review

Natural genetic variation as a tool in understanding the role of CETP in lipid levels and disease

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Abstract Since the identification of cholesteryl ester transfer protein (CETP), its role in the modulation of HDL levels and cardiovascular disease has been debated. With the early detection of genetic variants followed by the finding of families deficient in CETP, genetic studies have played a large role in the attempts to understand the association of CETP with lipids and disease; however, results of these studies have often led to disparate conclusions. With the availability of a greater variety of genetic polymorphisms and larger studies in which disease has been examined, it is now possible to compare the breadth of CETP genetic studies and draw better conclusions. The most broadly studied polymorphism is TaqIB for which over 10,000 individuals have been genotyped and had HDL levels determined. When these studies are subjected to a meta-analysis, the B2B2 homozygotes are found to have higher HDL levels than B1B1 homozygotes (0.12 mmol/l, 95% CI = 0.11–0.13, $P < 0.0001$). A similar analysis of the I405V polymorphism yields 0.05 mmol/l higher HDL levels in 405VV homozygotes than in 405II homozygotes (95% CI = 0.03–0.07, $P < 0.0001$). The implications of these studies for cardiovascular disease will be addressed.—Boekholdt, S. M., and J. F. Thompson. **Natural genetic variation as a tool in understanding the role of CETP in lipid levels and disease. *J. Lipid Res.* 2003.** 44: **1080–1093.**

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Since the discovery of cholesteryl ester transfer protein (CETP) and its identification as a modulator of HDL levels, there has been much speculation about its role in human disease [as reviewed in refs. (1–7)]. There are well-documented biochemical studies that demonstrate CETP's ability to affect lipid transport, and there is good evidence from many systems that CETP affects HDL levels in animals and humans. However, while high levels of HDL are well known to be protective for cardiovascular disease, the uncertainty surrounding the detailed mechanism(s) for HDL's positive effects has led to controversy over whether the high HDL levels induced either by genetic deficiency of CETP or by therapeutic inhibition of CETP would be beneficial or not. Animal model studies have been of limited value in addressing the question, because many species do not express a functional CETP protein, do not normally carry the bulk of their cholesterol on LDL, and do not develop atherosclerotic lesions over the same time frame as humans. Thus, most animal models cannot be relied upon to provide strong evidence for CETP's role in disease. However, HDL levels in normal human populations have been shown to have a significant genetic component (8), and associated studies have therefore been carried out to obtain a more direct measure of CETP's impact on HDL and disease.

The natural genetic variation at the CETP locus can be used to help understand its impact on disease, but such studies must be interpreted cautiously because of the large number of potentially confounding influences. The human genetic studies described thus far have generated conflicting conclusions, indicating the need for a careful analysis so that the disparate results can be evaluated. The types of problems typically encountered in genetic association studies directed at cardiovascular and other complex diseases have been summarized previously (9, 10).

Before analyzing CETP studies, it is necessary to place the gene in biological perspective. Even when CETP mass/activity assays are carried out to determine the role of a given polymorphism, there are limitations, because it is assumed that plasma CETP activity is responsible for the gene's effects when protein levels in tissues could potentially have an impact on disease as well. Furthermore, only one aspect of CETP activity is usually measured (transfer of a specific lipid from HDL to LDL or the reverse) when CETP can, in fact, carry out many lipid transfer reactions among many types of particles. The activity of each transfer reaction performed by CETP isoforms with varying

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amino acid sequences could be differentially affected. When CETP mass is measured instead of activity, there is the further complication that antibodies may not recognize each protein isoform with the same affinity. Thus, even when CETP mass/activity is measured, a complete picture may not be provided.

If CETP determinations are not made, one potential surrogate is the measurement of various lipid parameters to determine if they have been affected by a given genetic polymorphism. The initial rationale for CETP's involvement in atherosclerosis was the observation of its ability to impact HDL. Since HDL is readily measured in clinical samples, these measurements are generally, though not always, reported along with the genetic data. CETP could potentially affect other measurable aspects of lipid particles, including LDL-triglyceride levels and LDL-HDL size. Furthermore, studies in which HDL levels are used as a selection criterion or studies in which results are adjusted for HDL levels must be interpreted knowing that the beneficial effects of lowering CETP are being potentially eliminated by these choices. Similarly, one must be aware that selection criteria based on LDL or disease may also induce bias in a population's CETP genetic diversity. Superimposed on all these issues is the polygenic nature of cardiovascular disease: even if CETP plays a major role in disease in one population, it may not have the same impact in a genetically divergent population.

To simplify discussion, genetic variation at the CETP locus will be segregated into categories. First, alterations that cause multiple amino acid changes or significant truncations will be discussed. These changes tend to be rare but have the most significant and consistent effect on CETP activity. Alterations that induce a single amino acid change will then be presented. The single amino acid changes vary substantially in their effect on CETP activity and lipid parameters. Finally, changes that do not affect the coding sequence of CETP but could potentially affect expression levels of the protein will be discussed. Two single-nucleotide polymorphisms (SNPs), Taq1B, which does not alter the sequence of CETP, and I405V, which results in a single amino acid change, have been studied sufficiently often that a formal meta-analysis could be performed to better characterize their effects across multiple populations.

While there are undoubtedly DNA polymorphisms in the CETP gene that have not yet been reported, the gene has been sufficiently well studied that most if not all common coding SNPs and promoter variations have been identified. It is thus timely to assess previous work and place it in context with the current state of knowledge, both to understand what has been accomplished to date and to focus future resources.

MULTIPLE AMINO ACID CHANGES/TRUNCATIONS

Several mutations have been identified that result in a premature translational stop in the protein. Seven such mutations that cause a loss of over 100 amino acids are listed in **Table 1** (11–17). Splicing defects can also lead to a significantly altered protein, as observed for three examples in Table 1 (18–20). While most of these mutations are sporadic and hence no individuals are observed in the homozygous state, G+1A/In14 occurs frequently enough in the Japanese population, typically at about 2%, that there are instances of homozygous individuals being studied. The reports listed in **Tables 2** and **3** examined anywhere from two to 38 individuals homozygous for the splicing defect, and none of these individuals was found to have any CETP activity or protein. The same studies examined two to 279 heterozygotes for CETP activity (14, 19–29). In the two largest studies, CETP activity for heterozygotes was 58–68% that of controls, suggesting there might be a slight compensation by the wild-type allele for the loss of the mutant allele, either by alterations in expression or reduced catabolism. The impact of the complete deficiency of CETP activity on HDL levels varied, but generally was associated with a 2- to 5-fold increase relative to controls. The impact on HDL levels in heterozygotes was much less dramatic, with a 25–80% increase typically observed. The other mutations listed in Table 1 are too rare for a thorough analysis to have been carried out, even among heterozygotes.

