting a particular gene for pharmaceutical development. Study of a third gene of interest for HDL-C, *APOC3*, may provide an opportunity to apply these lessons.

ApoC-III was first isolated and shown to be a noncompetitive inhibitor of LPL in 1972 [92]. The gene encoding apoC-III, *APOC3*, was cloned 11 years later [93], and soon after was shown to be organized in tandem with *APOA1*, *APOA4* and *APOA5* [94]. Preclinical data examining the relationship between apoC-III and HDL-C levels are somewhat limited. ApoC-III overexpression in mice led to increased TG and decreased apoA-I and HDL-C [95]. Conversely, a study of *APOC3* knockout mice found that they exhibited hypotriglyceridemia, although no significant changes in apoA-I or HDL-C levels were noted [96]. In a correlational study of 19 human subjects, HDL–apoC-III and HDL–apoA-I

concentrations were positively correlated, while there was an inverse correlation between HDL–apoC-III concentration and apoA-I catabolism, hypothesized to be due to apoC-III's inhibition of HL in addition to LPL [97]. A more recent study in 39 men with central obesity and 12 non-obese controls similarly found that subjects with central obesity had elevated apoC-III and HDL–apoC-III concentrations, along with a reduced fractional catabolic rate of HDL–apoC-III [98]. However, it also noted that HDL–apoC-III concentration itself was in fact associated with hypercatabolism of HDL–apoA-I, implying that apoC-III may play a role in lowering HDL-C, perhaps due to an increase in unstable, TG-rich HDL particles, given the hypertriglyceridemia caused by increased apoC-III-mediated inhibition of LPL.

A number of studies examined plasma and lipoprotein apoC-III concentrations and concluded that they are directly related to cardiovascular risk. A series of nested case–control studies determined that apoC-III content of VLDL and LDL was a significant predictor of subsequent coronary events [99], and that LDL with apoC-III levels were a strong predictor of recurrent coronary events in a population that had already had an MI [100]. A prospective cohort study of 633 patients with pre-existing CHD noted that elevated apoC-III levels (> 10.5 mg/dl above the median) were significantly associated with both total and cardiovascular mortality over a 5-year period [101], while another study found that for 739 CHD-free subjects who developed CHD over 10–14 years of follow-up and matched controls, LDL–apoC-III levels were significantly associated with CHD risk [102].

A trial of 162 male subjects postcoronary artery bypass grafting found that HDL–apoC-III levels were the primary risk factor predicting coronary artery disease progression in a combination colestipol/niacin-treated group; subjects without disease progression had high HDL–apoC-III and lower levels of apoC-III in VLDL and LDL [103]. Another trial of 270 hyperlipidemic subjects randomized to lovastatin or placebo noted that VLDL-associated apoC-III was a risk factor for progression of pre-existing mild-to-moderate atherosclerotic lesions [104]. A third 63-patient clinical trial noted that coronary artery disease progression, as measured by coronary angiography in both statin- and placebo-treated groups, was associated with apoC-III levels, as well as levels of apoC-III on VLDL and LDL [105]. Another study of 100 men who had undergone coronary angiography noted that CAD severity correlated with apoC-III concentration in heparin precipitate [106]. However, an earlier study found that apoC-III content was not linked with CAD progression [107]. In addition, a recent pooled analysis of two nested case–control studies comprising 634 CHD cases and 637 controls found that HDL–apoC-III was associated with a significantly increased risk of CHD, while apoC-III-free HDL was associated with significantly decreased risk [108]. Another paper showed increased apoC-III content on HDL in subjects with CHD than in those without; furthermore, apoC-III was shown to impair the endothelial antiapoptotic function of HDL [109].

Several genetic studies have investigated the relationship between apoC-III and HDL-C. Two subjects with very high HDL-C and apoA-I levels, as well as greatly decreased apoC-III levels, were found to be heterozygous for a K58E mutation [110]. Heterozygous carriers of an 8017G>C noncoding polymorphism (also known as SstI) had higher plasma apoC-III and TG concentrations, as well as smaller LDL peak particle diameter in a study of 91 healthy subjects [111]. A study of 1030 Chinese subjects noted that the same SstI SNP was

more common in women, but in men was associated with increased HDL-C [112]. The gene *GALNT2*, a locus linked with HDL-C levels [113], also warrants discussion. Its protein product is hypothesized to activate apoC-III, rendering it functional; two individuals with loss-of-function mutations in *GALNT2* were found to have elevated HDL-C and decreased glycosylation of apoC-III [114].

Studies of common genetic variants at the *APOC3* locus have suggested an association with CHD, although results are variable. One study found a weak, nonsignificant negative association of an 1100C>T SNP in *APOC3* with atherosclerosis in patients who had suffered MIs at a young age compared with age-matched controls [115]. The majority of studies have examined the -455T>C, -482C>T and 8017G>C (SstI) noncoding SNPs that are in linkage disequilibrium. Carriage of the minor allele at all three of these loci, termed the *APOC3**222 haplotype, has been associated with increased MI risk compared with wild-type status at all three loci in a case-control study of 3406 Costa Rican subjects [116]; however, a later study in the same population found that this increased cardiovascular risk, as well as higher apoC-III levels, were only seen in lean *APOC3**222 haplotype carriers and those with low fasting glucose. This effect was hypothesized to be due to insulin-mediated repression of apoC-III expression in wild-type individuals but not in mutation carriers or in subjects with insulin resistance [117]. Similarly, subjects homozygous for the -482C>T polymorphism in a study of 519 Turkish individuals had higher apoC-III levels only if they had abdominal obesity or insulin resistance; high circulating apoC-III levels were also noted to be predictive of CHD in this population, although the particular SNP itself was not [118]. However, another prospective cohort study of 498 subjects following high-throughput genotyping noted that the -482C>T polymorphism was significantly associated with major cardiac events, such as CHD-related death, nonfatal MI and revascularization [119]. In a study of 873 patients, 549 of whom had CHD and 270 had the metabolic syndrome, the -455T>C polymorphism by itself was associated with higher apoC-III levels as well as CHD; furthermore, patients with the metabolic syndrome were more likely to have increased apoC-III [120]. The same SNP has also been associated with lower HDL-C and acute coronary syndrome in a population of 229 Han Chinese cases compared with 254 healthy controls [121], although another study of 286 Han Chinese CHD cases and 325 controls found no relationship between either the -455T>C or -482C>T SNPs and CHD risk [122]. A study of 342 subjects with parental history of MI compared with 114 controls noted significantly higher apoB levels, but failed to find a difference in SstI polymorphisms between the two groups [123]. Another study in which 102 Italian subjects with established CHD and 104 controls were followed for 8 years found no association of the SstI locus with revascularization, MI or cardiovascular death [124]. Similarly, a prospective nested case-control study of 385 MI cases and 373 controls found no significant difference in SstI allele or genotype frequency between the two groups [125]. Another population study of 158 CHD patients, 35 patients with <70% stenosis on angiography and 151 CHD-free controls also failed to find any difference in SstI allele frequency between cases and controls [126], while a study of 219 diabetic and nondiabetic subjects found no association between SstI and either MI or diabetes [127]. However, a study of 200 Egyptian MI patients compared with 100 controls found that the SstI polymorphism was associated with a tripled risk of MI [128], and a larger study of 326 male MI patients and 361 controls noted a significant association of the SNP with MI [129].

