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CETP Polymorphism (TaqIB) Associates with Risk in Postinfarction Patients with High HDL Cholesterol and High CRP Levels:

Corsetti: TaqIB Cardiac Risk in High HDL-C/High CRP Patients

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Abstract

Objective—To investigate roles of inflammation and a cholesteryl ester transfer protein (CETP) polymorphism potentially related to recent findings demonstrating coronary risk with increasing HDL cholesterol (HDL-C).

Methods and Results—A novel graphical exploratory data analysis tool allowed examination of coronary risk in postinfarction patients relating to HDL-C and C-reactive protein (CRP). Results demonstrated a high-risk subgroup defined by high HDL-C and CRP exhibiting larger HDL particles and lower lipoprotein-associated phospholipaseA_2 (Lp-PLA_2) levels than lower-risk patients. Subgroup CETP-associated risk was probed using a functional *CETP* polymorphism (TaqIB, rs708272). Multivariable modeling revealed in the high-risk subgroup greater risk for B2 allele-carriers (less CETP activity) versus B1 homozygotes (hazard ratio 2.41, 95% CI 1.04-5.60, p=0.041). Within the high-risk subgroup, B2 allele-carriers had higher serum amyloid A levels than B1 homozygotes. Evidence is also presented demonstrating *CETP* genotypic differences in HDL subfraction distributions regarding nonHDL-C and Lp-PLA_2 potentially relating to impaired HDL remodeling.

Conclusions—Postinfarction patients with high HDL-C and CRP levels demonstrate increased risk for recurrent events. Future studies should aim at characterizing altered HDL particles from such patients and elucidating mechanistic details related to inflammation and HDL particle remodeling. Such patients should be considered in drug trials involving raising HDL-C.

Keywords

Atherosclerosis; cardiovascular diseases; inflammation; cholesteryl ester transfer protein; TaqIB

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Inverse association between high-density lipoprotein cholesterol (HDL-C) and cardiovascular disease (CVD) is well-established [1,2]. However, recent work indicates that higher HDL-C levels may not always be protective [3-7]. Consistent with this view is our identification in the Thrombogenic Factors and Recurrent Coronary Events (THROMBO) postinfarction study, of a subgroup of non-diabetic patients with high C-reactive protein (CRP) levels who show recurrent risk with increasing HDL-C [3,7]. Recently, we extended these studies to a healthy population (Prevention of Renal and Vascular End-Stage Disease study - PREVEND) to determine whether primary coronary risk acted similarly [8]. In addition to demonstrating a high-risk subgroup at high levels of HDL-C and CRP, the study also identified a second high-risk subgroup that was most apparent at high CRP levels but occurred at low levels of HDL-C as might be expected from many previous studies. In view of the high CRP levels in both high HDL-C subgroups and the importance of inflammation in atherogenesis [9,10], we speculated that HDL-C associated risk derived from inflammation and oxidative stress induced impairment of anti-inflammatory and anti-atherogenic features of HDL functionality [3,7].

HDL particle remodeling is a prominent feature of HDL metabolism whereby heterogeneous HDL particle subfractions continuously inter-convert through interactions with other lipoproteins, transfer proteins, and lipolytic enzymes, and as such, it plays a major role in cholesterol transport. An important step in HDL remodeling is the transfer of cholesteryl ester from cholesterol-rich HDL particles in exchange for triglycerides from apolipoprotein B (apoB)-containing cholesterol acceptor particles. This process is mediated by cholesteryl ester transfer protein (CETP) [11-13].

In view of the important role of CETP in HDL particle remodeling, compromise in CETP action would be expected to impair remodeling. This could result in accumulation of large cholesterol-rich HDL particles. Such particles, particularly in an inflammatory setting, might more readily undergo pro-atherogenic transformation. Thus, we hypothesized that compromised CETP activity would play a role in establishing the risk associated with high HDL-C and inflammation. To test this hypothesis, we probed effects of decreased CETP activity on recurrent coronary event risk in THROMBO patients with high HDL-C and CRP levels using the TaqIB (rs708272) polymorphism of *CETP*, a marker with well-characterized differences in CETP activity [11,12] and well-suited to serve as a probe [14,15].

Methods

Study Population

The study population was comprised of non-diabetic patients (N = 767) of the THROMBO postinfarction study [16]. Patients (with and without previous MI) were enrolled after index MI and followed for recurrence (average 26 months). Recurrent coronary events included: cardiac death, myocardial infarction, or unstable angina (hospitalization during follow-up with increase in either frequency or duration of angina symptoms, or development of new angina at rest - whichever occurred first with both requiring ischemic ECG changes without enzyme elevation). THROMBO was carried out with approval of and according to guidelines of Research Subjects Review Boards of participating institutions.

