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Separating the Mechanism-Based and Off-Target Actions of Cholesteryl Ester Transfer Protein Inhibitors With *CETP* Gene Polymorphisms

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Background—Cholesteryl ester transfer protein (CETP) inhibitors raise high-density lipoprotein (HDL) cholesterol, but torcetrapib, the first-in-class inhibitor tested in a large outcome trial, caused an unexpected blood pressure elevation and

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increased cardiovascular events. Whether the hypertensive effect resulted from CETP inhibition or an off-target action of torcetrapib has been debated. We hypothesized that common single-nucleotide polymorphisms in the *CETP* gene could help distinguish mechanism-based from off-target actions of CETP inhibitors to inform on the validity of CETP as a therapeutic target.

Methods and Results—We compared the effect of *CETP* single-nucleotide polymorphisms and torcetrapib treatment on lipid fractions, blood pressure, and electrolytes in up to 67 687 individuals from genetic studies and 17 911 from randomized trials. *CETP* single-nucleotide polymorphisms and torcetrapib treatment reduced CETP activity and had a directionally concordant effect on 8 lipid and lipoprotein traits (total, low-density lipoprotein, and HDL cholesterol; HDL2; HDL3; apolipoproteins A-I and B; and triglycerides), with the genetic effect on HDL cholesterol (0.13 mmol/L, 95% confidence interval [CI] 0.11 to 0.14 mmol/L) being consistent with that expected of a 10-mg dose of torcetrapib (0.13 mmol/L, 95% CI 0.10 to 0.15). In trials, 60 mg of torcetrapib elevated systolic and diastolic blood pressure by 4.47 mm Hg (95% CI 4.10 to 4.84 mm Hg) and 2.08 mm Hg (95% CI 1.84 to 2.31 mm Hg), respectively. However, the effect of *CETP* single-nucleotide polymorphisms on systolic blood pressure (0.16 mm Hg, 95% CI −0.28 to 0.60 mm Hg) and diastolic blood pressure (−0.04 mm Hg, 95% CI −0.36 to 0.28 mm Hg) was null and significantly different from that expected of 10 mg of torcetrapib.

Conclusions—Discordance in the effects of *CETP* single-nucleotide polymorphisms and torcetrapib treatment on blood pressure despite the concordant effects on lipids indicates the hypertensive action of torcetrapib is unlikely to be due to CETP inhibition or shared by chemically dissimilar CETP inhibitors. Genetic studies could find a place in drug-development programs as a new source of randomized evidence for drug-target validation in humans. **(*Circulation*. 2010;121:52-62.)**

Key Words: genetics ■ pharmacology ■ epidemiology ■ high-density lipoproteins

Higher concentrations of high-density lipoprotein (HDL) cholesterol are associated with a lower risk of coronary heart disease (CHD) independent of low-density lipoprotein (LDL) cholesterol.[1] HDL particles have antiatherogenic actions in vitro, and experimental elevation of HDL cholesterol concentration in some animal models attenuates atheroma formation.[2,3] Inhibitors of cholesteryl ester transfer protein (CETP), which mediates exchange of lipids between HDL particles and other lipoproteins, are a new class of drugs developed for their ability to raise HDL cholesterol. However, when the combination of a CETP inhibitor (torcetrapib) and a statin (atorvastatin) was compared with atorvastatin alone in the Investigation of Lipid Level Management to Understand Its Impact in Atherosclerotic Events (ILLUMINATE) trial,[4] the Data Safety Monitoring Board terminated the trial prematurely because of an unexpectedly higher rate of both cardiovascular and noncardiovascular events in the torcetrapib-treated patients.

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Whether the higher rate of cardiovascular events from torcetrapib treatment was a mechanism-based effect of CETP inhibition, which would be shared by other members of the same drug class, or an idiosyncratic (or off-target) action of the torcetrapib molecule is uncertain. It is important to distinguish between the two, because at least 2 other CETP inhibitors, anacetrapib and dalcetrapib, are in advanced stages of drug development.[5–7] Torcetrapib treatment has been associated with consistent and substantial elevations in blood pressure,[4,8–10] perhaps secondary to a mineralocorticoid-like effect, which could have contributed to the increased risk of cardiovascular events.[11] Although it has been proposed that the other CETP inhibitors do not share this blood pressure–elevating effect,[5,12] this is based on evidence from nonrandomized animal experiments and short-term dose-ranging studies in humans, both of which have limitations. Large, randomized outcome trials of anacetrapib or dalcetrapib would provide a definitive answer but could expose the trial participants to a potential hazard should the hypertensive effect be mechanism based rather than off target. On the other hand, the failure to further evaluate other members of this class in randomized trials could lead to the abandonment of a potentially valuable preventive therapy.

An alternative way of obtaining randomized evidence on the efficacy and safety of CETP inhibition in humans without the recruitment of new trial participants, prospective follow-up, or exposure to a drug is to study the effect of carriage of common alleles of the human *CETP* gene associated with reduced CETP levels and activity.[13] Genetic association studies are a type of natural randomized trial, because maternal and paternal alleles assort at random at conception.[14,15] In effect, a study of alleles of the *CETP* gene that reduce CETP activity is akin to a very long-term randomized intervention trial of a "clean" CETP inhibitor, free from the off-target effects of individual drug molecules. We therefore compared the effect of torcetrapib and carriage of common *CETP* alleles on lipids and lipoproteins, blood pressure, and other markers of cardiovascular risk in a large-scale, international, collaborative analysis to ascertain whether the increase in blood pressure seen in the clinical trials of torcetrapib was mechanism based or off target.