Rare and low-frequency nonsynonymous variants in *APOC3* of greater effect may be more informative with regard to atherosclerosis. One study identified an *APOC3* stopgain mutation (R19X) predicted to cause complete loss of protein function [130]. Approximately 5% of a population of 809 old order Amish subjects was found to be heterozygous for this variant. In a subset of 20 related subjects, heterozygotes had apoC-III levels less than half of those of non-carrier relatives ($p = 0.0002$). Analysis of the entire population found that carriers had higher HDL-C (67 vs 55 mg/dl; $p = 9.0 \times 10^{-7}$) and lower LDL-C (116 vs 140 mg/dl; $p = 0.02$) and total cholesterol (191 vs 209 mg/dl; $p = 0.001$) when compared with noncarriers. Heterozygotes also had lower TG levels both when fasting as well as after an oral fat challenge. Importantly, coronary artery calcification scoring by CT scanning found that mutation carriers were much less likely to have detectable coronary artery calcification (OR: 0.35; 95% CI: 0.21–0.60), a marker of coronary atherosclerosis, or to have a score at or above their ethnicity-, sex- and age-specific 75th percentile (OR: 0.38; 95% CI: 0.19–0.77; $p = 0.003$). This finding was interpreted by the authors as indicative of a cardioprotective effect from partial apoC-III deficiency [130].

The low-frequency R19X mutation causing reduced apoC-III mass and activity is associated with increased HDL-C, as well as reduced TGs and LDL-C. This mutation also appears to be associated with reduced coronary atherosclerosis, although more data is required to confirm that this is the case. Taken together, these points suggest that therapy to reduce or inhibit apoC-III might not only raise HDL-C, but also reduce CHD risk. However, apoC-III – unlike CETP and EL – is a structural lipoprotein that may be difficult to inhibit utilizing a small-molecule approach. Consequently, attention has turned toward targeting apoC-III with biological approaches. One example, ISI-APOCIII_{RX}, is an apoC-III antisense oligonucleotide that has been studied as a potential intervention for Type 1 diabetes and shown to lower apoC-III levels in a rat model of that condition [131]. According to unpublished reports, the drug decreased apoC-III levels up to 78% in a Phase I study of 32 subjects and is currently undergoing a Phase II trial [203,204]. Similar gene silencing approaches have been carried out through the use of antisense oligonucleotides and RNAi directed against apoB-100 and PCSK9, as have been reviewed elsewhere [132].

Conclusion

Three monogenic causes of elevated HDL-C – CETP (encoded by *CETP*), EL (encoded by *LIPG*) and apoC-III (encoded by *APOC3*) – have received a great deal of attention over the past decade. Identification of these genes and analysis of rare genetic variants in them have allowed researchers to increase understanding of HDL metabolism significantly. However, development of related therapeutics to decrease cardiovascular risk has been less straightforward.

Despite the failure of two CETP inhibitors, the effect of potent CETP inhibition on cardiovascular end points is still unclear. The case of EL provides an example of the ability of population-level genetic studies to give information on cardiovascular disease end points. Finally, the mounting evidence that apoC-III levels are inversely related not only to HDL-C levels, but also to atherosclerosis and cardiovascular disease make it, unlike EL, a promising target for drug discovery.

The growing body of evidence that the clinical measure HDL-C does not reflect the functionality of HDL should guide further investigation into single genes thought to be relevant to HDL metabolism. Functionality measures such as cholesterol efflux potential – shown to be inversely related to CIMT and angiographic coronary atherosclerotic disease [133] – appear to be better metrics of a particular mutation's effect on reverse cholesterol transport and HDL-mediated athero protection than simple HDL-C levels. This investigational approach, when combined with studies of atherosclerosis and CHD, may prove fruitful for the investigation of other promising HDL-related genes, such as *SCARB1*, *LIPC* and *LPL*. Variation in *SCARB1*, encoding the HDL receptor scavenger receptor-BI, has been shown to lead to increased HDL-C in a population of 18 individuals heterozygous for a loss-of-function P297S mutation, although without obvious effect on carotid atherosclerosis [134]. Mutations in *LIPC*, which encodes HL, and in *LPL*, have been implicated in CHD in a recent Mendelian randomization study [135]; both genes warrant continued study.

The three genes discussed in this review illustrate both the potential benefits and pitfalls of single-gene studies in advancing HDL-related cardiovascular therapeutics. Such studies should continue to prove indispensable in advancing knowledge of the role HDL-C plays in reverse cholesterol transport and in identifying targets for drug development. In order to lead to efficacious pharmaceuticals, studies of newly identified genes of interest must focus not only on lipid levels such as HDL-C, but also on the effect of genetic variants on measures such as HDL functionality, atherosclerosis, stroke, MI and CHD-related death, as well as noncardiovascular outcomes.

Future perspective

Single-gene studies will continue to contribute important knowledge about HDL metabolism and potential therapeutic targets. The role of large-scale genetic studies in identifying promising genes and variants will grow in importance. Novel genes and mutations affecting HDL metabolism will continue to be identified through sequencing. In-depth phenotypic evaluations of subjects carrying these variants will enable careful characterization of their effects and potential utility as drug targets. In addition, Mendelian randomization studies will allow assessment of the relationship between these genes and CHD.

Acknowledgments

The authors wish to acknowledge P Zanoni and S Khetarpal for their suggestions regarding this paper.

DJ Rader serves as a consultant to several companies that are developing therapies targeting HDL, including Amgen, AstraZeneca, Bristol-Myers-Squibb, CSL, Eli Lilly, Johnson & Johnson, Merck & Co, Regulus and Novartis. This paper was supported in part by the National Center for Research Resources, grant TL1RR024133, and is now at the National Center for Advancing Translational Sciences, grant TL1R000138. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. This paper was also supported in part by a Doris Duke Charitable Foundation Clinical Research Mentorship.