Blood Markers

Fasting blood specimens were drawn two months after index MI. Apolipoprotein B (apoB), total cholesterol, lipoprotein-associated phospholipase A_2 (Lp-PLA_2), apolipoprotein A-I (apoA-I), HDL-C, triglyceride, glucose, insulin, lipoprotein(a) (Lp(a)), plasminogen activator inhibitor-1 (PAI-1), CRP, von Willibrand factor antigen (vWF), fibrinogen, D-

dimer, factor VII, and factor VIIa were quantified as described previously [17]. Serum amyloid A (SAA) was analyzed using high-sensitivity immunonephelometry. Gradient gel electrophoresis was used to separate HDL particles according to size [18] with approximate size ranges (nm): H2b, 9.7-12.9; H2a, 8.8-9.7; H3a, 8.2-8.8; H3b, 7.8-8.2; and H3c, 7.2-7.8; subfraction cholesterol concentrations were estimated as fractional absorbance x HDL-C.

Genotyping

Genotyping the *CETP* TaqIB polymorphism utilized a melting curve analysis method described previously [19] based on G-nucleobase quenching to determine binding affinity to polymerase chain reaction-amplified *CETP* sequence of 5′-FAM labeled probe (TCTGAACCCTAACTCGAAC) complementary to the TaqIB1 allele.

Statistical Analyses

Outcome event mapping, a graphical exploratory data analysis tool, was used to identify high-risk subgroups [3,7,16,20]. Briefly, the approach begins with 3-dimensional scatter plots of outcome events (z-axis) over a bivariate risk domain (x-y plane) of two continuous biomarker variables. Patient outcome is coded as: 0 (no outcome event), or 1 (outcome event). Biomarker variables are rank-transformed to more evenly distribute patients over the bivariate risk domain. Next, a smoothing algorithm is applied to the scatter plot resulting in a surface (outcome event map) with height over the bivariate plane interpretable as estimated outcome rate. Peaks in mappings signal high-risk subgroups; specific delineation of high-risk subgroups is achieved by identifying all patients within the footprint of a region demarcated by a contour line of constant risk positioned at peak base.

Statistical and graphical analyses were performed using Statistica 8.0 (StatSoft, Inc., Tulsa, OK). Significant differences ($p<0.05$) between and among populations were assessed using Mann-Whitney U and Kruskal-Wallis (Bonferroni correction for post-hoc testing) tests. Chi-square test was used to assess differences between polymorphism variant distributions. Correlation was assessed by Spearman correlation coefficient. Cox proportional hazards multivariable regression was used to follow outcomes over time as a function of the dichotomized TaqIB polymorphism and biomarkers dichotomized as highest quartile versus combined 3 lower quartiles. Multivariable models were constructed including those biomarkers and adjustment for those clinical covariates found to be significant in Cox univariate analysis ($p<0.10$). Significance in final multivariable models was at the $p<0.05$ level.

Results

Study Population

Clinical and laboratory characterization of the study population were reported previously [17]. Briefly, patients were on average 58 years old, 77% male, 79% white, and overweight with high triglycerides and slightly low HDL-C levels. TaqIB genotyping was available for 680 (88.7%) of the 767 patients of the study population which was in Hardy-Weinberg equilibrium with respect to the polymorphism (B1B1 - 34.3%, B1B2 - 51.3%, and B2B2 - 14.4%). Mean HDL-C levels for genotype variants were: B1B1, 0.98±0.29 mmol/l; B1B2, 1.00±0.28 mmol/l; and B2B2, 1.05±0.25 mmol/l (homozygotes significantly different, $p=0.011$). HDL median diameter and subfraction proportions were not different for genotype variants.

Risk Mapping as a Function of HDL-C and CRP

To investigate effects of high HDL-C/inflammation on recurrent coronary event risk, an outcome event mapping was generated as a function of HDL-C and CRP (Figure 1). The

mapping shows a major high-risk peak at high levels of HDL-C and CRP as well as a secondary peak at lower levels of HDL-C and CRP. The contour line corresponding to the mean outcome event rate (0.159) of the total population served as a basis for defining a high-risk subgroup [16] corresponding to the major peak (N=166) and a background subgroup comprising remaining lower risk patients (N=601). Univariate Cox regression analysis was performed comparing high-risk patients to the background subgroup (hazard ratio 1.81, 95% CI 1.24-2.65, p=0.0022).

Table 1 presents results comparing clinical and laboratory parameters between the groups. The high-risk subgroup versus remaining patients demonstrated more female and black patients; higher levels of HDL-C, apoA1, total cholesterol, and inflammatory markers (CRP, VWF, fibrinogen, D-dim, and SAA); and lower levels of triglycerides and Lp-PLA_2. To assess whether female enrichment of the high-risk subgroup resulted from the known association of higher levels of HDL-C and CRP in women, separate Cox univariate analyses were run for high-risk female versus background patients (hazard ratio 1.87, 95% CI 1.12-3.11, p=0.016) and high-risk male versus background patients (hazard ratio 1.32, 95% CI 1.04-1.68, p=0.022). Results indicated significant risk in each case. Table 2 presents comparison results for HDL median particle diameter and percentage of HDL-C in HDL subfractions. Overall, results demonstrated larger HDL particles in the high-risk subgroup.

Clinical and Biomarker Characterization of TaqIB Effects

Comparing high-risk and background subgroups, TaqIB distributions were not different (B1B1 - 32.9%, B1B2 - 50.3%, and B2B2 - 16.8% versus 34.6%, 51.6%, and 13.8%, respectively, p = 0.66). To assess the potential of a dosage effect of the polymorphism on recurrent coronary event rates, Table 3 gives as a function of genotype: recurrence rates (percentages), number of patients, and p-values for the comparison of recurrence rates in the high-risk subgroup versus the background subgroup. The table reveals increasing risk in the high-risk subgroup relative to the background subgroup with B2 allele dose.