Methods

Search Strategy and Selection Criteria

Randomized Controlled Trials

Randomized controlled trials evaluating the effect of torcetrapib on markers of cardiovascular risk or clinical outcomes were identified from PubMed and EMBASE up to the end of November 2007 with the use of the US National Library of Medicine's Medical Subject Headings and the free-text terms "torcetrapib" or "CETP inhibitor" in combination with "randomized controlled trial." For inclusion in the main analyses, studies had to be randomized, parallel-design studies in adults that examined the effect of treatment with torcetrapib (alone or in combination) with a suitable comparator. Studies were included if they had been

published as full-length articles or letters in peer-reviewed journals in any language. Randomized studies were further subdivided into shorter dose-finding studies of <1 year's duration and longer clinical trials of >1 year's duration and analyzed separately.

Genetic Studies

PubMed and EMBASE were searched up to November 2007 for studies in humans evaluating any polymorphism in the *CETP* gene. The search included the Medical Subject Headings and free-text terms "cholesteryl ester transfer protein" or "CETP" in combination with "polymorphism*," "mutation*," "allele*," "gene*," "Taq1B," "−629C>A," or "I405V," with no limits or restrictions. We supplemented information from published studies with unpublished genetic data obtained through a large collaborative network of investigators that allowed access to information on a wider range of traits of interest, enabled more precise estimation of genetic effect sizes, and minimized the scope for reporting and publication bias. (For further details, see the online-only Data Supplement.)

Generation of Tabular Data

Two of the authors (A.D.H. and R.S.) extracted data, and disagreements were resolved by discussion with a third author (J.P.C.). For randomized controlled trials, information was extracted on treatment regimen and comparator, as well as pretreatment and posttreatment measures of a wide range of cardiovascular risk markers (see the online-only Data Supplement for further details). The relationship between torcetrapib dose and effect on these variables, if available, was also recorded from dose-ranging studies. For genetic studies, study-level information was either extracted from published studies by 2 authors or requested from principal investigators (see the online-only Data Supplement).

Statistical Analysis

Randomized Clinical Trials of Torcetrapib

The effect of torcetrapib on different lipid fractions, blood pressure, and other cardiovascular traits was assessed by calculation of the difference in the change in mean values between active and control arms. Study-specific estimates were weighted by the inverse of the variance and pooled by random-effects meta-analysis to generate summary estimates.

Genetic Studies

Primary analyses were based on the *CETP* gene variants commonly referred to as TaqI B (rs708272) and −629C>A (rs1800775), which were the most widely typed variants. The 2 are in linkage disequilibrium (r^2 measure of association 0.73 in individuals of European descent[16]; online-only Data Supplement Figure I), which allows information on the 2 variants to be treated jointly in a pooled analysis. Additional analyses involved the I405V variant (rs5882). For continuous outcomes, the mean difference and 95% confidence interval (CI) by genotype category were obtained from each study and then pooled with a random-effects model to obtain a summary mean difference and 95% CI. Individuals homozygous for the common TaqIB (or −629C) allele served as the reference group throughout, and this group was designated B1B1, with heterozygous individuals and individuals homozygous for either rare allele designated B1B2 and B2B2, respectively, to preserve the convention introduced in prior studies. For binary outcomes, results were expressed as an odds ratio and 95% CI. To assess the robustness of the findings, stratified analyses were conducted according to study-level characteristics. In a subset of studies, predefined stratified analysis of individual-level data was performed to investigate the effect of *CETP* genotype on HDL cholesterol by quartiles of systolic, diastolic, and pulse pressure and by LDL cholesterol quartile to gain insight into the potential for effect modification by blood pressure–lowering or cholesterol-lowering medications. Deviation from Hardy-Weinberg equilibrium was assessed in each study. Heterogeneity was assessed with a χ^2 test. The I^2 measure[17] and 95% CI were used to describe the extent of variability across studies. Additional information on the statistical analysis is provided in the online-only Data Supplement. All analyses were conducted with Stata 9.0 (StataCorp LP, College Station, Tex).

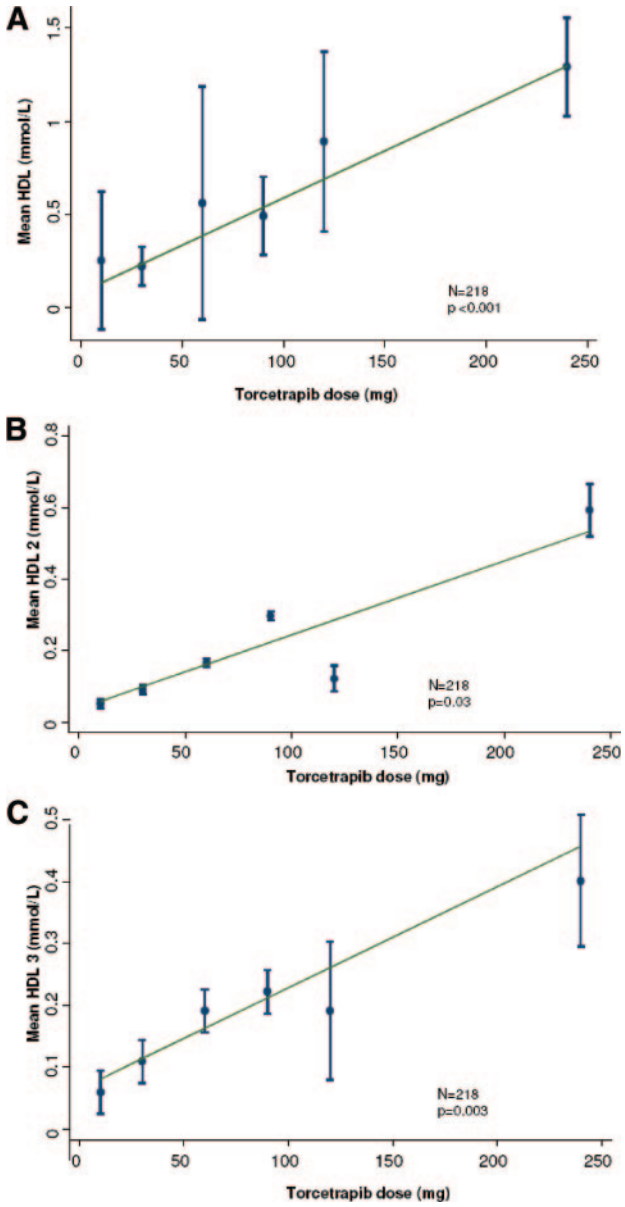

Figure 1. A through C, Relationship between torcetrapib dose and HDL cholesterol and HDL2 and HDL3 subfractions. *P* values refer to the results of a meta-regression, and N refers to the total number of individuals in the 3 dose-ranging studies that contributed to this analysis.

