Genetic and biochemical risks factors in coronary artery disease
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CETP gene variation; relation to lipid parameters and cardiovascular risk

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Abstract

Purpose of review
Over the past decade lowering of LDL-c levels has been established as the foundation for preventing coronary artery disease (CAD), but substantial additional risk reduction remains to be gained by modifying risk factors other than LDL-c. Raising HDL-c levels by inhibiting activity of the cholesteryl ester transfer protein (CETP) is a prime target. Research on naturally occurring variants in the CETP gene has yielded numerous insights that have been relevant for understanding the lipoprotein metabolism, and crucial to the development of pharmacological CETP inhibition.

Recent findings
This review discusses a number of recently published studies, including a haplotype analysis of the CETP promoter region confirming that not the TaqIB variant, but the −629 C>A variant is instrumental in determining CETP activity, as previously suggested. In addition, we discuss a recent meta-analysis which confirms that the I405V and TaqIB variants are indeed associated with lower CETP activity and higher HDL-c levels. Also, we review two sub-analyses of large randomized controlled pravastatin trials which found no evidence for a proposed pharmacogenetic interaction between the CETP TaqIB variant and pravastatin treatment.

Summary
The currently available evidence suggests that several genetic variants in the CETP gene are associated with altered CETP plasma levels and activity, HDL-c plasma levels, LDL and HDL particle size, and perhaps the risk of CAD. No evidence exists for a pharmacogenetic interaction between the CETP TaqIB variant and pravastatin efficacy.
Introduction
Among numerous genetic and lifestyle traits, dyslipidaemia is one of the most prominent risk factors for coronary artery disease (CAD). In the past decade, lowering low-density lipoprotein cholesterol (LDL-c) has been established as the principal target for therapeutic intervention in dyslipidaemia, and in fact now constitutes the very foundation of CAD prevention. Randomized controlled trials in both the primary and secondary prevention setting have unequivocally shown that treatment with statins effectively lowers LDL-c levels and reduces CAD by approximately 30%. This figure indicates, however, that another 70% remains to be gained through modulation of cardiovascular risk factors other than LDL-c. Since prospective epidemiological studies have clearly shown that low high-density lipoprotein cholesterol (HDL-c) levels are a strong and independent risk factor for CAD, pharmacological intervention to raise HDL-c enjoys significant interest and stands in the centre of attention in the arena of drug development. We and others have recently shown that pharmacological inhibition of cholesteryl ester transfer protein (CETP) is an effective method to raise HDL-c. However, the uncertainty surrounding the mechanisms by which HDL exerts its atheroprotective effects, has led to controversy about whether high HDL-c levels induced by pharmacological CETP inhibition would indeed result in a reduced risk of CAD.

CETP plays a central role in HDL-c metabolism by shuttling cholesteryl esters from HDL particles to apolipoprotein (apo) B-containing particles, partly in exchange for triglycerides. Expression of the simian or human CETP gene in mice, which are CETP-deficient by nature, resulted in a dose-related reduction of HDL-c levels and significantly more early atherosclerotic lesions in the proximal aorta. By contrast, in the setting of hypertriglyceridemia, CETP expression was either anti-atherogenic or did not promote atherosclerosis, indicating that, at least in rodents, the metabolic setting apparently determines whether CETP is atheroprotective or not. In addition, the extent to which these results can be extrapolated to human biology remains unclear because mice do not physiologically express CETP, do not normally carry the bulk of their cholesterol in LDL, and do not develop atherosclerotic lesions during their natural lifespan. In summary, the role of CETP in human atherosclerosis has not been fully elucidated, which contrasts with the fact that, to date, two pharmacological CETP inhibitors are being tested in human trials. In the quest to unravel the role of CETP in determining lipid parameters and cardiovascular risk in humans, variants in the CETP gene have always played a substantial role. This report summarizes and discusses the recent advances in this field of research.