Prior to more definitive assessment of the role of CETP action regarding HDL-C associated risk, TaqIB polymorphism effects on clinical and biomarker levels in the high-risk subgroup were assessed for all parameters of Tables 1 and 2. Compared to B1 homozygotes, B2 allele carriers were significantly older (60.4±10.5 years versus 55.4±12.1 years, p=0.021); and they had significantly higher levels of triglycerides (2.05±1.03 mmol/l versus 1.88±1.29 mmol/l, p=0.044) and SAA (median and interquartile range: 0.740 mg/dl (0.440-1.29 mg/dl) versus 0.490 mg/dl (0.310-0.910 mg/dl, p=0.016)).

Risk Association of TaqIB within the High-Risk Subgroup

To assess the role of CETP action regarding risk within the high-risk subgroup, the TaqIB polymorphism was used as a probe based upon well-characterized allele-associated differences in CETP activity [11,12]. Thus, multivariable Cox proportional hazards modeling was performed using as predictor variables the dichotomized polymorphism (B2 allele carriers versus B1 homozygotes) and dichotomized biomarkers (Table 1) found to be significant in univariate Cox analysis. Models were adjusted for univariate significant clinical covariates with the following assessed: age, gender, race, prior MI, BMI, smoking, ejection fraction during index MI (< 0.30/≥ 0.30), pulmonary congestion, claudication, and hypertension. Using this approach, models required adjustment only for BMI; qualifying biomarkers included apoB, Lp(a), D-dimer, and Lp-PLA_2. The regression model within the high-risk subgroup so constructed demonstrated the TaqIB polymorphism as a significant predictor of risk (hazard ratio 2.41, 95% CI 1.04-5.60, p=0.041). Of the biomarkers, only apoB was significant (hazard ratio 2.64, 95% CI 1.31-5.30, p=0.006). Polymorphism risk association was graphically illustrated using surface mappings for B1 homozygotes (Figure

2A) and B2 carriers (Figure 2B). The mapping for B2 carriers shows a major peak similar to the high-risk peak of Figure 1; whereas the mapping for B1 homozygotes does not. Forcing age, gender, race and statin use (Table 1) into multivariable models gave essentially the same results regarding TaqIB and apoB. To assess interaction between the polymorphism and apoB, separate Cox models were run for low apoB patients and high apoB patients. Results demonstrated non-significance for the TaqIB hazard ratio in low apoB patients (p=0.29); whereas a trend emerged (p=0.055) for high apoB patients indicating focused TaqIB associated risk.

TaqIB Differential Functional Effects

To demonstrate TaqIB-associated functional effects, we monitored HDL particle subfractions as a function of cholesterol-acceptor levels (VLDL, IDL, and LDL) approximated as nonHDL-C levels. Median nonHDL-C level was used to dichotomize subgroups. Means, SD's, and percentage differences in HDL subfractions for high and low nonHDL-C patients in the high-risk and background subgroups are given in Table 4 as a function of TaqIB genotypes. For B1B1 patients in the high-risk subgroup, the table shows large subfraction differences for high versus low nonHDL-C patients deriving from smaller amounts of large HDL particles (H2b) and larger amounts of smaller particles (especially H3b) as a function of high nonHDL-C. For B2 allele carriers in the high-risk subgroup, corresponding differences were smaller and non-significant. Differences in the entire background subgroup were generally small and resembled those of B2 allele carriers in the high-risk subgroup. Similar analyses were also performed using triglycerides instead of nonHDL-C to approximate cholesterol acceptor levels. Marked differences among TaqIB genotypes were not demonstrated.

Regarding previous findings related to SAA (high levels in high-risk subgroup for B2 carriers) and Lp-PLA_2 (low levels in high risk subgroup), further studies were undertaken to assess potential roles for these parameters in altered HDL remodeling. Spearman correlation coefficients of HDL-C subfraction concentrations with SAA and Lp-PLA_2 in the high-risk subgroup as a function of TaqIB were generated giving: for SAA - essentially no significant correlation (results not shown); and for Lp-PLA_2 - values in B1 homozygotes for H2b, H2a, H3a, H3b, and H3c of −0.41*, −0.39*, −0.15, 0.24, and 0.49*, respectively; and values in B2 carriers of −0.38*, −0.14, −0.12, 0.08, and 0.17, respectively (* denotes $p < 0.05$).

Discussion

In a non-diabetic postinfarction population using a novel graphical exploratory data analysis tool, a subgroup of patients at high risk for recurrent coronary events was identified at high HDL-C and CRP levels. Subgroup patients had low levels of Lp-PLA_2 and large HDL particles. These results parallel findings from a primary coronary events study (PREVEND) that also identified a high-risk subgroup at high HDL-C and CRP levels with presumptive evidence of large HDL particles [8]. Thus in both primary and secondary coronary event studies, high-risk subgroups with large HDL particles have been identified at high levels of HDL-C and CRP. Unlike PREVEND [8], the current study did not show a prominent high-risk subgroup at low HDL-C. This may relate to different pathophysiologic mechanisms underlying primary and secondary coronary risk development.