Consistency Between *CETP* Gene Effects and Equivalent Torcetrapib Dose

To determine the consistency of the observed effect of *CETP* genotype on cardiovascular traits with the expected effects for a comparable dose of torcetrapib, the shape of the dose–effect relationship for torcetrapib was evaluated from dose-ranging trials by use of the reported continuous outcomes HDL, HDL2, and HDL3, as well as apolipoprotein A-I (apoA-I) and apolipoprotein B (apoB). Despite careful searching, no quantitative information on the relationship between torcetrapib dose and blood pressure from these trials was available in a form that could be used in the analysis. Having confirmed a linear dose–response relationship for the available variables (Figures 1A through 1C), we used the summary effect

Table 1. Effect of Torcetrapib (60 mg) and *CETP* Genotype on Lipids and Lipoproteins

Comparison: Lipids and Lipoproteins	Randomized Controlled Trials, Torcetrapib 60 mg No. of Studies (Individuals)	Summary Mean Difference (95% CI)	*P*	Genetic Studies, B1B2 vs B1B1 No. of Studies (Individuals)	Summary Mean Difference (95% CI)	*P*	Genetic Studies, B2B2 vs B1B1 No. of Studies (Individuals)	Summary Mean Difference (95% CI)	*P*
HDL cholesterol, mmol/L	4 (17 911)	0.78 (0.68–0.87)	<0.001	30 (54 971)	0.06 (0.05–0.07)	<0.001	30 (34 432)	0.13 (0.11–0.14)	<0.001
ApoA1,* g/L	1 (15 067)	0.30 (0.30–0.31)	<0.001	11 (22 909)	0.03 (0.02–0.04)	<0.001	11 (14 739)	0.06 (0.05–0.08)	<0.001
Total cholesterol, mmol/L	4 (17 911)	0.18 (0.10–0.25)	<0.001	29 (54 135)	0.01 (−0.01–0.02)	0.48	29 (33 970)	0.05 (0.03–0.07)	<0.001
LDL cholesterol, mmol/L	4 (17 911)	−0.54 (−0.64–−0.43)	<0.001	27 (51 860)	−0.01 (−0.03–0.00)	0.07	27 (32 424)	−0.03 (−0.05–0.01)	<0.01
Triglycerides, mmol/L	4 (17 911)	−0.12 (−0.18–−0.07)	<0.001	28 (52 084)	−0.04 (−0.06–−0.02)	<0.001	28 (32 589)	−0.06 (−0.10–−0.02)	<0.01
ApoB,* g/L	1 (15 067)	−0.11 (−0.11–−0.10)	<0.001	11 (22 909)	−0.01 (−0.02–0.00)	0.05	11 (14 739)	−0.02 (−0.03–0.01)	<0.01
HDL2, mmol/L	NA	NA	NA	2 (3086)	0.02 (0.01–0.02)	0.001	2 (1856)	0.03 (0.01–0.04)	0.01
HDL3, mmol/L	NA	NA	NA	2 (3086)	0.04 (0.02–0.05)	<0.001	2 (1856)	0.06 (0.02–0.11)	0.01
Apo-AII, mg/L	NA	NA	NA	3 (8661)	0.28 (0.26–0.31)	<0.001	3 (5632)	0.29 (0.26–0.32)	<0.001

NA indicates not applicable.

Differences between continuous traits are for values reported at the end of the randomized trials unless otherwise indicated.

*Data obtained after 3 months.

of a 60-mg dose of torcetrapib on HDL cholesterol (the measure with the most data) from the meta-analysis of randomized trials and the summary effect of *CETP* genotype on HDL cholesterol from the meta-analysis of genetic studies (1) to express the effect of carriage of the B2 variant as a torcetrapib dose equivalent and (2) to estimate the effect of this dose of torcetrapib on blood pressure and other traits. A simulation model that incorporated the variance in the effect estimates of the genotype and drug effects was used to obtain the CIs (see online-only Data Supplement for details). The observed gene effect was compared with the effect of a comparable dose of torcetrapib by means of a *z* test.[18] More details are provided in the online-only Data Supplement.

Results

Randomized Controlled Trials of Torcetrapib

Dose–Response Relationship of Torcetrapib on HDL

Three studies (median size 40 participants, range 19 to 162 participants) with a mean study duration of 5.3 (standard deviation 3.1) weeks enabled the exploration of the effect of different doses of torcetrapib on HDL cholesterol and its subfractions (HDL2 and HDL3).[19–21] Over the dose range studied (10 to 240 mg daily), torcetrapib produced a linear, dose-dependent increase in HDL cholesterol ($P<0.001$ from meta-regression), HDL2 ($P=0.03$), and HDL3 ($P=0.003$), with no evidence of a threshold effect (Figures 1A through 1C).