References

- Papers of special note have been highlighted as:
- of interest

■■■of considerable interest

1. Ford ES, Ajani UA, Croft JB, et al. Explaining the decrease in U.S. deaths from coronary disease, 1980–2000. *N. Engl. J. Med.* 2007; 356(23):2388–2398. [PubMed: 17554120]
2. Boden WE, O'Rourke RA, Teo KK, et al. Optimal medical therapy with or without PCI for stable coronary disease. *N. Engl. J. Med.* 2007; 356(15):1503–1516. [PubMed: 17387127]
3. Frye RL, August P, Brooks MM, et al. A randomized trial of therapies for Type 2 diabetes and coronary artery disease. *N. Engl. J. Med.* 2009; 360(24):2503–2515. [PubMed: 19502645]
4. Abifadel M, Varret M, Rabes JP, et al. Mutations in *PCSK9* cause autosomal dominant hypercholesterolemia. *Nat. Genet.* 2003; 34(2):154–156. [PubMed: 12730697]
5. Cohen J, Pertsemlidis A, Kotowski IK, Graham R, Garcia CK, Hobbs HH. Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in *PCSK9*. *Nat. Genet.* 2005; 37(2):161–165. [PubMed: 15654334]
6. 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(12):1264–1272. [PubMed: 16554528]
7. Roth EM, McKenney JM, Hanotin C, Asset G, Stein EA. Atorvastatin with or without an antibody to *PCSK9* in primary hypercholesterolemia. *N. Engl. J. Med.* 2012; 367(20):1891–1900. [PubMed: 23113833]
8. Wilson PW, Castelli WP, Kannel WB. Coronary risk prediction in adults (the Framingham Heart Study). *Am. J. Cardiol.* 1987; 59(14):G91–G94.
9. Larach DB, Degoma EM, Rader DJ. Targeting high density lipoproteins in the prevention of cardiovascular disease? *Curr. Cardiol. Rep.* 2012
10. Motazacker MM, Peter J, Treskes M, Shoulders CC, Kuivenhoven JA, Hovingh GK. Evidence of a polygenic origin of extreme high-density lipoprotein cholesterol levels. *Arterioscler. Thromb. Vasc. Biol.* 2013; 33(7):1521–1528. [PubMed: 23685560]
11. Barter PJ, Brewer HB Jr, Chapman MJ, Hennekens CH, Rader DJ, Tall AR. Cholesteryl ester transfer protein: a novel target for raising HDL and inhibiting atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* 2003; 23(2):160–167. [PubMed: 12588754]
12. Maugeais C, Tietge UJ, Broedl UC, et al. Dose-dependent acceleration of high-density lipoprotein catabolism by endothelial lipase. *Circulation.* 2003; 108(17):2121–2126. [PubMed: 14517167]
13. Hesler CB, Swenson TL, Tall AR. Purification and characterization of a human plasma cholesteryl ester transfer protein. *J. Biol. Chem.* 1987; 262(5):2275–2282. [PubMed: 3818596]
14. ■ Brown ML, Inazu A, Hesler CB, et al. Molecular basis of lipid transfer protein deficiency in a family with increased high-density lipoproteins. *Nature.* 1989; 342(6248):448–451. [PubMed: 2586614] [Identification of two Japanese siblings with high HDL cholesterol (HDL-C) levels and genetic CETP deficiency.]
15. Inazu A, Brown ML, Hesler CB, et al. Increased high-density lipoprotein levels caused by a common cholesteryl-ester transfer protein gene mutation. *N. Engl. J. Med.* 1990; 323(18):1234–1238. [PubMed: 2215607]
16. Tato F, Vega GL, Tall AR, Grundy SM. Relation between cholesterol ester transfer protein activities and lipoprotein cholesterol in patients with hypercholesterolemia and combined hyperlipidemia. *Arterioscler. Thromb. Vasc. Biol.* 1995; 15(1):112–120. [PubMed: 7749806]
17. Kinoshita M, Teramoto T, Shimazu N, et al. CETP is a determinant of serum LDL-cholesterol but not HDL-cholesterol in healthy Japanese. *Atherosclerosis.* 1996; 120(1–2):75–82. [PubMed: 8645374]
18. McPherson R, Mann CJ, Tall AR, et al. Plasma concentrations of cholesteryl ester transfer protein in hyperlipoproteinemia. Relation to cholesteryl ester transfer protein activity and other lipoprotein variables. *Arterioscler. Thromb.* 1991; 11(4):797–804. [PubMed: 2065034]
19. Moulin P, Appel GB, Ginsberg HN, Tall AR. Increased concentration of plasma cholesteryl ester transfer protein in nephrotic syndrome: role in dyslipidemia. *J. Lipid Res.* 1992; 33(12):1817–1822. [PubMed: 1479291]