Since the frequency of G+1A/In14 in the Japanese population is relatively high, efforts to correlate its occurrence with disease have been attempted. Some of these studies have also included the D442G polymorphism (A+55G/Ex15) in the analysis. However, the latter SNP is not a null mutation and complicates the analysis due to its heterogeneous effects. The D442G SNP and most studies addressing it in combination with G+1A/In14 will be addressed later, because, except for one region of Japan, D442G is more common than G+1A/In14 and therefore plays a larger role in such studies.

One of the most useful populations for analyzing G+1A/In14 resides in the Omagari region of Japan, where

TABLE 1. Polymorphisms in the CETP gene: multiple amino acid changes/truncation

Location	Position	Function	Frequency	Reference
ΔGTGT+144/Ex1	144	V22X	rare	11
ΔC+45/Ex2	1103	A38X	rare	12
T+103G/Ex2	1161	Y57X	rare	13
G+65T/Ex6	9150	G181X	rare	14
C+103T/Ex9	11486	R268X	rare	15
A+178T/Ex9	11561	K293X	rare	16
C+46T/Ex10	13199	Q309X	rare	17
T+2G/In10	13206	Splice defect	rare	18
G+1A/In14	20291	Splice defect	rare	19
+T+3/In14	20293	Splice defect	rare	20

CETP, cholesteryl ester transfer protein. Nomenclature is as described (53). To the extent possible, all polymorphisms reported in the literature are included in this table, but not those reported only electronically. Because of its completeness through the CETP gene, GenBank sequence AC010550.7 is used as the numbering reference, with the start of transcription denoted as position +1. Each change is also referenced as to the change identified and its location in a given exon/intron. Frequency of a polymorphism is classified as rare (less than 1%), uncommon (1% to 5%), or common (more than 5%). Amino acid changes are listed under function.

TABLE 2. Effect of the Intron 14 splicing mutation and D442G on CETP and HDL levels: mutation G+1A/In14 splicing defect

CETP				HDL				
Ho	He	UF	Con	Ho	He	UF	Con	Reference
0 (5)	—	—	1.67 (30)	6.00 (5)	—	—	1.34 (30)	14
0 (2)	56 (5)	—	100 (19)	212 (2)	60 (5)	—	48 (19)	19
0 (22)	1.43 (28)	2.17 (26)	—	158 (22)	69 (28)	52 (26)	—	20
—	—	—	—	—	66.9 (17)	—	50.5 (3276)	21
0 (10)	1.4 (20)	2.3 (16)	2.2 (10)	4.24 (10)	1.71 (20)	1.37 (16)	1.16 (10)	22
0 (5)	7 (2)	—	25.6 (32)	3.85 (5)	2.49 (6)	—	1.51 (22)	23
—	—	—	—	6.10 (1)	2.77 (6)	—	1.49 (7)	24
0 (10)	11 (5)	—	25.6 (30)	176 (10)	100 (5)	—	59 (30)	25
0 (29)	1.4 (58)	—	2.4 (90)	4.03 (29)	2.72 (58)	—	1.55 (90)	26
—	—	—	—	—	63 (4)	—	47.4 (277)	27
0 (38)	67.9 (279)	—	100	4.43 (38)	2.09 (279)	—	1.34	28
—	—	—	—	—	1.1 (2)	—	1.41 (145)	29

Studies examining the effect of the splicing defect G+1A/In14 and the missense mutation D442G (A+55G/Ex15) are listed. In some cases, units for CETP could not be converted to standard values, so CETP and HDL levels were left in their original units, which may be found in the references provided. The number of individuals in each genotypic category is listed in parenthesis. Homozygotes (Ho), heterozygotes (He), unaffected family members (UF), and control (Con) values are provided when available. Studies in which compound heterozygotes were identified or in which heterozygotes were combined with homozygotes are not included.

27% of the population is heterozygous for G+1A/In14 and 0.6% is homozygous for this splicing defect (30). Both coronary heart disease (CHD) and longevity were examined as a function of G+1A/In14 in this population, but the number of CHD patients genotyped was relatively small, limiting the power of the study. In addition, D442G patients were combined with G+1A/In14 patients when CHD was examined, but were kept separate in the longevity component. Higher proportions of total D442G and G+1A/In14 were found in CHD patients, but the sample size was only 45 patients, 16 of whom had CHD ($P < .05$). When the frequency of G+1A/In14 alone was examined as a function of age, there was no significant variation in the occurrence of this mutation between the ages of 40 and 79. Only above age 80 was there a difference in G+1A/In14 frequency relative to younger ages. Of the 67 patients genotyped who were over age 80, only 10 were heterozygotes, a lower frequency than found in the younger ages up to age 79 ($P < .05$). Again, the very low number of patients over 80 who were genotyped limited the power of the study. The low cardiovascular death rate in Japan relative to other industrialized countries further complicates analysis. Larger studies to examine this in more detail may be warranted.

SINGLE AMINO ACID CHANGES

There are eight polymorphisms listed in **Table 4** that cause an altered amino acid sequence in the full-length protein (12, 31–35). Some of these sequence changes are fairly common and appear to have only slight effects on protein function (A373P, I405V, and R451Q), while others are quite rare and have severe deleterious effects on activity (L151P, R282C), or have not been characterized in detail (G314S and V469M). D442G is intermediate between

TABLE 3. Mutation: A+55G/Ex15 (D442G)