Effect of Torcetrapib on Lipid Profile, Blood Pressure, and Biomarkers

Four randomized trials (range 752 to 15 067 participants) with a mean duration of 21 (standard deviation 6) months that involved 17 911 participants in aggregate with a mean age of 55.4 (standard deviation 6.9) years evaluated the effect of torcetrapib 60 mg daily (in combination with atorvastatin) versus atorvastatin alone and were included in the main analysis.[4,8–10] Torcetrapib 60 mg daily increased HDL cholesterol by 0.78 mmol/L (95% CI 0.68 to 0.87 mmol/L), apoA-I by 0.30 g/L (95% CI 0.30 to 0.31 g/L), and total cholesterol by 0.18 mmol/L (95% CI 0.10 to 0.25 mmol/L). The same dose reduced LDL cholesterol by 0.54 mmol/L (95% CI −0.64 to −0.43 mmol/L), triglycerides by 0.12 mmol/L (95% CI −0.18 to −0.07 mmol/L), and apoB by 0.11 g/L (95% CI −0.11 to −0.10 g/L; Table 1; Figure IIa in the online-only Data Supplement). A pooled analysis of all 17 911 participants from the 4 trials indicated that torcetrapib 60 mg daily led to a mean increase in systolic blood pressure of 4.47 mm Hg (95% CI 4.10 to 4.84 mm Hg) and an increase in diastolic blood pressure of 2.08 mm Hg (95% CI 1.84 to 2.31 mm Hg). In the ILLUMINATE trial, the elevation in blood pressure was accompanied by a decrease in plasma potassium, an increase in sodium, and an increase in aldosterone concentration[4] (Table 2). In 3 trials[4,9,10] that included 17 159 participants, there was no effect of torcetrapib on C-reactive protein concentration (online-only Data Supplement Table I).

Genetic Studies

Study Details and CETP *Polymorphisms Evaluated*

A total of 31 studies (online-only Data Supplement references S1 to S39) and 67 687 individuals a mean of 55.8 (standard deviation 9.6) years old contributed information on at least 1 continuous outcome. Twenty-three studies with 60 316 individuals provided previously unpublished data. Of the unpublished studies, 21 studies (50 908 individuals) provided data on the rs708272 (Taq1B) polymorphism, and 2 studies (8535 participants) provided data only on the rs1800775 (−629C>A) polymorphism. Where studies provided data on both −629C>A and Taq1B, the latter was used for the primary analysis. Seven studies (21 353 individuals) also provided data on the rs5882 (I405V) polymorphism (online-only Data Supplement references S8, S10, S15, S16, S18–S20, S22, S25, S32, and S33), and these results are provided in the online-only Data Supplement. Study details are provided in online-only Data Supplement Tables II and III, respectively.

Effect of CETP *Genotypes on CETP Concentration, CETP Activity, and Lipids*

Six studies in individuals of European ancestry (5340 participants) provided information on the effect of *CETP* genotype on CETP concentration (online-only Data Supplement references S7, S8, S15, S28, S30, and S31), and 2 studies (858 participants; online-only Data Supplement references S15

Table 2. Effect of Torcetrapib (60 mg) and *CETP* Genotype on Blood Pressure and Circulating and Urinary Electrolytes and Creatinine

Comparison	Randomized Controlled Trials, Torcetrapib 60 mg No. of Studies (Individuals)	Summary Mean Difference (95% CI)	*P*	Genetic Studies, B1B2 vs B1B1 No. of Studies (Individuals)	Summary Mean Difference/Odds Ratio (95% CI)	*P*	Genetic Studies, B2B2 vs B1B1 No. of Studies (Individuals)	Summary Mean Difference (95% CI)	*P*
Blood pressure, mm Hg									
Systolic	4 (17 911)	4.47 (4.10–4.84)	<0.001	22 (47 841)	−0.27 (−0.64–0.10)	0.15	22 (30 047)	0.16 (−0.28–0.60)	0.46
Diastolic	4 (17 911)	2.08 (1.84–2.31)	<0.001	22 (47 841)	−0.23 (−0.43–−0.04)	0.02	22 (30 047)	−0.04 (−0.36–0.28)	0.80
Pulse pressure	NA	NA	NA	7 (29 411)	0.03 (−0.29–0.35)	0.88	7 (18 574)	−0.13 (−1.16–0.91)	0.81
Electrolytes and creatinine									
Plasma potassium, mmol/L†	1 (15 067)	−0.14 (−0.15–−0.13)	<0.001	6 (13 760)	0.00 (−0.01–0.01)	0.98	6 (8678)	−0.01 (−0.03–0.01)	0.39
Plasma sodium, mmol/L†	1 (15 067)	0.61 (0.51–0.71)	<0.001	6 (13 583)	−0.06 (−0.19–0.07)	0.35	6 (8554)	0.03 (−0.18–0.18)	0.98
Plasma creatinine, μmol/L†	1 (15 067)	−1.15 (−1.15–−0.75)	<0.001	4 (12 756)	−0.39 (−1.42–0.64)	0.45	4 (7956)	0.31 (−0.72–1.35)	0.55
Plasma bicarbonate, mmol/L†	1 (15 067)	0.35 (0.24–0.46)	<0.001	NA	NA	NA	NA	NA	NA
Plasma chloride, mmol/L†	1 (15 067)	0.07 (−0.02–0.16)	0.14	NA	NA	NA	NA	NA	NA
Urinary potassium, mmol/L	NA	NA	NA	1 (1599)	−1.84 (−5.15–1.47)	0.27	1 (1092)	−0.90 (−4.68–2.88)	0.64
Urinary sodium, mmol/L	NA	NA	NA	1 (1599)	2.29 (−2.46–7.04)	0.34	1 (1092)	3.60 (−1.99–9.19)	0.2
Urinary creatinine, mg/L	NA	NA	NA	1 (1599)	−0.26 (−0.91–0.39)	0.43	1 (1092)	−0.02 (−0.77–0.73)	0.96