20. de Grooth GJ, Smilde TJ, Van Wissen S, et al. The relationship between cholesteryl ester transfer protein levels and risk factor profile in patients with familial hypercholesterolemia. *Atherosclerosis*. 2004; 173(2):261–267. [PubMed: 15064100]
21. Barter PJ, Kastelein JJ. Targeting cholesteryl ester transfer protein for the prevention and management of cardiovascular disease. *J. Am. Coll. Cardiol.* 2006; 47(3):492–499. [PubMed: 16458126]
22. Tanigawa H, Billheimer JT, Tohyama J, Zhang Y, Rothblat G, Rader DJ. Expression of cholesteryl ester transfer protein in mice promotes macrophage reverse cholesterol transport. *Circulation*. 2007; 116(11):1267–1273. [PubMed: 17709636]
23. Foger B, Chase M, Amar MJ, et al. Cholesteryl ester transfer protein corrects dysfunctional high density lipoproteins and reduces aortic atherosclerosis in lecithin cholesterol acyltransferase transgenic mice. *J. Biol. Chem.* 1999; 274(52):36912–36920. [PubMed: 10601244]
24. Borggreve SE, Hillege HL, Dallinga-Thie GM. High plasma cholesteryl ester transfer protein levels may favour reduced incidence of cardiovascular events in men with low triglycerides. *Eur. Heart J.* 2007; 28(8):1012–1018. [PubMed: 17409111]
25. Kappelle PJ, Pertion F, Hillege HL, Dallinga-Thie GM, Dullaart RP. High plasma cholesteryl ester transfer but not CETP mass predicts incident cardiovascular disease: a nested case-control study. *Atherosclerosis*. 2011; 217(1):249–252. [PubMed: 21524747]
26. Robins SJ, Lyass A, Brocia RW, Massaro JM, Vasan RS. Plasma lipid transfer proteins and cardiovascular disease. The Framingham Heart Study. *Atherosclerosis*. 2013; 228(1):230–236. [PubMed: 23477743]
27. Ritsch A, Scharnagl H, Eller P, et al. Cholesteryl ester transfer protein and mortality in patients undergoing coronary angiography: the Ludwigshafen Risk and Cardiovascular Health study. *Circulation*. 2010; 121(3):366–374. [PubMed: 20065167]
28. Hirano K, Yamashita S, Kuga Y, et al. Atherosclerotic disease in marked hyperalphalipoproteinemia. Combined reduction of cholesteryl ester transfer protein and hepatic triglyceride lipase. *Arterioscler. Thromb. Vasc. Biol.* 1995; 15(11):1849–1856. [PubMed: 7583564]
29. Hirano K, Yamashita S, Nakajima N, et al. Genetic cholesteryl ester transfer protein deficiency is extremely frequent in the Omagari area of Japan. Marked hyperalphalipoproteinemia caused by *CETP* gene mutation is not associated with longevity. *Arterioscler. Thromb. Vasc. Biol.* 1997; 17(6):1053–1059. [PubMed: 9194754]
30. Zhong S, Sharp DS, Grove JS, et al. Increased coronary heart disease in Japanese–American men with mutation in the cholesteryl ester transfer protein gene despite increased HDL levels. *J. Clin. Invest.* 1996; 97(12):2917–2923. [PubMed: 8675707]
31. 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.* 2004; 45(5):948–953. [PubMed: 14967821]
32. Bruce C, Sharp DS, Tall AR. Relationship of HDL and coronary heart disease to a common amino acid polymorphism in the cholesteryl ester transfer protein in men with and without hypertriglyceridemia. *J. Lipid Res.* 1998; 39(5):1071–1078. [PubMed: 9610775]
33. Agerholm-Larsen B, Nordestgaard BG, Steffensen R, Jensen G, Tybjaerg-Hansen A. Elevated HDL cholesterol is a risk factor for ischemic heart disease in white women when caused by a common mutation in the cholesteryl ester transfer protein gene. *Circulation*. 2000; 101(16):1907–1912. [PubMed: 10779455]
34. Kakko S, Tamminen M, Paivansalo M, et al. Cholesteryl ester transfer protein gene polymorphisms are associated with carotid atherosclerosis in men. *Eur. J. Clin. Invest.* 2000; 30(1):18–25. [PubMed: 10619997]
35. Thompson A, Di Angelantonio E, Sarwar N. Association of cholesteryl ester transfer protein genotypes with CETP mass and activity, lipid levels, and coronary risk. *JAMA*. 2008; 299(23):2777–2788. [PubMed: 18560005]
36. Dullaart RP, Sluiter WJ. Common variation in the CETP gene and the implications for cardiovascular disease and its treatment: an updated analysis. *Pharmacogenomics*. 2008; 9(6):747–763. [PubMed: 18518852]

37. Voight BF, Peloso GM, Orho-Melander M, et al. Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. *Lancet*. 2012; 380(9841):572–580. [PubMed: 22607825] [Mendelian randomization study that failed to find decreased myocardial infarction risk in carriers of the LIPG N396S allele, despite significantly increased HDL-C; called into question the cardioprotective effect of endothelial lipase deficiency or inhibition.]
38. Kappelle PJ, Gansevoort RT, Hillege HJ, Wolffenbuttel BH, Dullaart RP, Group PS. Common variation in cholesteryl ester transfer protein: relationship of first major adverse cardiovascular events with the apolipoprotein B/apolipoprotein A–I ratio and the total cholesterol/high-density lipoprotein cholesterol ratio. *J. Clin. Lipidol.* 2013; 7(1):56–64. [PubMed: 23351584]
39. Ridker PM, Pare 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(1):26–33. [PubMed: 20031564]
40. 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. *J. Am. Coll. Cardiol.* 2012; 60(20):2041–2048. [PubMed: 23083790]
41. Sugano M, Makino N, Sawada S, et al. Effect of antisense oligonucleotides against cholesteryl ester transfer protein on the development of atherosclerosis in cholesterol-fed rabbits. *J. Biol. Chem.* 1998; 273(9):5033–5036. [PubMed: 9478952]
42. Rittershaus CW, Miller DP, Thomas LJ, et al. Vaccine-induced antibodies inhibit CETP activity in vivo and reduce aortic lesions in a rabbit model of atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* 2000; 20(9):2106–2112. [PubMed: 10978256]
43. Davidson MH, Maki K, Umporowicz D, Wheeler A, Rittershaus C, Ryan U. The safety and immunogenicity of a CETP vaccine in healthy adults. *Atherosclerosis*. 2003; 169(1):113–120. [PubMed: 12860257]
44. Morehouse LA, Sugarman ED, Bourassa PA. Inhibition of CETP activity by torcetrapib reduces susceptibility to diet-induced atherosclerosis in New Zealand White rabbits. *J. Lipid Res.* 2007; 48(6):1263–1272. [PubMed: 17325387]
45. Clark RW, Sutfin TA, Ruggeri RB, et al. Raising high-density lipoprotein in humans through inhibition of cholesteryl ester transfer protein: an initial multidose study of torcetrapib. *Arterioscler. Thromb. Vasc. Biol.* 2004; 24(3):490–497. [PubMed: 14739125]
46. Brousseau ME, Schaefer EJ, Wolfe ML, et al. Effects of an inhibitor of cholesteryl ester transfer protein on HDL cholesterol. *N. Engl. J. Med.* 2004; 350(15):1505–1515. [PubMed: 15071125]
47. McKenney JM, Davidson MH, Shear CL, Revkin JH. Efficacy and safety of torcetrapib, a novel cholesteryl ester transfer protein inhibitor, in individuals with below-average high-density lipoprotein cholesterol levels on a background of atorvastatin. *J. Am. Coll. Cardiol.* 2006; 48(9):1782–1790. [PubMed: 17084250]
48. Davidson MH, McKenney JM, Shear CL, Revkin JH. Efficacy and safety of torcetrapib, a novel cholesteryl ester transfer protein inhibitor, in individuals with below-average high-density lipoprotein cholesterol levels. *J. Am. Coll. Cardiol.* 2006; 48(9):1774–1781. [PubMed: 17084249]
49. Nissen SE, Tardif JC, Nicholls SJ, et al. Effect of torcetrapib on the progression of coronary atherosclerosis. *N. Engl. J. Med.* 2007; 356(13):1304–1316. [PubMed: 17387129]
50. Kastelein JJ, van Leuven SI, Burgess L, et al. Effect of torcetrapib on carotid atherosclerosis in familial hypercholesterolemia. *N. Engl. J. Med.* 2007; 356(16):1620–1630. [PubMed: 17387131]
51. Bots ML, Visseren FL, Evans GW, et al. Torcetrapib and carotid intima-media thickness in mixed dyslipidaemia (RADIANCE 2 study): a randomised, double-blind trial. *Lancet*. 2007; 370(9582):153–160. [PubMed: 17630038]
52. Barter PJ, Caulfield M, Eriksson M, et al. Effects of torcetrapib in patients at high risk for coronary events. *N. Engl. J. Med.* 2007; 357(21):2109–2122. [PubMed: 17984165] [ILLUMINATE trial of torcetrapib; halted prematurely due to increased risk of adverse cardiovascular events and all-cause mortality in the torcetrapib-treated group.]
53. Rader DJ. Illuminating HDL – is it still a viable therapeutic target? *N. Engl. J. Med.* 2007; 357(21):2180–2183. [PubMed: 17984168]