Further studies in the high-risk subgroup were performed with TaqIB, a functional *CETP* polymorphism, to probe early stages of HDL metabolism. Genotype distributions in the study population were similar to values in healthy populations indicating absence of genotype-related survival effects for postinfarction patients [11,12]. For blood markers, B2 allele carriers (less CETP activity) had higher levels of triglycerides and SAA. Multivariable modeling indicated only TaqIB (B2 allele carriers at higher risk) and high apoB levels as

significant risk predictors. Support for TaqIB functional effects was suggested by genotypic differences in HDL subfraction distributions as a function of nonHDL-C (rough measure of cholesterol-acceptor particles). Further support resulted from correlation studies of Lp-PLA_2 with HDL subfractions showing for B1 homozygotes cholesterol shift with higher Lp-PLA_2 from large to small particle subfractions, especially to H3c,. This was consistent with the reported preferential association of Lp-PLA_2 in HDL particles with the H3c subfraction [21]. Such a shift was not seen in B2 allele-carriers.

The B2 allele (decreased CETP activity, increased HDL-C) associated with risk in the high-risk subgroup. Recent meta-analyses of TaqIB suggest lower B2-associated CVD risk [11,12]; while other studies suggest higher risk [12]. Such differences have been attributed to study type with population-based studies demonstrating high B2 allele-associated risk and high-risk population studies demonstrating low B2 allele-associated risk [12].

It is of interest to note recent efforts to reduce CVD risk by raising HDL-C levels. There are striking similarities with current study findings and clinical trials with the drug, torcetrapib, [22-26]. In both cases, CVD events were associated with raised HDL-C levels. While off-target effects of torcetrapib, especially increased blood pressure, could not be excluded as a factor [22], this is irrelevant in the current study as reduced CETP activity was not by pharmacologic means. Indeed, a recent study comparing torcetrapib and the B2 allele revealed concordant effects on blood lipids and lipoproteins but increased blood pressure only for torcetrapib [15]. The current approach allows identification of high-risk individuals embedded in larger patient populations. Excluding such patients may allow validation of the use of such drugs in selected populations.

We believe that B2 allele-associated risk derives in some part from pro-atherogenic transformation of HDL [9,27,28]. Although our finding of differential effects on HDL subfraction distributions in B2 allele carriers versus B1 homozygotes as a function of nonHDL-C demonstrated interaction of TaqIB genotypes with a measure of cholesterol-acceptor particle levels, definitive conclusions regarding a potential role for impaired HDL particle remodeling in the establishment of B2-associated risk were not possible from such results as neither CETP mass nor activity were actually measured. The inflammatory environment and CETP activity could have direct effects on HDL as well as indirect effects involving prolonged residence time of altered HDL in the inflammatory milieu of the high-risk subgroup [9,29]. Alterations of HDL constituents could potentially result especially for apoA1, paraoxonase, Lp-PLA_2, lecithin:cholesterol acyl-transferase (LCAT), and SAA [9,27,28,30]. Consistent with this notion are our findings in the high-risk subgroup of lower levels of Lp-PLA_2; and in B2 allele carriers higher levels of SAA, an apolipoprotein closely associated with HDL [31-34]. High SAA levels displace apoA1 in HDL resulting in loss of apoA1 functionality and impaired activity of LCAT, paraoxonase, and also Lp-PLA_2 [27] (especially notable in view of our finding of lower Lp-PLA_2 in the high-risk subgroup). That lower Lp-PLA_2 levels were associated with the high-risk subgroup contrary to the generally held belief that high Lp-PLA_2 levels are pro-atherogenic may be reflective of the notion that Lp-PLA_2 in LDL is pro-atherogenic; whereas Lp-PLA_2 in HDL is anti-atherogenic [21,30]. Other pro-atherogenic effects of SAA on HDL include increased cholesterol delivery to vascular walls as SAA-enriched HDL binds strongly to vascular proteoglycans facilitating cholesterol retention and further HDL alteration [31,33].

Study limitations included lack of direct evidence of impaired HDL particle remodeling in association with the B2 allele. Also, no direct evidence was provided on the role of inflammation in dysfunctional HDL although high SAA in B2 carriers was highly suggestive. Additional risk factor data (exercise, diet, ethanol, mental status, and social support) were lacking. Specifically focused future studies could be oriented at addressing all

these issues as well as replicating current study findings. Strengths of the study included use of an exploratory data analysis tool that can identify risk-based subgroups anywhere in a bivariate risk domain.

Study results demonstrate that high levels of HDL-C and CRP define a subgroup of postinfarction patients at high risk for recurrent coronary events. Additionally, the B2 allele of the TaqIB polymorphism of *CETP* was demonstrated to be associated with recurrent events in the high-risk subgroup. Future studies should aim to examine specific mechanisms leading to HDL dysfunction and to characterize resultant particles. Such patients should be considered in future drug trials involving raising HDL-C levels as this could aid in validating valuable approaches by excluding such patients.