NA indicates not applicable.
*Data obtained after 3 months.
†Data from ILLUMINATE only.
Differences between continuous traits are at end of the randomized trials unless otherwise indicated.

and S18–S20) provided information on the effect on CETP activity. A further 5 studies (1867 participants) contributed data from individuals of Japanese origin (online-only Data Supplement Figure III and references S34 through S39). A graded effect of genotype on CETP concentration and activity was evident in both populations. People of European ancestry who were homozygous for the B2 allele had lower CETP concentrations (−0.47 μg/mL, 95% CI −0.67 to −0.26 μg/mL) and lower CETP activity (−17.00 nmol · mL^{-1} · h^{-1}, 95% CI −18.52 to −15.49 nmol · mL^{-1} · h^{-1}) than people homozygous for the B1 allele (online-only Data Supplement Figures IIIa and IIIb). In 31 studies with 67 687 participants, B2-homozygous individuals had higher concentrations of HDL cholesterol (0.13 mmol/L, 95% CI 0.11 to 0.14 mmol/L; Figure 2). The link between genotype and HDL cholesterol was consistent in analyses stratified by study size, sex, presence of CHD, and ancestry and across quartiles of LDL cholesterol, systolic and diastolic blood pressure, and pulse pressure (online-only Data Supplement Figures IIIc and IV). In addition, B2-homozygous individuals exhibited higher concentrations of total cholesterol (0.05 mmol/L, 95% CI 0.03 to 0.07 mmol/L) and apoA-I (0.06 g/L, 95% CI 0.05 to 0.08 g/L) and lower concentrations of LDL cholesterol (−0.03 mmol/L, 95% CI −0.05 to −0.01 mmol/L), triglycerides (−0.06 mmol/L, 95% CI −0.10 to −0.02 mmol/L), and apoB (0.02 g/L, 95% CI −0.03 to −0.01 g/L). In 2 studies, individuals homozygous for the B2 allele had higher circulating concentrations of both the larger HDL2 particles (0.03 mmol/L, 95% CI 0.01 to 0.04 mmol/L) and smaller HDL3 particles (0.06 mmol/L, 95% CI 0.02 to 0.11 mmol/L; Table 1). Heterozygous subjects exhibited lipid and lipoprotein concentrations approximately intermediate between those found in homozygous subjects, consistent with an additive effect of each copy of the variant allele (Table 1; per-allele data available on request). The effect of variant *CETP* alleles on lipid and lipoprotein profile thus reproduced the direction of effect of treatment with torcetrapib in clinical trials for 8 separate lipid and lipoprotein traits (Table 1; Figure 3A; online-only Data Supplement Figures 2a and 2b). Using a simulation model and assuming a linear dose–response relationship (Figure 1), we estimated that the effect on HDL in B2-homozygous individuals corresponded to a

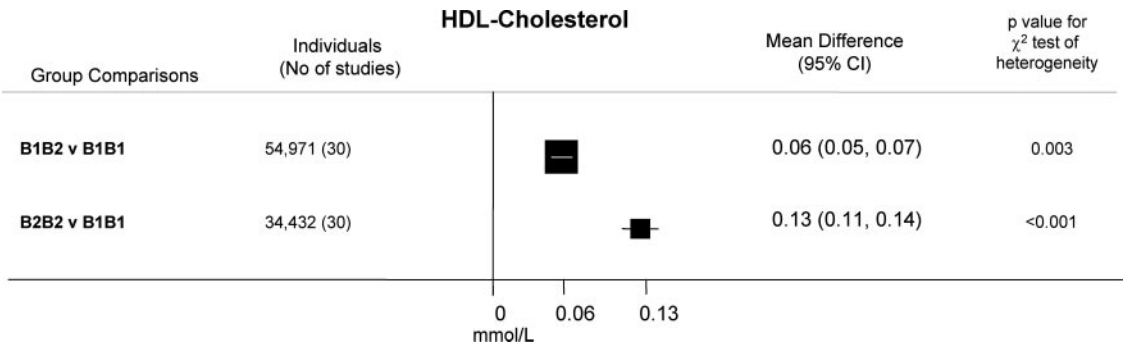

Figure 2. Effect of *CETP* genotype on HDL cholesterol in individuals of European ancestry. The B1B1 genotype is used as the reference group. The numbers refer to the total number of individuals that contribute to the comparisons shown.