Categories
The CETP gene locus is highly polymorphic, and most if not all common coding variants as well those in the upstream promoter region have
been identified. We have classified the CETP sequence variations into three arbitrary categories. First, some mutations cause aberrant mRNA processing or introduce premature stop codons (non-sense mutations) often causing premature protein truncation, or alternatively have a large detrimental impact on normal protein function (D442G). These mutations cause partial or complete CETP deficiency, and are therefore interesting targets to study the functionality of CETP but, due to their rarity, studying their impact on cardiovascular risk has proven to be difficult. Second, some point mutations underlie amino acid substitutions (mis-sense mutations) with milder effects on CETP function, such as the I405V variant. These changes vary in their effect on CETP activity and lipid parameters, but some of these are sufficiently frequent to assess their impact on cardiovascular risk in population-based studies. Third, a number of genetic variants are located in untranslated regions of the CETP gene or, alternatively, change codons such that the amino acid sequence of the protein remains unchanged (silent mutations). Most of these are fairly common, and some of them convey a relatively small but potentially important effect on CETP function, lipid and lipoprotein parameters, as well as cardiovascular risk.

**Mutations associated with partial or complete CETP deficiency**

The literature is fragmented because it consists of a number of case reports or small case series on rare mutations, least rare of which is the (+1)G>A mutation located in intron 14 (for an extensive summary, see reference 23*). In order to increase statistical power, these mutations are often analyzed together with the D442G mutation in exon 15, which causes partial CETP deficiency. The heterogeneous effects of these mutations complicate the interpretation of these analyses. In complete absence of CETP function, the failure to transfer cholesteryl esters from HDL to other lipoproteins leads to an accumulation of cholesteryl esters in the HDL fraction, and is generally associated with a 2- to 5-fold increase of HDL-c relative to controls. Among heterozygotes for these mutations, CETP activity was generally 50-70% of that in controls, suggesting increased expression of the wild-type allele and/or reduced CETP protein catabolism. The effects of these mutations on HDL-c varied substantially (25-80% increase from control). Heterozygosity for the D442G variant has a considerable impact on CETP activity, often called partial CETP deficiency, and is usually analyzed in concert with the penetrant non-sense and splice site mutations. D442G homozygotes retain significant CETP activity, typically 25-50% that of the control population, indicating that the protein is only partially defective. Heterozygotes usually have 60-85% of the wild-type activity. The impact on HDL-c is moderate, with homozygotes having slightly increased levels (usually <10%). Only the (+1)G>A and D442G variants occur at a frequency high enough to study an association with cardiovascular risk. A marked increase in HDL-c combined with a decrease in LDL-c is anticipated to substantially reduce CAD risk in CETP deficient subjects. However, this relationship has proven to be
complex and confusing: longevity, an increased CAD risk and a decreased CAD risk have all been reported.\textsuperscript{24,38-42} The largest clinical study, performed among 3,469 Japanese-American men in Honolulu, initially reported an increased CAD risk, but this was only true for men with intermediate HDL-c levels.\textsuperscript{26} However, it has recently been suggested that after extended follow-up the increased CAD risk was no longer statistically significant,\textsuperscript{43} but these data have not yet been formally published. In summary, the relationship between CETP deficiency, either in homozygous or heterozygous form, and the risk of CAD remains obscure. Its low prevalence, and its geographical limitation to mainly Japan in combination with the low cardiovascular mortality rate in this country relative to other industrialized countries, impede our understanding of CETP function and atherosclerosis in the Western world. We have recently identified a novel CETP splice site mutation (IVS7+1) in Caucasians of Dutch descent.\textsuperscript{44} Preliminary results indicate that mean carotid intima media thickness (a surrogate marker for atherosclerosis) in 25 heterozygotes for this mutation is similar to that of family controls. These data suggest that these individuals are not at increased risk of atherosclerosis, as was previously suggested.