CETP				HDL				
Ho	He	UF	Con	Ho	He	UF	Con	Reference
—	—	—	—	1.77 (2)	1.99 (16)	—	1.72 (206)	11
0.93 (4)	1.44 (47)	2.17 (26)	—	86 (4)	91 (47)	52 (26)	—	20
—	—	—	—	54.5 (60)	55.4 (170)	—	50.5 (3276)	21
10.7 (1)	19.7 (7)	—	25.6 (32)	4.53 (2)	1.97 (27)	—	1.51 (22)	23
—	—	—	—	2.29 (3)	2.27 (11)	—	1.49 (7)	24
6.4 (3)	18.3 (9)	—	25.6 (30)	111 (3)	96 (9)	—	59 (30)	25
—	—	—	—	—	57.9 (18)	—	47.4 (277)	27
43.5 (14)	61.2 (153)	—	100	2.82 (14)	2.36 (153)	—	1.34	28
—	—	—	—	115 (3)	117 (34)	—	117 (80)	36
—	2.0 (21)	—	2.6 (65)	—	1.58 (21)	—	1.69 (65)	37
—	1.8 (19)	—	2.2 (183)	—	—	—	—	38
—	—	—	—	—	36 (11)	—	34 (193)	39
—	—	—	—	—	34 (10)	—	35 (60)	40
—	—	—	—	—	1.44 (13)	—	1.19 (12)	41
—	3.6 (15)	—	9.5 (20)	—	46 (16)	—	48 (363)	42 (Chinese)
—	—	—	—	—	72 (9)	—	63 (130)	42 (Japanese)
—	—	—	—	68.0 (1)	53.8 (30)	—	45.7 (239)	43

TABLE 4. Polymorphisms in the CETP gene: single amino acid changes

Location	RFLP	Position	Function	Frequency	Reference	dbSNP
T+64C/Ex5		8030	L151P	rare	31	
C+145T/Ex9		11528	R282C	rare	31	
G+10A/Ex11		16153	G314S	rare	32	rs5881
G+21C/Ex12	StuI	19231	A373P	uncommon	33	rs5880
G+16A/Ex14	(FokI)	20233	I405V	common	34	rs5882
A+55G/Ex15		21433	D442G	rare/uncommon	35	rs2303790
G+82A/Ex15	TaqI	21460	R451Q	uncommon	12	rs1800777
G+49A/Ex16		21693	V469M	rare	32	rs5887

RFLP, restriction length fragment polymorphism; SNP, single-nucleotide polymorphism. Nomenclature is as described (53). To the extent possible, all polymorphisms reported in the literature are included in this table, but not those reported only electronically. Because of its completeness through the CETP gene, GenBank sequence AC010550.7 is used as the numbering reference, with the start of transcription denoted as position +1. Each change is also referenced as to the change identified and its location in a given exon/intron. Frequency of a polymorphism is classified as rare (less than 1%), uncommon (1% to 5%), or common (more than 5%). If the frequency varies across populations, the overall frequency is given followed by the frequency in a specific ethnic group in which it is more common. For some polymorphisms, the identity of the RFLP is listed. Amino acid changes are listed under function. dbSNP identifiers are provided for those SNPs available at the time of publication.

these extremes. It occurs commonly only in Asian populations and has a demonstrable impact on CETP activity.

Many studies have reported on the CETP and/or lipid levels for D442G homo- and heterozygotes (9, 20, 21, 23–25, 27, 28, 36–43). In vitro, the specific activity of CETP from cells producing G442 is 45% that of cells producing wild-type D442 protein (35). The mutant form was also found to possibly have dominant negative effects on the wild-type protein, suggesting that generalization of results with this mutation should be done cautiously. All of the homozygotes studied have significant CETP activity, typically 25% to 50% that of the control population, indicating that the protein is partially defective in vivo but is capable of carrying out a significant part of its normal function. D442G heterozygotes usually have 60% to 85% of the wild-type activity, although one study noted only 38% (42). In the two largest studies, heterozygotes retained 61–66% of control activity. The impact on HDL is much smaller, with homozygotes in one of the largest studies having less than a 10% increase in HDL relative to controls (21). In the study with the largest number of heterozygotes, patients were initially selected on the basis of hyperalphalipoproteinemia, thus their HDL levels may not be fully representative of patients with this mutation (28). In most smaller studies, heterozygotes also generally have elevated HDL levels, usually in the 10% to 80% range, although four studies actually noted a decreased HDL level among homozygotes (36) or heterozygotes (37, 40, 42). The study that measured the largest drop in heterozygote CETP activity actually had a decrease in HDL levels in a Chinese population but not in a Japanese population (42), illustrating the difficulties in working with small test populations. In none of these studies were a significant number of other polymorphisms monitored.

The significant residual activity of D442G distinguishes it from the null G+1A/In14 mutation that was discussed above. Despite these very different characteristics, the two mutations are frequently combined when assessing the effect of CETP deficiency on lipids and disease. If the reported results allow one to distinguish the effects of the two mutations as well as separating the impact of homo- versus heterozygous changes, they have been included below.

The largest clinical study in which D442G was examined took place in Honolulu among 3,469 Japanese-American men (21). The initial results of this study indicated that the D442G (along with G+1A/In14) mutation was associated with increased risk for CHD ($P = .049$), but this was only true among men with intermediate levels of HDL (41–60 mg/dl). This study has continued with more extensive follow up of these individuals. While additional results have not been fully reported yet, D442G may not be a risk factor in this population, particularly among high-HDL individuals (7, 44). Other studies on D442G individuals have noted the risk of CHD going in both directions, but not in a statistically significant manner. For example, among 474 Taiwanese patients and controls, the cardiovascular risk for DG genotypes was 1.69-fold higher than DD, with no significant effect on HDL (45), while a study in Japan found that 92 individuals with DG or GG had no cases of CHD, and three of 196 with the wild-type DD did have CHD (46). Among Chinese, the rate of D442G in normal controls versus CHD patients (8:200 vs. 7:200) was not significantly different (47). In this study, HDL was significantly higher among those with D442G. Thus, D442G slightly increases HDL levels in most of the populations studied (it occurs at a significant rate only among Asians), but its impact on disease is not as easily ascertained. If there is a clinical impact of D442G on disease, the effect is small.

Two of the more common amino acid changes, A373P and R451Q, are often studied together because they are in such high linkage disequilibrium. Virtually all people with the less-frequent 451Q allele (3–4% in European populations) also have the less-frequent 373P allele (about 5% in European populations). Only one of 658 Danes with the less-common 451Q did not also have the less-common 373P (48). No detailed studies on the functional consequences of the single A373P change or the double A373P/R451Q changes have been carried out on the iso-

lated proteins, so it is not clear whether CETP's biochemical activity is affected by these amino acid changes.