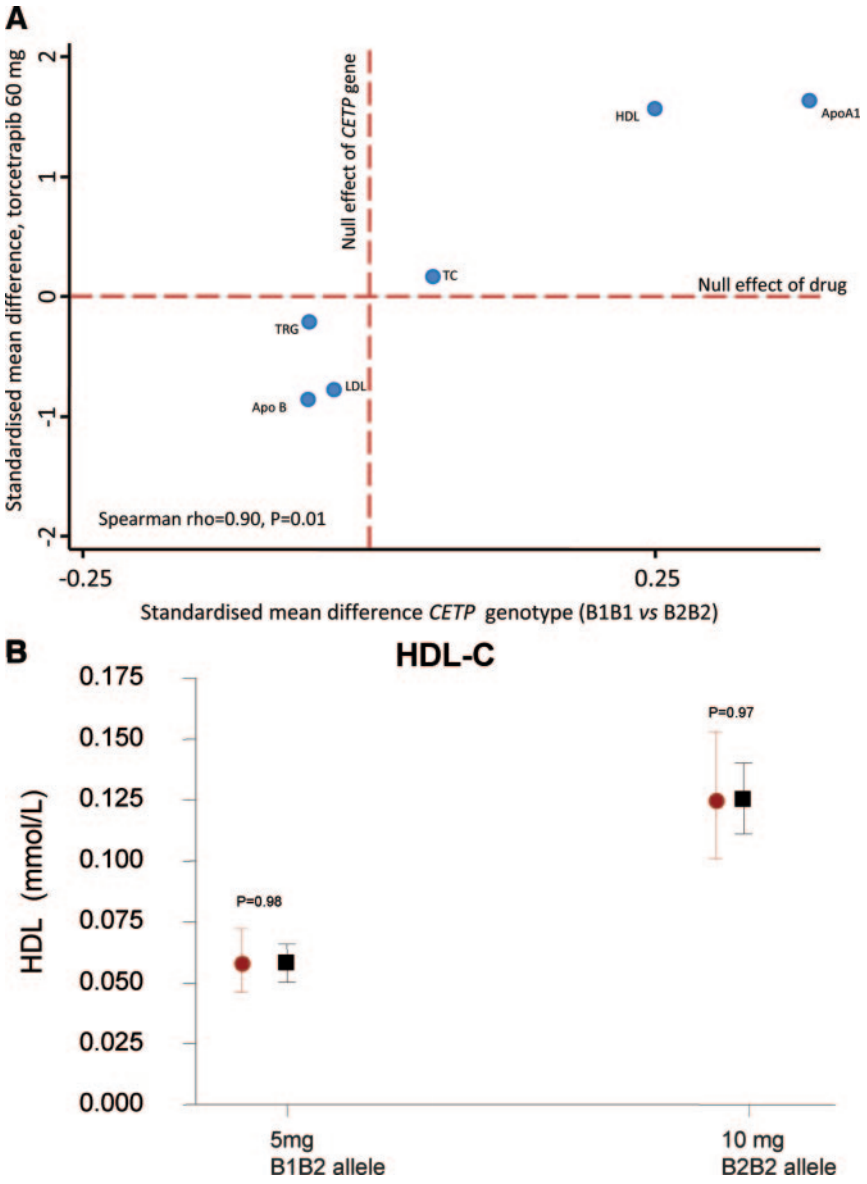

Figure 3. A, Effect of torcetrapib and *CETP* gene variants on 6 lipid traits evaluated in both genetic studies and randomized trials. B, Observed effects of the *CETP* gene and expected effects of a 5- and 10-mg dose of torcetrapib dose on HDL cholesterol.

dose of torcetrapib of 9.7 mg (95% CI 8.18 to 11.41 mg), and for heterozygous individuals, it corresponded to a dose of 4.5 mg (95% CI 3.71 to 5.38 mg), ie, to a torcetrapib dose of approximately 10 and 5 mg, respectively (Figure 3B).

Effect of *CETP* Genotypes on Blood Pressure and Electrolytes

Twenty-two studies (58 948 individuals) provided information on *CETP* genotypes and systolic and diastolic blood pressure, including previously unpublished information from 20 studies (54 936 individuals). *CETP* genotype had no effect on systolic and diastolic blood pressure; the mean differences in comparisons between homozygous subjects were 0.16 mm Hg (95% CI −0.28 to 0.60 mm Hg) and −0.04 mm Hg (95% CI −0.36 to 0.28 mm Hg) for systolic and diastolic blood pressure, respectively. Mean differences in systolic and diastolic blood pressure between heterozygous individuals (B1B2) and those homozygous for the B1 allele were −0.27 mm Hg (95% CI −0.64 to 0.10 mm Hg) and −0.23 mm Hg (95% CI −0.43 to −0.04 mm Hg), respectively (Figure 4A). The null findings were again consistent in analyses stratified by study size, sex, presence of preexisting CHD, ancestral origin, and allele types (Figures 4A and 4B; online-only Data Supplement Figures Va and Vb). The expected effect on blood pressure of a 10-mg daily dose of torcetrapib was estimated to be 0.72 mm Hg (95% CI 0.60 to 0.87 mm Hg) and 0.33 mm Hg (95% CI 0.27 to 0.41 mm Hg) for systolic and diastolic blood pressure, respectively, assuming a linear relationship between torcetrapib dose and blood pressure, and this was significantly different from the observed genetic effect on blood pressure (Figures 5A and 5B). Unlike torcetrapib treatment, *CETP* genotype was not associated with serum sodium, potassium, or creatinine concentration or with urinary sodium or potassium concentration (Table 2; Figures 5C and 5D). Individuals with variant CETP alleles were also no more likely to receive antihypertensive medications (odds ratio 0.98, 95% CI 0.80 to 1.21; online-only Data Supplement Table I).