54. Okamoto H, Yonemori F, Wakitani K, Minowa T, Maeda K, Shinkai H. A cholesteryl ester transfer protein inhibitor attenuates atherosclerosis in rabbits. *Nature*. 2000; 406(6792):203–207. [PubMed: 10910363]
55. Huang Z, Inazu A, Nohara A, Higashikata T, Mabuchi H. Cholesteryl ester transfer protein inhibitor (JTT-705) and the development of atherosclerosis in rabbits with severe hypercholesterolaemia. *Clin. Sci. (Lond.)*. 2002; 103(6):587–594. [PubMed: 12444911]
56. de Grooth GJ, Kuivenhoven JA, Stalenhoef AF. Efficacy and safety of a novel cholesteryl ester transfer protein inhibitor, JTT-705, in humans: a randomized Phase II dose-response study. *Circulation*. 2002; 105(18):2159–2165. [PubMed: 11994249]
57. Fayad ZA, Mani V, Woodward M, et al. Safety and efficacy of dalcetrapib on atherosclerotic disease using novel non-invasive multimodality imaging (dal-PLAQUE): a randomised clinical trial. *Lancet*. 2011; 378(9802):1547–1559. [PubMed: 21908036]
58. Schwartz GG, Olsson AG, Abt M, et al. Effects of dalcetrapib in patients with a recent acute coronary syndrome. *N. Engl. J. Med*. 2012; 367(22):2089–2099. [PubMed: 23126252] [Dal-OUTCOMES trial of dalcetrapib; stopped early due to futility, as no reduced risk of recurrent myocardial infarction was seen in the dalcetrapib-treated group, despite significantly increased HDL-C.]
59. Ranalletta M, Bierilo KK, Chen Y, et al. Biochemical characterization of cholesteryl ester transfer protein inhibitors. *J. Lipid Res*. 2010; 51(9):2739–2752. [PubMed: 20458119]
60. Krishna R, Anderson MS, Bergman AJ, et al. Effect of the cholesteryl ester transfer protein inhibitor, anacetrapib, on lipoproteins in patients with dyslipidaemia and on 24-h ambulatory blood pressure in healthy individuals: two double-blind, randomised placebo-controlled Phase I studies. *Lancet*. 2007; 370(9603):1907–1914. [PubMed: 18068514]
61. 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*. 2009; 157(2):352–360.e2. [PubMed: 19185645]
62. Cannon CP, Shah S, Dansky HM, et al. Safety of anacetrapib in patients with or at high risk for coronary heart disease. *N. Engl. J. Med*. 2010; 363(25):2406–2415. [PubMed: 21082868]
63. 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(19):2099–2109. [PubMed: 22089718]
64. Jaye M, Lynch KJ, Krawiec J, et al. A novel endothelial-derived lipase that modulates HDL metabolism. *Nat. Genet*. 1999; 21(4):424–428. [PubMed: 10192396] [Initial cloning and characterization of endothelial lipase; overexpression studies in mice led to decreased HDL-C and apoA-I levels.]
65. Hirata K, Dichek HL, Cioffi JA, et al. Cloning of a unique lipase from endothelial cells extends the lipase gene family. *J. Biol. Chem*. 1999; 274(20):14170–14175. [PubMed: 10318835]
66. Yancey PG, Kawashiri MA, Moore R, et al. In vivo modulation of HDL phospholipid has opposing effects on SR-BI- and ABCA1-mediated cholesterol efflux. *J. Lipid Res*. 2004; 45(2):337–346. [PubMed: 14594995]
67. Nijstad N, Wiersma H, Gautier T, van der Giet M, Maugeais C, Tietge UJ. Scavenger receptor BI-mediated selective uptake is required for the remodeling of high density lipoprotein by endothelial lipase. *J. Biol. Chem*. 2009; 284(10):6093–6100. [PubMed: 19136670]
68. Ishida T, Choi S, Kundu RK, et al. Endothelial lipase is a major determinant of HDL level. *J. Clin. Invest*. 2003; 111(3):347–355. [PubMed: 12569160]
69. Jin W, Millar JS, Broedl U, Glick JM, Rader DJ. Inhibition of endothelial lipase causes increased HDL cholesterol levels in vivo. *J. Clin. Invest*. 2003; 111(3):357–362. [PubMed: 12569161]
70. Ma K, Cilingiroglu M, Otvos JD, Ballantyne CM, Marian AJ, Chan L. Endothelial lipase is a major genetic determinant for high-density lipoprotein concentration, structure, and metabolism. *Proc. Natl Acad. Sci. USA*. 2003; 100(5):2748–2753. [PubMed: 12601178]
71. Qiu G, Hill JS. Endothelial lipase promotes apolipoprotein AI-mediated cholesterol efflux in THP-1 macrophages. *Arterioscler. Thromb. Vasc. Biol*. 2009; 29(1):84–91. [PubMed: 18988890]