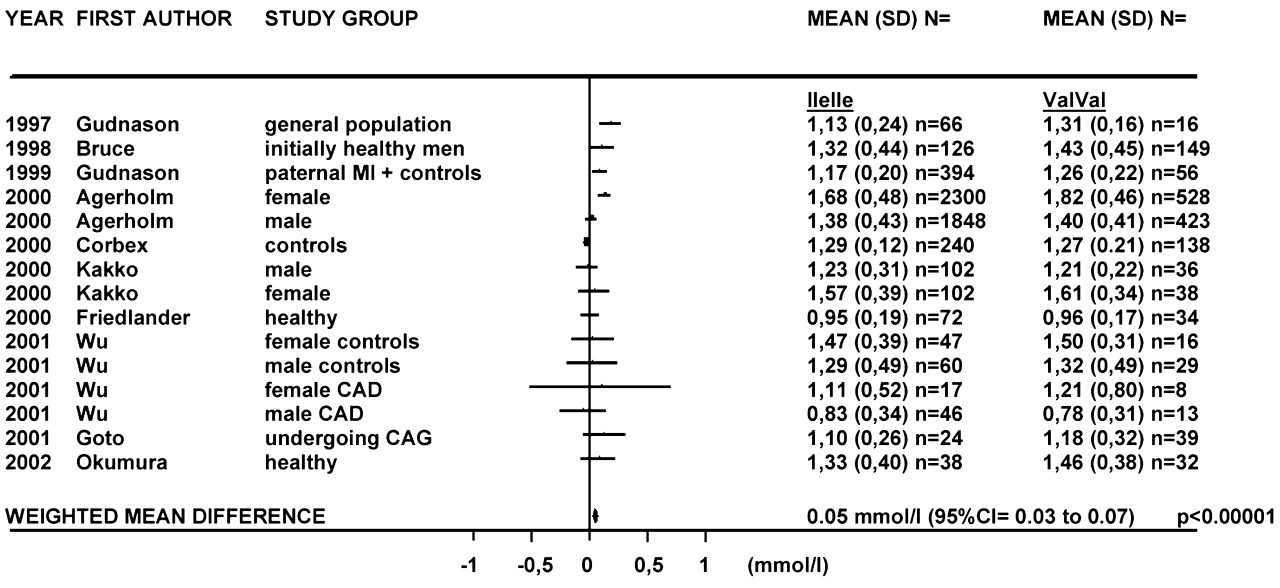
There have been two large studies of A373P heterozygotes that yield somewhat different results. When 1,236 French/Irish cases/controls were examined in the Etude Cas Temoin sur l'Infarctus du Myocarde study, CETP levels were measured and no effect of the mutation was observed (49). Not surprisingly, no effect on HDL levels was observed either, leading to the expected lack of effect on myocardial infarction (MI). In contrast, a larger study of 9,166 healthy Danes (48) found 10% lower HDL levels with 373P homozygotes. A more modest HDL lowering was observed in heterozygotes. CETP mass/activity was not measured. No association of the A373P mutation was observed with ischemic heart disease (IHD) unless the population was stratified by HDL levels and separated by gender. The effect was only apparent in premenopausal women and postmenopausal women who had not been treated with hormone replacement therapy. As mentioned earlier, the stratification by HDL is exactly the type of adjustment that eliminates the beneficial impact of CETP, so must be used cautiously for evaluating the true benefits/risks of CETP. The same two studies also examined the effect of the R451Q mutation that is present in a significant subset of the A373P population. With the French/Irish population, no association with CETP, HDL, or disease was found. With the Danish population, the association with HDL was similar (10% lowering) to what was seen with A373P, and similar disease impacts were observed.

Other studies do not clarify these discrepant results. Smaller studies on a Finnish population detected an association with CETP activity. One study showed no significant effect on HDL (50), while another showed an effect only in women (51). The heterozygous R451Q population had higher CETP and less intima-media thickness (IMT) (52). This effect was observed only in men ($P = 0.007$); in fact, the opposite trend was observed in women but the effect was not statistically significant. HDL level was observed not to be correlated with IMT in these populations, suggesting that, if the CETP effect on IMT is real, it may not be mediated via HDL. Another study on 110 healthy Americans found no association of R451Q with either CETP or HDL levels (53). Thus, the A373P and R451Q polymorphisms are associated with an effect of less than 10% on CETP activity, and only very weak impacts on disease are observed. Even this possible impact requires subsetting of populations or adjustment for other factors, providing little insight into the etiological role of CETP in cardiovascular disease.

The final common polymorphism resulting in an amino acid change is I405V. This is a very common SNP, as the less-common allele is present in all populations examined and generally occurs at a frequency of over 25%. In multiple studies, those with the less-common 405V allele have lower CETP levels, differing by 9–23% between the two homozygous states. The effect is less marked on HDL, with observations ranging from −6% to +10% (49, 51, 53–59). Comparing 405I to 405V frequency in a high-HDL population versus a low-HDL population showed a significant difference in allelic frequency in the two groups (60).

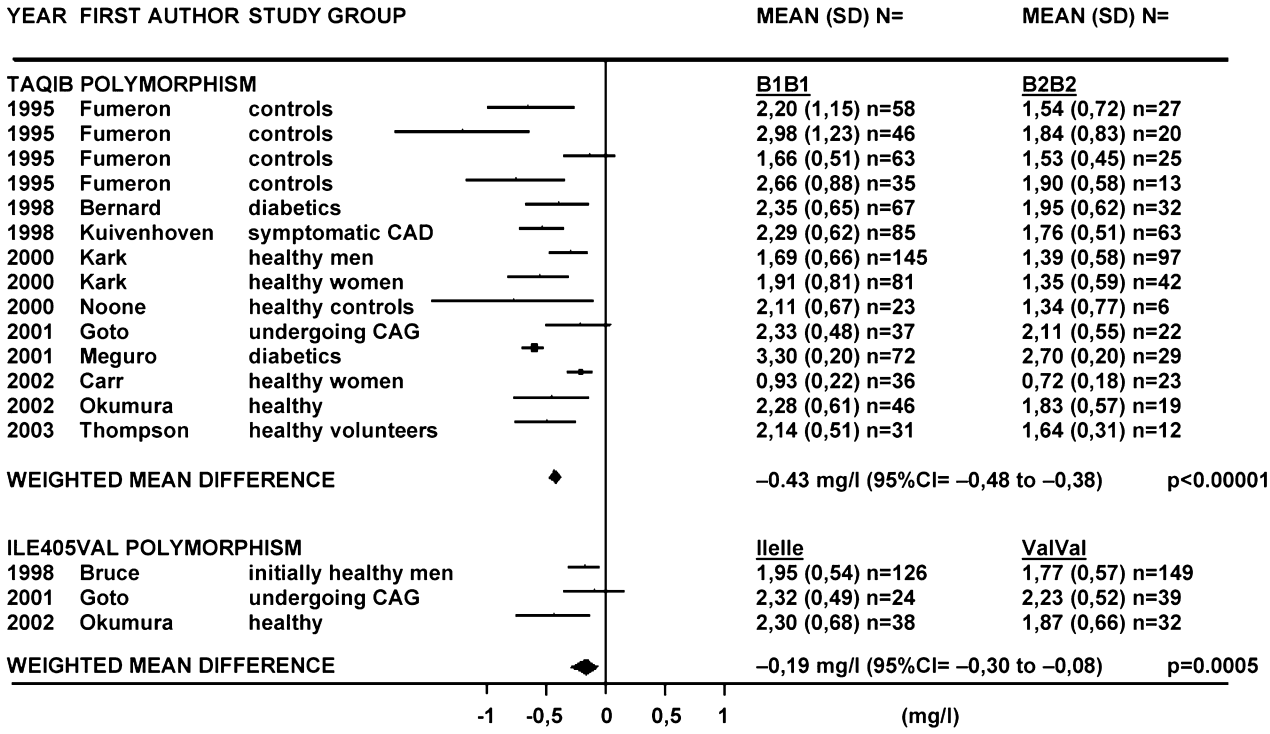
To better characterize the I405V polymorphism, a formal meta-analysis was carried out. We identified all popu-