Condensed Abstract

A subgroup of postinfarction patients at high risk for recurrent coronary events was identified at high HDL cholesterol and high C-reactive protein levels. Within the subgroup, the TaqIB polymorphism of *CETP*, well-known to demonstrate differences in transfer protein activity, was found to be associated with recurrent risk.

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References

1. Singh IM, Shishehbor MH, Ansell BJ. High-density lipoprotein as a therapeutic target - a systematic review. JAMA 2007;298:786–798. [PubMed: 17699012]
2. Joy T, Hegele RA. Is raising HDL a futile strategy for atheroprotection? Nat Rev Drug Discovery 2008;7:143–155.
3. Corsetti JP, Zareba W, Moss AJ, Rainwater DL, Sparks CE. Elevated HDL is a risk factor for recurrent coronary events in a subgroup of non-diabetic postinfarction patients with hypercholesterolemia and inflammation. Atherosclerosis 2006;187:191–197. [PubMed: 16242700]
4. deGoma EM, Leeper NJ, Heidenreich PA. Clinical significance of high-density lipoprotein cholesterol in patients with low low-density lipoprotein cholesterol. J Am Coll Cardiol 2008;51:49–55. [PubMed: 18174036]
5. van der Steeg WA, Holme I, Boekholdt SM, Larsen ML, Lindahl C, Stroes ESG, Tikkanen MJ, Wareham NJ, Faergeman O, Olsson AG, Pedersen TR, Khaw KT, Kastelein JJP. High-density lipoprotein cholesterol, high-density lipoprotein particle size, and apolipoprotein A-I: significance for cardiovascular risk. J Am Coll Cardiol 2008;51:634–642. [PubMed: 18261682]
6. van Acker BAC, Botma GJ, Zwinderman AH, Kuivenhoven JA, Dallinga-Thie GM, Sijbrands EJG, Boer JMA, Seidell JC, Jukema JW, Kastelein JJP, Jansen H, Verhoeven AJM. High HDL-C does not protect against coronary artery disease when associated with combined cholesteryl ester transfer protein and hepatic lipase gene variants. Atherosclerosis 2008;200:161–167. [PubMed: 18164013]
7. Corsetti JP, Ryan D, Moss AJ, Zareba W, Sparks CE. NAD(P)H oxidase polymorphism (C242T) and high HDL-C associate with recurrent coronary events in post infarction patients. Atherosclerosis 2007;196:461–468. [PubMed: 17214994]
8. Corsetti JP, Gansevoort RT, Sparks CE, Dullaart RPF. HDL Protection against Primary Cardiac Risk is Lost with Inflammation. Eur J Clin Invest. 2010 In Press.
9. Kontush A, Chapman MJ. Functionally defective high-density lipoprotein: a new therapeutic target at the crossroads of dyslipidemia, inflammation, and atherosclerosis. Pharmacological Reviews 2006;58:342–374. [PubMed: 16968945]