72. Wiersma H, Gatti A, Nijstad N, Kuipers F, Tietge UJ. Hepatic SR-BI, not endothelial lipase, expression determines biliary cholesterol secretion in mice. *J. Lipid Res.* 2009; 50(8):1571–1580. [PubMed: 19252221]
73. Brown RJ, Lagor WR, Sankaranarayanan S. Impact of combined deficiency of hepatic lipase and endothelial lipase on the metabolism of both high-density lipoproteins and apolipoprotein B-containing lipoproteins. *Circ. Res.* 2010; 107(3):357–364. [PubMed: 20558822]
74. deLemos AS, Wolfe ML, Long CJ, Sivapackianathan R, Rader DJ. Identification of genetic variants in endothelial lipase in persons with elevated high-density lipoprotein cholesterol. *Circulation.* 2002; 106(11):1321–1326. [PubMed: 12221047]
75. Vergeer M, Cohn DM, Boekholdt SM, et al. Lack of association between common genetic variation in endothelial lipase (LIPG) and the risk for CAD and DVT. *Atherosclerosis.* 2010; 211(2):558–564. [PubMed: 20466371]
76. Yamakawa-Kobayashi K, Yanagi H, Endo K, Arinami T, Hamaguchi H. Relationship between serum HDL-C levels and common genetic variants of the endothelial lipase gene in Japanese school-aged children. *Hum. Genet.* 2003; 113(4):311–315. [PubMed: 12884003]
77. Paradis ME, Couture P, Bosse Y, et al. The T111I mutation in the EL gene modulates the impact of dietary fat on the HDL profile in women. *J. Lipid Res.* 2003; 44(10):1902–1908. [PubMed: 12867537]
78. Razzaghi H, Santorico SA, Kamboh MI. Population-based resequencing of LIPG and ZNF202 genes in subjects with extreme HDL levels. *Front. Genet.* 2012; 3:89. [PubMed: 22723803]
79. Jensen MK, Rimm EB, Mukamal KJ, et al. The T111I variant in the endothelial lipase gene and risk of coronary heart disease in three independent populations. *Eur. Heart J.* 2009; 30(13):1584–1589. [PubMed: 19411665]
80. Edmondson AC, Brown RJ, Kathiresan S, et al. Loss-of-function variants in endothelial lipase are a cause of elevated HDL cholesterol in humans. *J. Clin. Invest.* 2009; 119(4):1042–1050. [PubMed: 19287092]
81. Khetarpal SA, Edmondson AC, Raghavan A. Mining the LIPG allelic spectrum reveals the contribution of rare and common regulatory variants to HDL cholesterol. *PLoS Genet.* 2011; 7(12):e1002393. [PubMed: 22174694]
82. McCoy MG, Sun GS, Marchadier D, Maugeais C, Glick JM, Rader DJ. Characterization of the lipolytic activity of endothelial lipase. *J. Lipid Res.* 2002; 43(6):921–929. [PubMed: 12032167]
83. Miksztowicz V, McCoy MG, Schreier L, et al. Endothelial lipase activity predicts high-density lipoprotein catabolism in hemodialysis: novel phospholipase assay in postheparin human plasma. *Arterioscler. Thromb. Vasc. Biol.* 2012; 32(12):3033–3040. [PubMed: 23104846]
84. Tatematsu S, Francis SA, Natarajan P, et al. Endothelial lipase is a critical determinant of high-density lipoprotein-stimulated sphingosine 1-phosphate-dependent signaling in vascular endothelium. *Arterioscler. Thromb. Vasc. Biol.* 2013; 33(8):1788–1794. [PubMed: 23723371]
85. Rao SP, Riederer M, Lechleitner M, et al. Acyl chain-dependent effect of lysophosphatidylcholine on endothelium-dependent vasorelaxation. *PLoS ONE.* 2013; 8(5):e65155. [PubMed: 23741477]
86. Ishida T, Choi SY, Kundu RK, et al. Endothelial lipase modulates susceptibility to atherosclerosis in apolipoprotein-E-deficient mice. *J. Biol. Chem.* 2004; 279(43):45085–45092. [PubMed: 15304490]
87. Ko KW, Paul A, Ma K, Li L, Chan L. Endothelial lipase modulates HDL but has no effect on atherosclerosis development in apoE^{-/-} and LDLR^{-/-} mice. *J. Lipid Res.* 2005; 46(12):2586–2594. [PubMed: 16199802]
88. Mank-Seymour AR, Durham KL, Thompson JF, Seymour AB, Milos PM. Association between single-nucleotide polymorphisms in the endothelial lipase (LIPG) gene and high-density lipoprotein cholesterol levels. *Biochim. Biophys. Acta.* 2004; 1636(1):40–46. [PubMed: 14984737]
89. Singaraja RR, Sivapalaratnam S, Hovingh K. The impact of partial and complete loss-of-function mutations in endothelial lipase on high-density lipoprotein levels and functionality in humans. *Circ. Cardiovasc. Genet.* 2013; 6(1):54–62. [PubMed: 23243195]
90. Goodman KB, Bury MJ, Cheung M, et al. Discovery of potent, selective sulfonylfuran urea endothelial lipase inhibitors. *Bioorg. Med. Chem. Lett.* 2009; 19(1):27–30. [PubMed: 19058966]