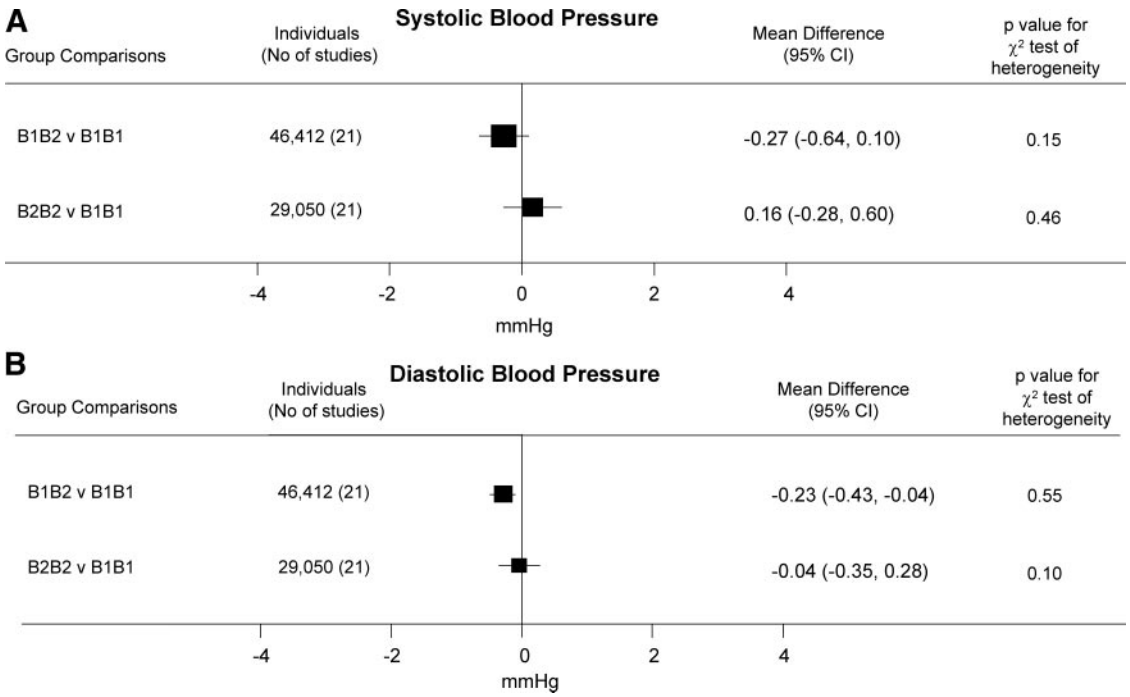

Figure 4. Effect of *CETP* genotype on systolic (A) and diastolic (B) blood pressure in populations of European descent. Weighted mean difference is given, with the B1B1 genotype used as the reference genotype. The numbers refer to the total number of individuals that contribute to the comparisons shown.

Effect of *CETP* Genotypes on Variables Unrelated to CETP Inhibition

There was no link between *CETP* genotypes and variables unrelated to CETP function, including age, body mass index, or smoking habit (online-only Data Supplement Table I). There was also no consistent association with blood glucose or with C-reactive protein concentration, consistent with data from clinical trials of torcetrapib (online-only Data Supplement Table I).

Discussion

Main Findings and Interpretation

We found concordance in the effect of common variants in the *CETP* gene and pharmacological inhibition of CETP by torcetrapib on 8 continuous lipid and lipoprotein markers evaluated in both randomized trials and genetic studies (HDL cholesterol, HDL2, HDL3, LDL cholesterol, triglycerides, total cholesterol, apoA-I, and apoB). The only continuous traits for which the effect of genotype and drug were consistently discordant were systolic and diastolic blood pressure and the electrolytes sodium and potassium. This large-scale randomized evidence in humans supports the interpretation that the blood pressure–elevating effect of torcetrapib (and the connected effect on electrolytes) is mechanistically unrelated to CETP inhibition. The findings have important implications, specifically for the development of other CETP inhibitors and more generally for the potential use of genetic variants to inform drug development.

Other Sources of Evidence on the Same Question

Our interpretation that the hypertensive effect of torcetrapib is off target receives additional support from other lines of evidence. First, treatment with the CETP inhibitors anacetrapib and dalcetrapib has not been associated with blood pressure elevation, although the studies thus far have been relatively small in size and of short duration.[5,7] Second, torcetrapib (but not anacetrapib) has been reported to cause a blood pressure increase in several animal models,[12] including species that do not express CETP. Third, a recent study[22] indicated that torcetrapib treatment elevates aldosterone concentration, with corresponding effects on sodium and potassium concentration, and these electrolyte changes were not observed in a short-term dose-ranging study of anacetrapib.[7] These findings, from the separate lines of investigation, each with differing limitations and sources of error, provide reassurance that the hypertensive effect of torcetrapib is off target and therefore unlikely to be shared by other CETP inhibitors.

CETP Inhibition and Prevention of CHD

The higher blood pressure among individuals in the torcetrapib arm of the ILLUMINATE trial might explain the higher rate of cardiovascular events, but there may also be other explanations. CETP inhibition might interfere with reverse cholesterol transport and generate an HDL particle of abnormal size and function,[23] a mechanism-based adverse effect. Prior small mechanistic studies have suggested torcetrapib treatment increased the concentration of both large HDL2 and small HDL3 particles but that the effect on HDL2 was proportionately greater. However, this differential effect was only seen at a dose of torcetrapib 4 times as large as the dose used in the large-scale clinical trials.[19] Genetic data on the effect of *CETP* genotype on HDL subtype were limited, but in the present analysis, there was no clear evidence of a