10. Rocha V, Libby P. Obesity, inflammation, and atherosclerosis. Nature Reviews Cardiology 2009;6:399–409.
11. Thompson A, Di Angelantonio E, Sarwar N, Erqou S, Salcheen D, Dullaart RPF, Keavney B, Ye Z, Danesh J. Association of cholesteryl ester transfer protein genotypes with CETP mass and activity, lipid levels, and coronary Risk. JAMA 2008;299:2777–2788. [PubMed: 18560005]
12. Dullaart RPF, Sluiter W. Common variation in the CETP gene and the implications for cardiovascular disease and its treatment: an updated analysis. Pharmacogenomics 2008;9:747–763. [PubMed: 18518852]
13. Masson D, Jiang XC, Lagrost l, Tall AR. The role of plasma lipid transfer proteins in lipoprotein metabolism and atherogenesis. J Lipid Res 2009;50:S201–S206. [PubMed: 19023137]
14. Boekholdt SM, Thompson JF. Natural genetic variation as a tool in understanding the role of CETP in lipid levels and disease. J Lipid Res 2003;44:1080–1093. [PubMed: 12639975]
15. Sofat R, Hingorani AD, Smeeth L, Humphries SE, Talmud PJ, Cooper J, Shah T, Dandhu MS, Ricketts SL, Boekholdt M, Wareham N, Khaw KT, Kumari M, Kivimaki M, Marmot M, Asselbergs FW, van der Harst P, Dullaart RPF, Navis G, van Veldhuisen DJ, Van Gilst WH, Thompson JF, McCaskie P, Palmer LJ, Arca M, Quagliarini F, Gaudio C, Cambien F, Nicaud V, Poirer O, Gudnason V, Isaacs A, Witteman JCM, van Duijn CM, Pencina M, Vasan RS, D'Agostino RB, Ordovas J, Li TY, Kakko S, Kauma H, Savolainen MJ, Kesaniemi YA, Sandhofer A, Paulweber B, Sorli JV, Goto A, Yokoyama S, Okumura K, Horne BD, Packard C, Freeman D, Ford I, Sattar N, McCormack V, Lawlor DA, Ebrahim S, Smith GD, Kastelein JJP, Deanfield J, Casas JP. Separating the mechanism-based and off-target actions of cholesteryl ester transfer protein inhibitors with CETP gene polymorphisms. Circulation 2010;121:52–62. [PubMed: 20026784]
16. Corsetti JP, Zareba W, Moss AJ, Sparks CE. Serum Glucose and Triglyceride Determine High-Risk Subgroups in Non-Diabetic Postinfarction Patients. Atherosclerosis 2005;183:293–300. [PubMed: 15925371]
17. Corsetti JP, Rainwater DL, Moss AJ, Zareba W, Sparks CE. High lipoprotein-associated phospholipase A_2 is a risk factor for recurrent coronary events in postinfarction patients. Clin Chem 2006;52:1331–1338. [PubMed: 16709622]
18. Rainwater DL. Electrophoretic separation of LDL and HDL subclasses. Method Mol Bio 1998;110:137–151.
19. Goldenberg I, Moss AJ, Block R, Ryan D, Corsetti JP, McNitt S, Eberly SW, Zareba W. Polymorphism in the cholesteryl ester transfer protein gene and the risk of early onset myocardial infarction among cigarette smokers. Annals of Noninvasive Electrocardiology 2007;12:364–374. [PubMed: 17970962]
20. Corsetti JP, Ryan D, Moss AJ, Rainwater DL, Zareba W, Sparks CE. Plasminogen activator inhibitor-1 polymorphism (4G/5G) predicts recurrence in nonhyperlipidemic postinfarction patients. Arterioscler Thromb Vasc Biol 2008;28:548–554. [PubMed: 18096824]
21. Tellis CC, Tselepis AD. The role of lipoprotein-associated phospholipase A_2 in atherosclerosis may depend on its lipoprotein carrier in plasma. Biochim et Biophys Acta 2009;1791:327–338.
22. Tall AR. CETP inhibitors to increase HDL cholesterol levels. N Engl J Med 2007;356:1364–1366. [PubMed: 17387130]
23. Nissen SE, Tardif JC, Nicholls SJ, Revkin JH, Shear CL, Duggan WT, Ruzyllo W, Bachinsky WB, Lasala GP, Tuzcu EM, ILLUSTRATE Investigators. Effect of torcetrapib on the progression of coronary atherosclerosis. N Engl J Med 2007;356:1304–1316. [PubMed: 17387129]
24. Kastelein JJ, van Leuven SI, Burgess L, Evans GW, Kuivenhoven JA, Barter PJ, Revkin JH, Grobbee DE, Riley WA, Shear CL, Duggan WT, Bots ML, RADIANCE 1 Investigators. Effect of torcetrapib on carotid atherosclerosis in familial hypercholesterolemia. N Engl J Med 2007;356:1620–1630. [PubMed: 17387131]
25. Bots ML, Visseren FL, Evans GW, Riley WA, Revkin JH, Tegeler CH, Shear CL, Duggan WT, Vicari RM, Grobbee DE, Kastelein JJ, RADIANCE 2 Investigators. Torcetrapib and carotid intima-media thickness in mixed dyslipidaemia (RADIANCE 2 study): a randomized, double-blind trial. Lancet 2007;370:153–160. [PubMed: 17630038]

26. Barter PJ, Caulfield M, Eriksson M, Grundy SM, Kastelein JJ, Komajda M, Lopez-Sendon J, Mosca L, Tardif JC, Waters DD, Shear CL, Revkin JH, Buhr KA, Fisher MR, Tall AR, Brewer B, ILLUMINATE Investigators. Effects of torcetrapib in patients at high risk for coronary events. N Engl J Med 2007;357:2109–2122. [PubMed: 17984165]
27. Norata GD, Pirillo A, Catapano AL. Modified HDL: biological and physiopathological consequences. Nutrition, Metabolism & Cardiovascular Diseases 2006;16:371–386.
28. Ansell BJ, Fonarow GC, Fogelman AM. The paradox of dysfunctional high-density lipoprotein. Curr Opin Lipidol 2007;18:427–434. [PubMed: 17620860]
29. Van Lenten BJ, Hama SY, de Beer FC, Stafforini DM, McIntyre TM, Prescott SM, La Du BN, Fogelman AM, Navab M. Anti-inflammatory HDL becomes pro-inflammatory during the acute phase response. J Clin Invest 1995;96:2758–2767. [PubMed: 8675645]
30. Florentin M, Liberopoulos EN, Wierzbicki AS, Mikhailidis. Multiple actions of high-density lipoprotein. Current Opinion in Cardiology 2008;23:370–378. [PubMed: 18520722]
31. Chait A, Han CY, Oram JF, Heinecke JW. Lipoprotein-associated inflammatory proteins: markers or mediators of cardiovascular disease? J Lipid Res 2005;46:389–403. [PubMed: 15722558]
32. van der Westhuyzen DR, Cai L, de Beer MC, de Beer FC. Serum anyloid A promotes cholesterol efflux mediated by scavenger receptor B-1. J Biolog Chem 2005;280:35890–35895.
33. O'Brien KD, Chait A. Serum amyloid A: the "other" inflammatory protein. Curr Atherosclerosis Reports 2006;8:62–68.
34. van der Westhuyzen DR, de Beer FC, Webb NR. HDL cholesterol transport during inflammation. Curr Opin Lipidol 2007;18:147–151. [PubMed: 17353662]

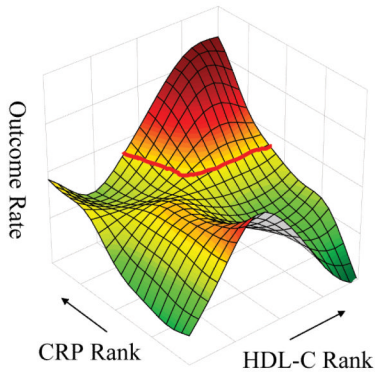

Figure 1.