Single amino acid substitutions
Eight point mutations underlie single amino acid substitutions.\textsuperscript{15,45-49} Some of these are fairly common and appear to have only slight effects on protein function (A373P, R451Q, and I405V), while others are quite rare and have severe deleterious effects on activity (L151P, R282C), or have not been characterized in detail (G314S and V469M). The D442G variant has been discussed in the previous section.

In the current section we will specifically discuss the common I405V variant, which occurs at a frequency of over 25% in the studied populations. Homozygotes for the 405V variant have 9-23% lower CETP levels. The relationship with HDL-c levels is less evident, with observations ranging substantially.\textsuperscript{50-58} However, in a recent meta-analysis, we determined that 405V homozygotes have 0.05 mmol/l (95%CI=0.03-0.07) higher HDL-c levels than 405I homozygotes.\textsuperscript{27} Despite this small but significant association with HDL-c levels, the studies relating this variant to CAD risk have not found consistent results. A large-scale study with sufficient statistical power to detect the mild effect of this single genetic marker could give better insight. Such a study would require sufficient power to assess interactions with other characteristics because evidence exists that the effect of this variant depends on the metabolic context. For instance, in the setting of hypertriglyceridaemia, the 405V variant was found to be associated with low CETP activity but with a higher prevalence of CAD.\textsuperscript{58} This observation may be explained by the fact that the actual rate of transfer of cholesteryl esters out of HDL is determined not only by the amount of active CETP, but even more by the concentration of the acceptors of the transfer reaction (e.g. triglyceride-rich lipoproteins like chylomicron remnants and VLDL). This implicates that the metabolic context may determine whether CETP
is pro-atherogenic or anti-atherogenic in humans, as well as in rodents as described previously.

This context-dependency is again underlined by a recent report in Ashkenazi Jews, a population where exceptional longevity occurs more frequently than in most other populations. Homozygosity for the 405V variant was significantly more frequent in individuals with exceptional longevity and their offspring, and in addition, these individuals had significantly larger HDL and LDL particles. Despite the fact that numerous studies have investigated the relationship between this variant and CAD risk in various populations, no consensus exists on the results. It is therefore quite remarkable that in this particular Jewish population, the effect of the I405V variant seems so strong. In this respect, it is noteworthy that lipoprotein subclasses have also been found to associate with another CETP gene variant, known as the TaqIB polymorphism. The B2 allele for this variant, which will be discussed in further detail below, was shown to associate not only with lower CETP activity and higher HDL-c levels, but also with larger, and thus less atherogenic HDL and LDL particle size. This is consistent with our recent observation that in patients with familial hypercholesterolemia, significant correlations exist between CETP concentration and both HDL and LDL particle size. This observation is also consistent with previous reports that increased LDL size was associated with low CETP plasma levels and with the TaqIB B2 allele. Thus, the 405V variant is associated with lower CETP levels and this translates not only into quantitative changes in lipoprotein profiles including slightly higher HDL-c levels, but also into beneficial qualitative changes in LDL and HDL particle composition.

Mutations in non-coding regions of the CETP gene
Large variation exists at the CETP gene locus that does not alter the amino acid sequence of the protein. These variants tend to be quite common. The single variant that has received most scrutiny was originally identified as a restriction fragment length polymorphism identified by the enzyme TaqIB. Because of its reported association with CETP and HDL-c plasma levels in the absence of a functional effect on amino acid sequence or promoter region, this variant has been suspected to constitute a marker for another functional variant. The -629C>A variant is a good candidate to explain the association between the TaqIB genotype and HDL-c levels because strong linkage disequilibrium exists between these variants, and since the -629C>A variant has been shown to modify CETP promoter activity. We have recently shown that amongst 5 tightly linked polymorphisms in and around the promoter region, including TaqIB, only the -629C>A variant was independently associated with HDL-c levels. However, not all studies are consistent in this regard.