TABLE 5. Effect of I405V on HDL



Weighted mean differences (WMDs) for the individual study groups and a pooled WMD for the outcome HDL cholesterol in 405ValVal homozygotes compared with wild-type 405IleIle homozygotes as reference group. Square size is proportional to the weight of the study group in the pooled analysis; bar length is proportional to 95% confidence intervals.

TABLE 6. Effect of I405V and Taq1B on CETP mass



WMDs for the individual study groups and a pooled WMD for the outcome CETP mass in TaqIB B2B2 homozygotes compared with wild-type B1B1 homozygotes as reference group, and for 405ValVal homozygotes compared with 405IleIle homozygotes as reference group. Square size is proportional to the weight of the study group in the pooled analysis; bar length is proportional to 95% confidence intervals.

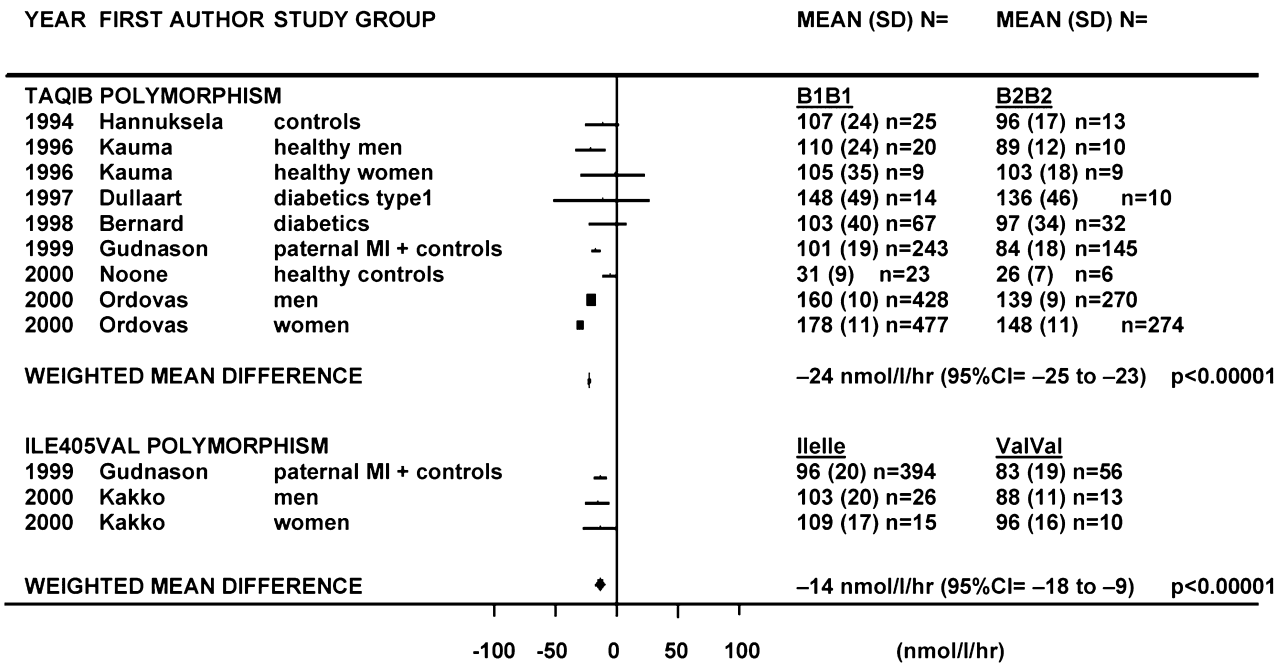
lation-based studies on CETP polymorphisms and their association with either lipid parameters, CETP mass, or CETP activity. The literature was scanned by a formal search of MEDLINE electronic database. Search terms that were used were both MESH terms and (part of) the text words "cholesteryl ester transfer protein" or "CETP" in combination with "polymorphism," "mutation," or "genetics." The search results were subsequently limited to "human" and "English language." Reference lists of retrieved articles were scanned for additional potentially relevant publications. In addition, for each retrieved publication, an electronic "cited reference search" was performed (Web of Science version 4.1.1, Institute for Scientific Information 2000), identifying all papers citing the index publication. If the presented data could not be used in the meta-analysis, the principal investigator was contacted and asked to provide unreported data.

We limited our analysis to the two most extensively studied CETP polymorphisms, i.e., the TaqIB and I405V polymorphisms. We also limited our meta-analysis to the outcomes HDL, CETP mass, and CETP activity. Other outcomes, such as LDL and triglycerides, were not included because these data are under-reported in the articles, and are therefore likely to be subject to publication bias. IHD was not used as an outcome because of the considerable heterogeneity among the reported disease states, which included MI, coronary angiography, diabetic macroangiopathy, unstable angina, etc. Studies were included if they reported data on both a CETP polymorphism and a lipid parameter in a sample of unrelated individuals.