91. O'Connell DP, LeBlanc DF, Cromley D, Billheimer J, Rader DJ, Bachovchin WW. Design and synthesis of boronic acid inhibitors of endothelial lipase. *Bioorg. Med. Chem. Lett.* 2012; 22(3): 1397–1401. [PubMed: 22225633]
92. Brown WV, Baginsky ML. Inhibition of lipoprotein lipase by an apoprotein of human very low density lipoprotein. *Biochem. Biophys. Res. Commun.* 1972; 46(2):375–382. [PubMed: 5057882] [Initial isolation of the apoC-III protein and characterization as a noncompetitive inhibitor of lipoprotein lipase.]
93. Karathanasis SK, McPherson J, Zannis VI, Breslow JL. Linkage of human apolipoproteins A-I and C-III genes. *Nature.* 1983; 304(5924):371–373. [PubMed: 6308458]
94. Karathanasis SK. Apolipoprotein multigene family: tandem organization of human apolipoprotein AI, CIII, and AIV genes. *Proc. Natl Acad. Sci. USA.* 1985; 82(19):6374–6378. [PubMed: 3931073]
95. Ito Y, Azrolan N, O'Connell A, Walsh A, Breslow JL. Hypertriglyceridemia as a result of human apo CIII gene expression in transgenic mice. *Science.* 1990; 249(4970):790–793. [PubMed: 2167514]
96. Maeda N, Li H, Lee D, Oliver P, Quarfordt SH, Osada J. Targeted disruption of the apolipoprotein C-III gene in mice results in hypotriglyceridemia and protection from postprandial hypertriglyceridemia. *J. Biol. Chem.* 1994; 269(38):23610–23616. [PubMed: 8089130]
97. Le NA, Gibson JC, Ginsberg HN. Independent regulation of plasma apolipoprotein C-II and C-III concentrations in very low density and high density lipoproteins: implications for the regulation of the catabolism of these lipoproteins. *J. Lipid Res.* 1988; 29(5):669–677. [PubMed: 3411241]
98. Chan DC, Nguyen MN, Watts GF, Barrett PH. Plasma apolipoprotein C-III transport in centrally obese men: associations with very low-density lipoprotein apolipoprotein B and high-density lipoprotein apolipoprotein A-I metabolism. *J. Clin. Endocrinol. Metab.* 2008; 93(2):557–564. [PubMed: 18000086]
99. Sacks FM, Alaupovic P, Moye LA, et al. VLDL, apolipoproteins B, CIII, and E, and risk of recurrent coronary events in the Cholesterol and Recurrent Events (CARE) trial. *Circulation.* 2000; 102(16):1886–1892. [PubMed: 11034934]
100. Lee SJ, Campos H, Moye LA, Sacks FM. LDL containing apolipoprotein CIII is an independent risk factor for coronary events in diabetic patients. *Arterioscler. Thromb. Vasc. Biol.* 2003; 23(5): 853–858. [PubMed: 12637336]
101. Olivieri O, Martinelli N, Girelli D, et al. Apolipoprotein C-III predicts cardiovascular mortality in severe coronary artery disease and is associated with an enhanced plasma thrombin generation. *J. Thromb. Haemost.* 2010; 8(3):463–471. [PubMed: 20002542]
102. Mendivil CO, Rimm EB, Furtado J, Chiuve SE, Sacks FM. Low-density lipoproteins containing apolipoprotein C-III and the risk of coronary heart disease. *Circulation.* 2011; 124(19):2065–2072. [PubMed: 21986282]
103. Blankenhorn DH, Alaupovic P, Wickham E, Chin HP, Azen SP. Prediction of angiographic change in native human coronary arteries and aortocoronary bypass grafts. Lipid and nonlipid factors. *Circulation.* 1990; 81(2):470–476. [PubMed: 2404631]
104. Hodis HN, Mack WJ, Azen SP, et al. Triglyceride- and cholesterol-rich lipoproteins have a differential effect on mild/moderate and severe lesion progression as assessed by quantitative coronary angiography in a controlled trial of lovastatin. *Circulation.* 1994; 90(1):42–49. [PubMed: 8026027]
105. Alaupovic P, Mack WJ, Knight-Gibson C, Hodis HN. The role of triglyceride-rich lipoprotein families in the progression of atherosclerotic lesions as determined by sequential coronary angiography from a controlled clinical trial. *Arterioscler. Thromb. Vasc. Biol.* 1997; 17(4):715–722. [PubMed: 9108785]
106. Koren E, Corder C, Mueller G, et al. Triglyceride enriched lipoprotein particles correlate with the severity of coronary artery disease. *Atherosclerosis.* 1996; 122(1):105–115. [PubMed: 8724117]
107. Genest JJ Jr, Bard JM, Fruchart JC, Ordovas JM, Wilson PF, Schaefer EJ. Plasma apolipoprotein A-I, A-II, B, E and C-III containing particles in men with premature coronary artery disease. *Atherosclerosis.* 1991; 90(2–3):149–157. [PubMed: 1684707]

108. Jensen MK, Rimm EB, Furtado JD, Sacks FM. Apolipoprotein C-III as a potential modulator of the association between HDL-cholesterol and incident coronary heart disease. *J. Am. Heart Assoc.* 2012; 1(2) pii: jah3-e000232.
109. Riwanto M, Rohrer L, Roschitzki B, et al. Altered activation of endothelial anti- and proapoptotic pathways by high-density lipoprotein from patients with coronary artery disease: role of high-density lipoprotein proteome remodeling. *Circulation.* 2013; 127(8):891–904. [PubMed: 23349247]
110. von Eckardstein A, Holz H, Sandkamp M, Weng W, Funke H, Assmann G. Apolipoprotein C-III(Lys58----Glu). Identification of an apolipoprotein C-III variant in a family with hyperalphalipoproteinemia. *J. Clin. Invest.* 1991; 87(5):1724–1731. [PubMed: 2022742]
111. Herron KL, Lofgren IE, Adiconis X, Ordovas JM, Fernandez ML. Associations between plasma lipid parameters and *APOC3* and *APOA4* genotypes in a healthy population are independent of dietary cholesterol intake. *Atherosclerosis.* 2006; 184(1):113–120. [PubMed: 16326171]
112. Yin RX, Li YY, Lai CQ. Apolipoprotein A1/ C3/A5 haplotypes and serum lipid levels. *Lipids Health Dis.* 2011; 10:140. [PubMed: 21854571]
113. Teslovich TM, Musunuru K, Smith AV, et al. Biological, clinical and population relevance of 95 loci for blood lipids. *Nature.* 2010; 466(7307):707–713. [PubMed: 20686565]
114. Holleboom AG, Karlsson H, Lin RS, et al. Heterozygosity for a loss-of-function mutation in *GALNT2* improves plasma triglyceride clearance in man. *Cell Metab.* 2011; 14(6):811–818. [PubMed: 22152306]
115. Peacock RE, Hamsten A, Johansson J, Nilsson-Ehle P, Humphries SE. Associations of genotypes at the apolipoprotein AI-CIII-AIV, apolipoprotein B and lipoprotein lipase gene loci with coronary atherosclerosis and high density lipoprotein subclasses. *Clin. Genet.* 1994; 46(4):273–282. [PubMed: 7834891]
116. Ruiz-Narvaez EA, Yang Y, Nakanishi Y, Kirchdorfer J, Campos H. *APOC3/A5* haplotypes, lipid levels, and risk of myocardial infarction in the Central Valley of Costa Rica. *J. Lipid Res.* 2005; 46(12):2605–2613. [PubMed: 16192625]
117. Ruiz-Narvaez EA, Sacks FM, Campos H. Abdominal obesity and hyperglycemia mask the effect of a common *APOC3* haplotype on the risk of myocardial infarction. *Am. J. Clin. Nutr.* 2008; 87(6):1932–1938. [PubMed: 18541587]
118. Onat A, Erginel-Unaltuna N, Coban N, Cicek G, Yuksel H. *APOC3-482C>T* polymorphism, circulating apolipoprotein C-III and smoking: interrelation and roles in predicting Type-2 diabetes and coronary disease. *Clin. Biochem.* 2011; 44(5–6):391–396. [PubMed: 21185820]
119. Andreassi MG, Adlerstein D, Carpeggiani C. Individual and summed effects of high-risk genetic polymorphisms on recurrent cardiovascular events following ischemic heart disease. *Atherosclerosis.* 2012; 223(2):409–415. [PubMed: 22717531]
120. Olivieri O, Bassi A, Stranieri C, et al. Apolipoprotein C-III, metabolic syndrome, and risk of coronary artery disease. *J. Lipid Res.* 2003; 44(12):2374–2381. [PubMed: 14563827]
121. Ding Y, Zhu MA, Wang ZX, Zhu J, Feng JB, Li DS. Associations of polymorphisms in the apolipoprotein *APOA1-C3-A5* gene cluster with acute coronary syndrome. *J. Biomed. Biotechnol.* 2012; 2012:509420. [PubMed: 22675253]
122. Yu J, Huang J, Liang Y, et al. Lack of association between apolipoprotein C3 gene polymorphisms and risk of coronary heart disease in a Han population in East China. *Lipids Health Dis.* 2011; 10:200. [PubMed: 22054125]
123. Boer JM, Feskens EJ, Kuivenhoven JA, et al. Parental history of myocardial infarction: lipid traits, gene polymorphisms and lifestyle. *Atherosclerosis.* 2001; 155(1):149–156. [PubMed: 11223436]
124. Baroni MG, Berni A, Romeo S, et al. Genetic study of common variants at the *ApoE*, *ApoAI*, *ApoCIII*, *ApoB*, lipoprotein lipase (*LPL*) and hepatic lipase (*LIPC*) genes and coronary artery disease (CAD): variation in *LIPC* gene associates with clinical outcomes in patients with established CAD. *BMC Med. Genet.* 2003; 4:8. [PubMed: 12964943]
125. Liu S, Song Y, Hu FB, et al. A prospective study of the *APOA1* XmnI and *APOC3* SstI polymorphisms in the *APOA1/C3/A4* gene cluster and risk of incident myocardial infarction in men. *Atherosclerosis.* 2004; 177(1):119–126. [PubMed: 15488874]