Outcome event mapping of estimated coronary event rate over the risk domain of HDL-C and CRP ranks for the study population. Full-scale outcome rate along z-axis is 0.40. The red iso-contour at peak base corresponds to mean event rate for the total population (0.159). For reference, HDL ranks 480, 660, and 730 correspond approximately to: HDL-C of 1.04 mmol/l (40mg/dL), 1.30 mmol/l (50 mg/dL), and 1.55 mmol/l (60 mg/dL), respectively; while CRP ranks 195 and 465 correspond to CRP of 1 mg/L and 3 mg/L, respectively.

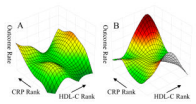

Figure 2.

Outcome event mappings of estimated coronary event rates over the risk domain of HDL-C and CRP ranks as a function of the dichotomized TaqIB polymorphism of *CETP* for: A. B1 homozygotes and B. B2 allele carriers. Full-scale outcome rate along z-axis is 0.50. See Figure 1 legend for correspondence of HDL-C and CRP ranks with levels.

Table 1

Mean and standard deviations for background and high HDL-C/high CRP high-risk subgroups.

Parameter	Background Subgroup (N = 601)	High HDL-C/High CRP Subgroup (N = 166)	p value
Recurrence Rate (%)	13.8	23.5	0.0025
Males (%)	82.9	56	< 0.000001
Race (% White)	81.5	67.5	0.000096
Prior MI (%)	17.5	14.1	0.30
Statins (%)	40.4	39.8	0.88
	Mean±SD	Mean±SD	
Age (years)	58.3±12.1	58.4±11.1	0.75
BMI (kg/m2)	27.5±4.55	27.7±5.95	0.34
Blood Marker			
HDL-C (mmol/l)	0.94±0.25	1.21±0.29	< 0.000001
ApoA1 (g/l)	1.15±0.24	1.29±0.26	< 0.000001
ApoB (g/l)	1.22±0.28	1.25±0.28	0.17
Chol (mmol/l)	5.05±1.14	5.33±1.13	0.0021
Lp-PLA_2 (μmol/min/ml)	27.0±5.95	23.8±5.74	< 0.000001
Trig (mmol/l)	2.31±1.33	2.00±1.15	0.00148
Glucose (mmol/l)	5.00±1.11	5.03±1.4	0.49
Lp(a) (micromol/l)	0.85±0.81	0.94±0.83	0.15
CRP (mg/l)	3.04±5.14	9.14±9.68	< 0.000001
VWF (%)	136±55	166±78	0.000001
Fibr (g/l)	3.35±0.76	3.89±0.89	< 0.000001
d-dim (μg/l)	448±397	562±464	0.000036
SAA (mg/dl)	0.47±0.78	2.64±10.74	< 0.000001

Table 2

Mean and standard deviations of HDL particle diameter and percentage of HDL-C in HDL subfractions in background and high HDL-C/high CRP high-risk subgroups.

Parameter	Background Subgroup (N = 601)	High HDL-C/High CRP Subgroup (N = 166)	p value
HDL Median Diameter (nm)	8.75±0.26	8.94±0.33	< 0.000001
Subfraction Percentages			
H2b	23.8±7.7	28.2±9.9	< 0.000001
H2a	22.2±3.9	24.6±3.9	< 0.000001
H3a	26.0±3.9	24.4±5.4	0.00058
H3b	18.0±4.9	14.2±4.7	< 0.000001
H3c	9.9±3.5	8.4±3.8	< 0.000001

Table 3

Coronary event recurrence rates (percentages) and numbers of patients as a function of the *CETP* TaqIB genotype for the total study population, and the background and high-risk subgroups. Also given are p-values for the comparison of recurrence rates between the background and high-risk subgroups.

Genotype	Total Population (N)	Background Subgroup (N)	High-Risk Subgroup (N)	p-value
B1B1	13.7 (233)	13.4 (186)	14.9 (47)	0.80
B1B2	16.3 (349)	13.7 (277)	26.4 (72)	0.0097
B2B2	17.3 (98)	12.2 (74)	33.3 (24)	0.018

Table 4

Means and SD's of HDL subfraction cholesterol (expressed both as percentages of total HDL-C and as concentrations) for patients below and above median nonHDL-C in the high-risk and background subgroups as a function of genotypes. Also included are corresponding percentage differences (% Diff) in HDL-C subfraction cholesterol for high nonHDL-C patients in comparison to low nonHDL-C patients