Data were independently extracted and entered into separate databases by two investigators. The results were compared, and disagreements were resolved by consensus. Although the methods used to determine CETP activity vary among the studies, the data were included in a meta-analysis assuming identical measurements for the genotypes within each study. Weighted mean differences with corresponding 95%CIs were calculated. The raw data from each population described separately were entered as a separate stratum. Analyses were performed according to a recessive model in which mutant homozygotes are compared against wild-type homozygotes in order to obtain maximal contrast. Tests for heterogeneity were performed with each meta-analysis. Data were analyzed using Review Manager version 4.1 (The Cochrane Collaboration 2000).

When 405V is compared with 405I, there is a modest but highly significant association with both HDL levels (**Table 5**) and CETP mass/activity (**Tables 6**, **7**). 405V mass is 0.19 mg/l less ($P = 0.0005$), and 405V activity is 14 nmol/l/h less ($P < 0.00001$) than the more common 405I. The lowered CETP for 405V homozygotes is associated with a 5 mmol/l increase in HDL relative to 405I ($P < 0.00001$). Thus, the combination of multiple studies al-

TABLE 7. Effect of I405V and Taq1B on CETP activity



WMDs for the individual study groups and a pooled WMD for the outcome CETP activity in TaqIB B2B2 homozygotes compared with wild-type B1B1 homozygotes as reference group and for 405ValVal homozygotes compared with 405IleIle homozygotes as reference group. Square size is proportional to the weight of the study group in the pooled analysis; bar length is proportional to 95% confidence intervals.

lows one to more rigorously confirm this phenotype/genotype association.

Among the larger studies, limited disease outcomes can also be assessed. With 822 sons of MI victims (European Atherosclerosis Research Study), the lowered CETP is associated with a 7.5% HDL elevation, but no impact on CHD was observed (57). Among 4,983 healthy Danish women, a 4% HDL elevation was observed ($P < .001$), while no significant effect (+1.4%) was seen in 4,006 men (58). In this study, no IHD effect in men was observed. In women, the effect on IHD was not significant until after adjustment for HDL levels. When Japanese men living in Hawaii were examined, a 7.9% increase in HDL was observed, but there was no impact on CHD unless the population was stratified by triglycerides and other corrections included (59). In the Stanislas cohort, comparing homozygous II individuals with IV and VV is associated with a modest effect on IMT ($P < .1$), but only in men (61). CETP/HDL levels were not reported. These small effects on disease do not allow firm conclusions to be drawn. In all studies, the effects on LDL and triglycerides were small and frequently not reported due to a lack of statistical significance.

WILD-TYPE PROTEIN

In addition to the polymorphisms that result in altered protein sequence, there are many DNA changes that do not affect protein sequence. To expect such genomic changes to have a functional impact, they must alter the expression levels of the mRNA/protein or be in linkage disequilibrium with other changes that affect either expression or activity. The best-characterized noncoding mutation is the Taq1B SNP that occurs in Intron 1. The less-common allele of this SNP has been shown to be associated with lower CETP and higher HDL levels across numerous studies and populations with highly significant *P* values (45, 49, 53, 60, 62–99). Only in studies with small test populations has neither CETP nor HDL been affected in a statistically significant manner (43, 93–107). Since it is not immediately apparent how this SNP could modulate CETP expression, it has long been suspected that this SNP is linked with another change that more directly affects expression. Indeed, Taq1B has been found to be in strong linkage disequilibrium with the −629 promoter mutation that occurs in a transcription factor binding site and affects Sp1/Sp3 binding (108). It is not yet clear whether the −629 SNP is entirely responsible for the functional effects or whether other changes linked to Taq1B may also be involved (51, 53, 97, 109). For example, there is also a promoter VNTR located 1,946 bp upstream of the transcription start site that is linked to Taq1B and appears to have an independent effect on CETP and HDL levels (53, 110, 111). Other promoter polymorphisms have also been studied in combination with Taq1B and may also exert independent effects (97). The very high-linkage disequilibrium in this region makes distinguishing the effects of different polymorphisms difficult.

The Taq1B polymorphism is very frequent, with the heterozygous B1B2 state being the most common in most populations. Its association with CETP mass/activity varies depending on the population studied, but the less-common B2 allele generally is associated with a reduction in CETP activity and increased HDL level in the homozygous B2B2 state relative to the homozygous B1B1 state. As with I405V, the Taq1B studies were subjected to a formal meta-analysis as described above. Data were excluded if they reported data on subjects specifically selected by abnormal lipid criteria (68, 69, 77, 83, 88, 107), on alcoholics (75), on related individuals (91), and specifically on patients with non-CAD related disease (76, 78). Duplicate publications and publications with patient overlap were excluded. Some articles were not included in the meta-analyses because these data were not reported in the publications, and could not be obtained by contacting the investigators (63, 85, 96, 99).

Over 50 studies/sub-studies met the criteria for inclusion and were analyzed for CETP (Table 6, 7) and HDL levels (**Table 8**). In these studies, over 10,000 individuals were genotyped as homozygous for either B1 or B2. As with I405V, the disparate manner in which cardiovascular disease endpoints are reported prevents analysis of disease in a rigorous manner. Individuals with the homozygous B2B2 genotype had 0.43 mg/l lower CETP mass and 24 nmol/l/h lower activity than B1B1 homozygotes ($P < 0.00001$). In the trials analyzed, the B2-associated lowering of CETP mass/activity is accompanied by a 12 mmol/l increase in HDL ($P < 0.00001$). The direction of effects of Taq1B on LDL and triglycerides is significantly more variable than seen with HDL, and these data are frequently not reported due to lack of statistical significance, making a rigorous analysis of those parameters impossible.

The association of CETP lowering and HDL elevation with impact on disease varies dramatically depending on the nature and size of the population being studied. The studies examine widely different endpoints, so it is not possible to make the same kind of generalizations that can be made for CETP and HDL levels. Nonetheless, some patterns begin to emerge, especially among the larger studies. In one of the largest such studies [on offspring from the Framingham study, ref. (62)], 2,916 subjects were analyzed and the 14% lower CETP levels and 10% higher HDL were associated with a 30% lowering of risk for the B2B2 male offspring ($P = 0.035$). The number of affected women was too low to draw conclusions. When the risk of CHD was adjusted for HDL, the trend was still present but no longer significant ($P = 0.18$). Again, adjusting for HDL levels would be expected to eliminate the beneficial effect of CETP, so this is not surprising. In a study of 1,182 Icelanders (90), men with the B2B2 genotype had 15% higher HDL with a corresponding lowering of risk for CHD ($r = 0.69$, $P < 0.01$). In the Veterans Affairs HDL Intervention Trial of 852 men with low HDL (88), those with the B2B2 genotype had 5% higher HDL and a relative risk of 0.56 for CHD. However, this study did not achieve statistical significance ($P = 0.08$). When 119 Koreans with disease were compared with 106 controls, B1B1 homozygotes were at 1.97-fold greater risk for CAD ($P = 0.026$) (112). Nine hundred-twenty-five Japanese women were found to have more angina pectoris when they had the B1B1 genotype ($P = 0.03$), while the effect was not statistically significant in 583 men (113). Other studies that have attempted to associate this polymorphism with CHD have also been limited by the size of the population needed to address this issue, even in an at-risk population (49, 114, 115).