Numerous studies have investigated whether this variant associates with HDL-c levels and CAD risk. Virtually all of these studies are small
and usually underpowered to detect associations. In order to overcome this issue, we have recently performed a meta-analysis on this plethora of published studies. We found that individuals homozygous for the less-common B2 allele (in strong linkage disequilibrium with the −629A allele) have 0.12 mmol/l (95% CI = 0.11-0.13) higher HDL-c levels than B1B1 individuals. Another important drawback of small association studies is their limited statistical power to detect interactions with other parameters, which may be very relevant. In particular, for the TaqIB genotype interactions have been reported with sex, smoking, body mass index (BMI), and use of alcohol. In order to overcome these drawbacks, we also carried out a pooled analysis of individual patient data derived from large population-based studies. We observed a fully adjusted HDL-c difference between B1B1 homozygotes and B2B2 homozygotes of 0.10 (0.09-0.12) mmol/l, which is entirely consistent with our meta-analysis on all published studies. All individual association studies that had limited power to detect an association with the continuous parameter HDL-c levels, had even less power to detect an association with the categorical variable CAD. It is therefore not surprising that even among the larger studies, several did not detect an association with CAD risk. In our pooled analysis, we found that B2 homozygotes exhibited an odds ratio for CAD of 0.78 (95% CI = 0.66 - 0.93) relative to B1 homozygotes. This association disappeared after adjustment for HDL-c, suggesting that the relationship between TaqIB genotype and CAD risk is (largely) mediated by HDL-c levels. This is inconsistent with a recent study which suggested that the relationship between CETP variants and CAD risk was independent of HDL-c levels.

Interaction between TaqIB genotype and pravastatin efficacy
The cause of much of the attention for the TaqIB polymorphism in intron 1 has been the report that this genotype modifies the efficacy of pravastatin therapy. This study used progression of coronary atherosclerosis as assessed by consecutive coronary angiography as outcome. When clinical cardiovascular events were used as outcome, no statistically significant results were found. This was not surprising since the study was not powered to detect an effect of pravastatin on clinical events, let alone an interaction with a genetic variant. We have recently reported that this interaction may be largely explained by CETP plasma levels. Consistently, we showed that a similar interaction existed between CETP plasma levels and pravastatin in patients with familial hypercholesterolemia, when using intima-media thickness as a surrogate marker for atherosclerosis. To test whether this interaction could be confirmed in studies using cardiovascular events as outcome, two genetic sub-analyses have been performed in CARE and WOSCOPS, two large randomized placebo-controlled trials testing the efficacy of pravastatin. Neither of these could confirm the previously detected interaction between TaqIB genotype and pravastatin efficacy to reduce the risk of cardiovascular events. It must be kept in mind that the use of CETP plasma levels as a continuous parameter provides substantially
more statistical power than the use of a genetic marker in the CETP gene; first, because this genetic marker is only one of the parameters influencing CETP plasma levels, and second, because it is a categorical variable. The same is true for the use of continuous surrogate parameters for atherosclerosis, instead of the risk of cardiovascular events. Thus, whether this interaction in fact does not exist, or the statistical power to detect it was insufficient, remains unclear. Fortunately, both the TNT and IDEAL studies (with approximately 20,000 randomized patients) have established a substantial biobank including DNA samples, and pre-specified analyses involving these CETP-related parameters will be carried out when these trials are completed. Either way, the fact that very large studies would be required to detect if this interaction truly exists, indicates that its clinical relevance is probably limited.

Summary
The currently available evidence suggests that genetic defects causing partial or complete CETP deficiency have a strong effect on HDL-c levels. However, whether this translates into a consistent reduction in CAD risk, has not yet been established. Also, a substantial amount of data exists to the fact that common genetic variants at the CETP gene locus are associated with CETP plasma levels and activity, HDL-c plasma levels, LDL and HDL particle size, and the risk of CAD. Furthermore, two recent subanalyses of large randomized controlled trials suggest that no clinically relevant interaction exists between the CETP TaqIB genotype and the efficacy of pravastatin in reducing cardiovascular events.
References


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