	B1B1			B1B2			B2B2			B1B2 plus B2B2		
	Below NonHDL-C Median	Above NonHDL-C Median		Below NonHDL-C Median	Above NonHDL-C Median		Below NonHDL-C Median	Above NonHDL-C Median		Below NonHDL-C Median	Above NonHDL-C Median	
	Mean±SD	Mean±SD	% Diff	Mean±SD	Mean±SD	% Diff	Mean±SD	Mean±SD	% Diff	Mean±SD	Mean±SD	% Diff
High HDL-C Subgroup												
Percentage												
H2b	34.9±11.5	25.0±11.2	−28.4*	28.3±7.5	25.3±8.9	−10.6	31.1±12.9	27.1±6.4	−12.7	29.0±9.1	25.8±8.3	−11.1
H2a	24.7±3.4	24.8±3.7	0.3	23.8±4.7	25.2±4.2	6.1	24.7±4.1	25.8±3.4	4.3	24.1±4.5	25.3±4.0	5.2
H3a	22.0±5.3	25.5±6.9	15.6*	24.6±4.6	24.9±5.1	1.2	24.3±7.8	24.6±2.8	1.2	24.5±5.5	24.9±4.6	1.9
H3b	11.6±4.6	15.8±4.7	36.4*	14.4±4.9	15.3±5.2	5.8	12.8±4.5	14.1±3.5	10.4	14.0±4.8	15.0±4.8	6.6
H3c	6.8±2.7	9.0±3.9	32.3*	8.8±3.6	9.2±5.2	4.7	7.2±1.8	8.4±3.5	17.8	8.4±3.4	9.0±4.8	6.8
Concentration (mmol/l)												
H2b	0.485±0.331	0.291±0.154	−40.0*	0.353±0.156	0.31±0.149	−11.9	0.368±0.162	0.354±0.136	−3.6	0.356±0.157	0.322±0.146	−9.5
H2a	0.317±0.086	0.282±0.065	−10.9	0.3±0.134	0.301±0.084	0.4	0.296±0.077	0.329±0.084	11.3	0.299±0.121	0.308±0.084	3.0
H3a	0.272±0.050	0.288±0.088	5.8	0.301±0.092	0.29±0.057	−3.5	0.288±0.101	0.309±0.047	7.2	0.296±0.094	0.296±0.054	0.0
H3b	0.139±0.045	0.176±0.048	26.5*	0.172±0.062	0.177±0.056	2.9	0.149±0.049	0.174±0.035	16.8	0.166±0.059	0.176±0.051	6.2
H3c	0.084±0.030	0.101±0.042	21.3	0.104±0.048	0.107±0.053	2.7	0.083±0.018	0.102±0.032	23.2	0.099±0.043	0.106±0.048	6.9
Background Subgroup												
Percentage												
H2b	25.1±8.2	21.9±7.2	−12.9*	25.2±7.9	22.7±7.2	−10.0*	26.1±7.3	22.7±5.5	−13.1*	25.3±7.8	22.7±6.9	−10.4*
H2a	21.9±3.6	22.4±3.7	2.0	22.2±3.8	22.9±3.9	3.1	22.5±3.6	22.5±3.9	0.4	22.2±3.8	22.9±3.9	2.9
H3a	26±4.1	26.4±3.7	1.6	25.8±3.9	25.8±3.8	−0.1	26±4.3	26.4±2.9	1.7	25.9±4	25.9±3.7	0.3
H3b	17.5±5.4	18.9±4.5	8.0*	17.1±5	18.7±4.7	9.0*	16.4±4.6	18.3±4.5	11.5	17±4.9	18.6±4.6	9.1*
H3c	9.4±3.1	10.4±3.7	10.6	9.6±3.3	10±3.1	3.3	9±3.2	10±3.5	11.3	9.5±3.4	9.9±3.1	4.1

	B1B1			B1B2			B2B2			B1B2 plus B2B2		
	Below NonHDL-C Median	Above NonHDL-C Median		Below NonHDL-C Median	Above NonHDL-C Median		Below NonHDL-C Median	Above NonHDL-C Median		Below NonHDL-C Median	Above NonHDL-C Median	
	Mean±SD	Mean±SD	% Diff	Mean±SD	Mean±SD	% Diff	Mean±SD	Mean±SD	% Diff	Mean±SD	Mean±SD	% Diff
Concentration (mmol/l)												
H2b	0.236±0.123	0.216±0.12	−8.5	0.258±0.15	0.218±0.107	−15.6*	0.278±0.132	0.221±0.082	−20.5*	0.262±0.146	0.219±0.102	−16.4*
H2a	0.201±0.078	0.216±0.078	7.2	0.22±0.089	0.216±0.074	−2.1	0.236±0.086	0.22±0.075	−6.9	0.223±0.088	0.217±0.074	−2.8
H3a	0.233±0.071	0.25±0.069	7.2	0.248±0.072	0.238±0.059	−3.8	0.266±0.074	0.253±0.061	−4.9	0.252±0.072	0.241±0.059	−4.1
H3b	0.15±0.042	0.173±0.037	15.0*	0.157±0.033	0.168±0.037	7.4*	0.162±0.037	0.17±0.037	4.9	0.158±0.034	0.168±0.037	6.4*
H3c	0.081±0.024	0.095±0.033	18.3*	0.088±0.024	0.09±0.025	2.4	0.091±0.041	0.092±0.028	0.8	0.089±0.029	0.09±0.025	1.2

*$p < 0.05$.