In some populations, an association of Taq1B with other diseases or endpoints has been observed. Progression of atherosclerosis was found to be significantly associated ($P < 0.03$) with the B1 allele in a study of 807 Dutch men with CAD (64). French male diabetics are at increased risk of coronaropathies ($P = 0.025$) with the B1 allele, while there was no effect in females (67). Finns with gall bladder disease have a higher risk of gallstones with the B1 allele (76), and Japanese diabetics have a higher likelihood of microangiopathy ($P = 0.009$) with the B1 allele (73). Renal transplant recipients with the B1 genotype were more likely ($P = 0.045$) to suffer CHD (78). In these populations at high risk for cardiovascular disease, smaller populations could be used to see the beneficial effects of lowering CETP activity.

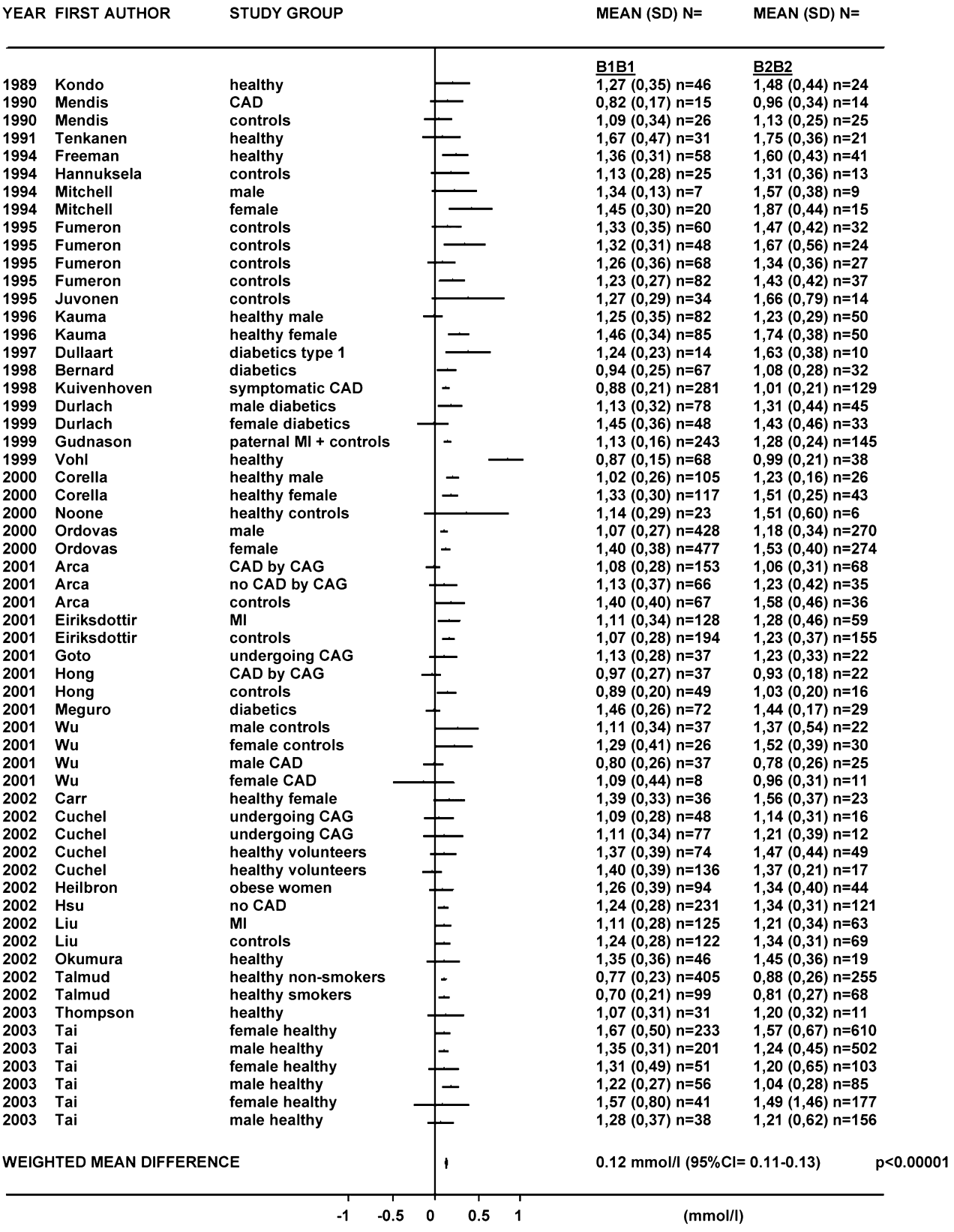
The Taq1B SNP can be considered a natural experiment in lowering CETP levels across a large swath of the population. Rather than affecting the specific activity or function of the protein as is the case with amino acid changes, the Taq1B SNP results in wild-type CETP with reduced expression, as this SNP is more tightly linked with promoter alterations than with any protein coding change. This is associated with a 12 mmol/l elevation in HDL in a sufficiently large fraction of the general population that an effect can be seen on cardiovascular disease, but only when the population studied is large enough or is sufficiently at risk.

Other polymorphisms with no effect on protein sequence have been identified but studied to a much lesser extent. The less-common allele of the MspI SNP in Intron 8 was found to associate with higher CETP ($P < 0.0001$) and lower HDL ($P < 0.000001$) than the common form (68). Attempts to associate this SNP with disease have not been reported thus far. A mutation in the 3′ untranslated region was found to have a significant association ($P = 0.002$) with CETP activity, but no effect on HDL (69). Other SNPs (BamHI in Intron 9, EcoNI in Intron 9, Taq1A in Intron 10) have also been studied, but do not appear to have significant effects on either CETP activity/mass or HDL (43, 49, 53, 68, 71, 75, 76, 106, 116). More changes have been identified throughout the gene, but remain less well characterized (**Table 9**). When a large population is segmented into high- and low-HDL populations, many SNPs that are not associated with HDL levels in a standard cross-sectional approach can be seen to be associated with HDL based on allelic frequency in the extreme populations (60).