126. Chhabra S, Narang R, Lakshmy R, et al. Apolipoprotein C3 SstI polymorphism in the risk assessment of CAD. *Mol. Cell. Biochem.* 2004; 259(1–2):59–66. [PubMed: 15124908]
127. Relvas WG, Izar MC, Helfenstein T, et al. Relationship between gene polymorphisms and prevalence of myocardial infarction among diabetic and non-diabetic subjects. *Atherosclerosis.* 2005; 178(1):101–105. [PubMed: 15585206]
128. Abd El-Aziz TA, Mohamed RH, Hashem RM. Association of lipoprotein lipase and apolipoprotein C-III genes polymorphism with acute myocardial infarction in diabetic patients. *Mol. Cell. Biochem.* 2011; 354(1–2):141–150. [PubMed: 21499891]
129. Sediri Y, Kallel A, Feki M, et al. Association of a DNA polymorphism of the apolipoprotein AI-CIII-AIV gene cluster with myocardial infarction in a Tunisian population. *Eur. J. Intern. Med.* 2011; 22(4):407–411. [PubMed: 21767760]
130. ■■■ Pollin TI, Damcott CM, Shen H, et al. A null mutation in human *APOC3* confers a favorable plasma lipid profile and apparent cardioprotection. *Science.* 2008; 322(5908):1702–1705. [PubMed: 19074352] [Identification of the *APOC3* R19X nonsense mutation in a population of old order Amish. Heterozygotes for this variant had increased HDL-C and decreased LDL cholesterol, total cholesterol and triglycerides, and less detectable coronary artery calcification. These results implied a cardioprotective effect of partial apoC-III deficiency.]
131. Holmberg R, Refai E, Hoog A, et al. Lowering apolipoprotein CIII delays onset of Type 1 diabetes. *Proc. Natl Acad. Sci. USA.* 2011; 108(26):10685–10689. [PubMed: 21670290]
132. Makinen PI, Yla-Herttuala S. Therapeutic gene targeting approaches for the treatment of dyslipidemias and atherosclerosis. *Curr. Opin. Lipidol.* 2013; 24(2):116–122. [PubMed: 23314926]
133. Khera AV, Cuchel M, de la Llera-Moya M, et al. Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. *N. Engl. J. Med.* 2011; 364(2):127–135. [PubMed: 21226578]
134. Vergeer M, Korporaal SJ, Franssen R, et al. Genetic variant of the scavenger receptor BI in humans. *N. Engl. J. Med.* 2011; 364(2):136–145. [PubMed: 21226579]
135. Varbo A, Benn M, Tybjaerg-Hansen A, Jorgensen AB, Frikke-Schmidt R, Nordestgaard BG. Remnant cholesterol as a causal risk factor for ischemic heart disease. *J. Am. Coll. Cardiol.* 2013; 61(4):427–436. [PubMed: 23265341]

Websites

201. REVEAL: Randomized evaluation of the effects of anacetrapib through lipid modification. <http://clinicaltrials.gov/show/NCT01252953>
202. A study of evacetrapib in high-risk vascular disease (ACCELERATE). <http://clinicaltrials.gov/show/NCT01687998>
203. Isis reports positive Phase 1 data demonstrating ISIS-APOCIIIIRx produces significant reductions in apoC-III protein levels and triglycerides. 2011. <http://ir.isispharm.com/phoenix.zhtml?c=222170&p=irolnewsArticle&ID=1636573>
204. Mechanistic study of ISIS ISIS-APOCIIIIRX in Type 2 diabetes. 2012. <http://clinicaltrials.gov/show/NCT01647308>

Executive summary

Background

- Loss-of-function mutations in *CETP*, *LIPG*, and *APOC3* (encoding CETP, endothelial lipase [EL], and apoC-III, respectively) are known monogenic causes of elevated HDL-cholesterol (HDL-C) levels.

CETP

- CETP activity correlates directly with LDL-cholesterol and inversely with HDL-C. Individuals with genetic CETP deficiency appear to have a modest reduction in coronary heart disease risk. However, clinical trials of the CETP inhibitors torcetrapib and dalcetrapib have failed to show a decrease in coronary heart disease incidence. Preclinical studies of CETP deficiency and CETP inhibitors alike may have been overly focused on seemingly favorable lipid profile changes instead of atherosclerotic burden and cardiovascular risk.

Endothelial lipase

- EL activity correlates inversely with HDL-C. However, animal studies have failed to conclusively show that decreased EL activity decreases atherosclerosis susceptibility. Furthermore, a large-scale genetic study did not show any association between a loss-of-function mutation in *LIPG* (N396S) and myocardial infarction risk, despite a substantial increase in HDL-C in mutation carriers. Development of pharmacological endothelial lipase inhibitors has slowed following publication of this study.

ApoC-III

- ApoC-III levels correlate directly with triglyceride levels and inversely with HDL-C. A number of preclinical and clinical studies convincingly show that apoC-III levels are also directly related with cardiovascular risk, that certain gain-of-function *APOC3* variants are associated with major cardiac events and that a common loss-of-function variant (R19X) is cardioprotective. These findings have increased interest in targeting apoC-III in order to decrease atherosclerosis and CHD risk.

Conclusion

- These three cases illustrate the importance of single-gene studies to future translational research and drug development relating to HDL-C. Such studies must focus not only on mutations and medications that lead to favorable lipid profile changes, but also on measures of atherosclerosis and cardiovascular disease risk. Population-wide genetic studies have proven useful in attaining such data.