SUMMARY

While dozens of studies have been carried out using genetic variation of CETP as a tool to understand the role of that gene, the conclusions that can be drawn about

TABLE 8. Effect of Taq1B on HDL



WMDs for the individual study groups and a pooled WMD for the outcome HDL cholesterol in TaqIB B2B2 homozygotes compared with wild-type B1B1 homozygotes as reference group. Square size is proportional to the weight of the study group in the pooled analysis; bar length is proportional to 95% confidence intervals.

TABLE 9. Polymorphisms in the CETP gene: polymorphisms that do not affect coding sequence

Location	RFLP	Position	Function	Frequency	Reference	dbSNP
G-2844T/Prom		−2,844		common	97	
G-2708A/Prom		−2,708		common	97	
T-2658C/Prom		−2,658		common	97	
C-2545A/Prom		−2,545		common	97	
VNTR-1946/Prom		−1,946	VNTR	common	110	
T-1932C/Prom		−1,932		common	97	
G-971A/Prom		−971		common	117	
C-631A/Prom		−631		common	108	rs1800776
C-629A/Prom		−629	Sp1/3 site	common	108	rs1800775
G-69A/Prom		−69	PEA3/ETS	rare	26	
G-38A/Prom		−38	Sp1/3 site	rare/common	53	
C+93A/Ex1		93		rare/uncommon	32	rs5884
G+202A/In1	HphI	347		common	118	rs711752
C+270T/In1		415		uncommon	119	
G+279A/In1	Taq1B	424		common	120	rs708272
C+39T/Ex7		9,412		rare?	32	rs5885
C+8T/In7		9,442		common	65	rs1532625
A+383G/In8	MspI	10,519		common	120	
C+595T/In8		10,731		common	53	
A+834T/In8	XbaI	10,970		common	53	rs289713
C+111T/Ex9		11,494		rare/common?	32	rs5883
A+29G/In9	BamHI	11,592		common	22	rs289714
A+188G/In9	EcoNI	11,751		common	121	rs158477
C+312A/In9		11,875		common	60	rs158478
G+626A/In9		12,189		common	60	rs158479
A+865G/In9		12,428		common	60	rs158617
T+876A/In9		12,439		common	60	rs289715
T+869C/In10		14,073		common	60	rs289718
T+878C/In10		14,082		common	60	rs289719
C+2180T/In10	Taq1A	15,384		common	120	
G+2389A/In10		15,593		common	60	rs291044
Δ12bp+199/In12		19,477–19,488		common	48	
C+408T/In12		19,686		common	65	rs1800774
C+35G/In13		19,768		rare	53	
G+51A/Ex14		20,268		rare?	32	rs5886
G+151A/In15	HphI	21,615		common	16	rs289741
G+159A/Ex16	NlaIII	21,803		common	53	rs1801706
G+9C/3′		21,903		common	53	rs289742
G+349T/3′		22,243		common	49	rs289744

Nomenclature is as described (53). To the extent possible, all polymorphisms reported in the literature are included in this table, but not those reported only electronically. Because of its completeness through the CETP gene, GenBank sequence AC010550.7 is used as the numbering reference, with the start of transcription denoted as position +1. Each change is also referenced as to the change identified and its location in a given exon/intron. Frequency of a polymorphism is classified as rare (less than 1%), uncommon (1% to 5%), or common (more than 5%). If the frequency varies across populations, the overall frequency is given followed by the frequency in a specific ethnic group in which it is more common. In a few cases, the original reference is unclear as to frequency and these are followed by "?." For some polymorphisms, the identity of RFLPs is listed. Promoter changes suspected to alter transcription factor binding are listed under function. For sequences upstream of the promoter VNTR, numbering is somewhat arbitrary because of the highly polymorphic nature of this region. Numbering used here is as provided in the original references. The VNTR is assigned a position of −1,946 for consistency with earlier work (111). dbSNP identifiers are provided for those SNPs available at the time of publication.

CETP's impact on disease in a normal population are limited. The most dramatic effects on CETP activity are found in individuals with either a complete lack of CETP or altered activity caused by a dominant negative or severely defective form of the enzyme. Thus, while those patients show that it is possible to live a normal life without CETP, they do little to shed light on how altering the level of CETP activity in the general population would impact cardiovascular disease. There is a second class of individuals who have an altered form of CETP with at least one, and sometimes two or three, amino acid changes that lead to an uncertain impact on disease. The effect on CETP activity and HDL levels is variable and not generally very large, making it difficult to see significant effects over background. Possibly the most useful genetic tool used thus far is the Taq1B SNP that results in a protein with wild-type sequence but altered levels of expression, similar to what would be observed in a population treated pharmacologically with a weak CETP inhibitor. The genotypes that are regularly associated with low CETP and high HDL are also generally, though not always, found to be associated with a protective effect for cardiovascular disease. However, when selection criteria or risk factor adjustment is carried out in a manner that limits the potential beneficial effects of CETP, the positive impact on disease can be obscured.

The very fact that a controversy exists about the message from CETP genetic studies is testament to the diffi-

culties in trying to draw solid conclusions from effects that are either small in magnitude or rare in occurrence. Even after tens of thousands of individuals have been genotyped, phenotyped, bled, and probed, it is necessary to bring together the entire body of literature to support the case that inhibition of CETP is a desirable therapeutic endpoint. That being said, there is no doubt that some would still question even that conclusion. Nonetheless, the preponderance of genetic studies reported thus far support the testing of therapeutics for the inhibition of CETP. Inhibition of greater than 20% would be expected to be necessary based on the marginal beneficial effects seen with the Taq1B SNP. Inhibition of 100% as observed in the homozygous G+1A/In14 mutation is probably neither necessary nor advisable for a beneficial effect. However, even complete loss of activity does not necessarily lead to adverse outcomes. The presence of cardiovascular disease is not overt, even in those individuals lacking any CETP activity whatsoever. While the genetic studies support the concept of inhibiting CETP for beneficial effects on cardiovascular disease, the degree of disease protection afforded by inhibition of CETP will only become apparent after clinical trials. For optimal therapeutic effect, any CETP inhibitor should raise HDL above 60 mg/dl based on extant genetic studies. Having such agents available for use in low HDL individuals could provide substantial public health benefits.

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