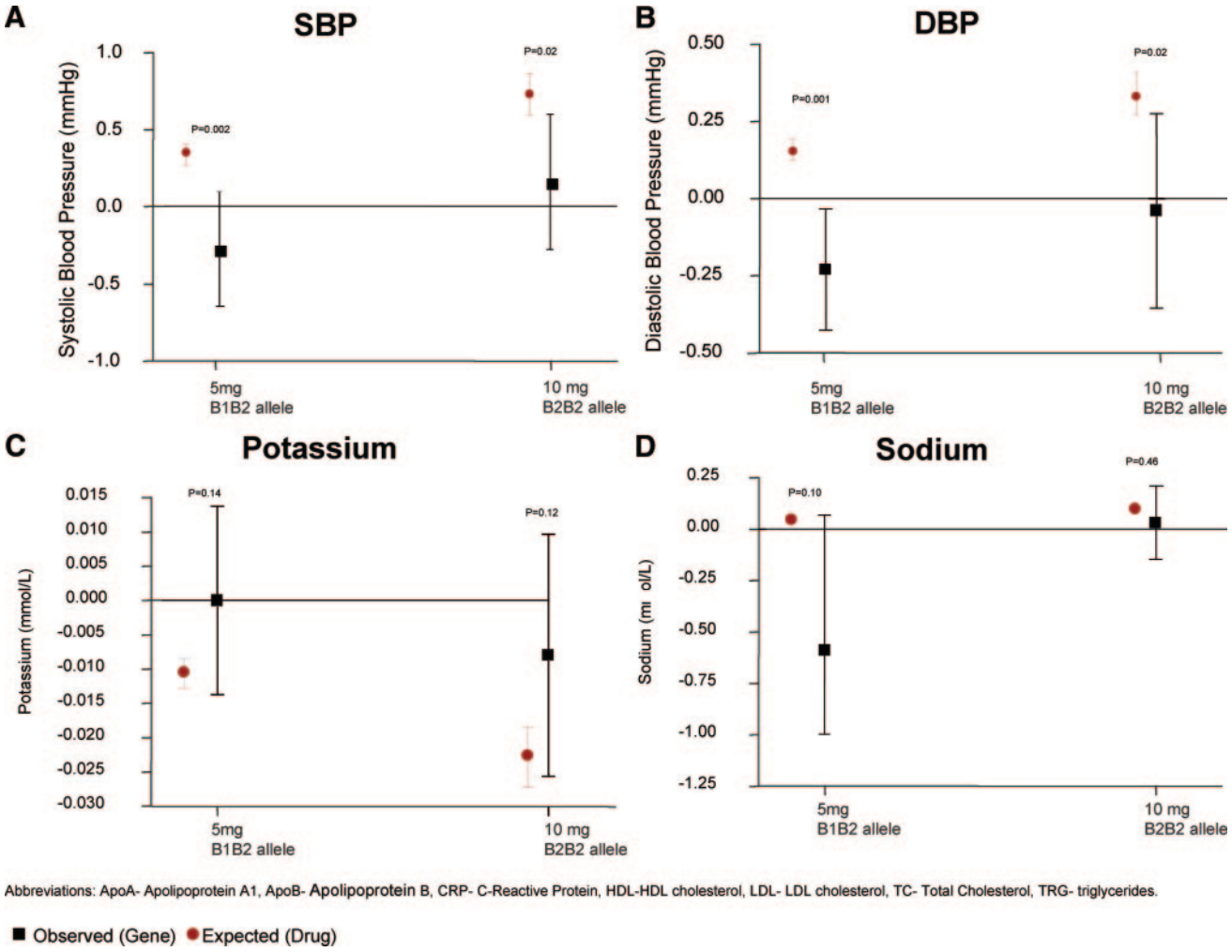

Figure 5. A through D, Observed effect of the *CETP* gene and expected effects of a 5- and 10-mg dose of torcetrapib on systolic (A) and diastolic (B) blood pressure, serum potassium (C), and sodium levels (D).

differential effect of *CETP* genotype on HDL subclasses. Although we have focused here on the effect of CETP genotypes on lipids, lipoproteins, and blood pressure to make direct comparison of the effect of pharmacological CETP inhibition and carriage of CETP alleles, a recent meta-analysis of studies that included 27 196 coronary cases and 55 338 controls and a genome-wide analysis from the Women's Genome Health Study both provided support for the CETP variants studied here being protective against CHD events.[24,25] Although this protective effect has not been consistent across all studies,[26] there has been no consistent signal for an increase in CHD risk from carriage of these alleles.

Potential Limitations

Although the findings are robust, our interpretation requires consideration in light of certain theoretical and practical limitations of the genetic approach we have used. *CETP* alleles are of much smaller effect than the most widely studied dose of torcetrapib, so it might be argued that the failure to detect an association between genotype and a continuous marker such as blood pressure could have arisen because of inadequate power, or perhaps the effect on blood pressure requires a suprathreshold degree of CETP inhibition. We attempted to maximize power and minimize the potential for a type II error by establishing a large genetic collaboration that included a substantial amount of previously unpublished information. Blood pressure was an outcome that had been widely recorded in the studies included in the present analysis (22 studies and 59 948 individuals) but was not widely reported, and so the findings should not be prone to bias. Although the investigation of the effect of *CETP* polymorphism on blood pressure was not the primary aim of any of the studies included here, blood pressure measurement was performed with validated devices and widely accepted methods. The study was also sufficiently powered to detect a blood pressure signal of the size expected of a 5- to 10-mg dose of torcetrapib (see the online-only Data Supplement). Indeed, 3 of these studies (14 109 individuals) contributed to the recent whole-genome analysis of blood pressure loci that identified single-nucleotide polymorphisms (SNPs) that altered blood pressure by ≈1 mm Hg/0.5 mm Hg, close to the effect size being sought in the present analysis.[27,28] With the available sample size, we also detected an effect of *CETP* genotype on triglycerides that was similar in size to that which would have been expected for blood pressure were this effect mechanism

based (online-only Data Supplement Figure IIa). We also triangulated the findings from randomized controlled trials with the genetic data (ie, we compared the expected effect of a 5- and 10-mg dose of torcetrapib with the observed genetic effect) rather than focusing solely on statistical tests in the genetic associations. Taken together, these analyses suggest that the null findings in relation to blood pressure are neither biased nor explained by inadequate sample size. Although we were unable to exclude a hypothetical nonlinear (threshold) relationship between CETP inhibition by torcetrapib and blood pressure because none of the dose-ranging studies of torcetrapib reported quantitative data on the dose–response effect in a form that could be extracted for analysis, the effect of torcetrapib on all lipid and lipoprotein traits evaluated was linear over the dose range studied. We therefore made the assumption that this was also true for blood pressure.

The randomized allocation of alleles in genetic studies differs from the randomized drug intervention in a clinical trial in that assignment of genotype occurs at conception and produces an effect across a lifetime, rather than in mid to late adulthood, when most randomized controlled trials are conducted. It is conceivable, therefore, that an adverse effect of a common genetic variant on blood pressure from early life may have led to developmental compensation by other systems.[15] If this were the case, a null association of *CETP* genotype with blood pressure seen in genetic studies might lead to unreliable inference on the likely effect of modification of CETP activity by a drug. However, there was no evidence that such developmental compensation was operating in the case of any of the 8 lipid traits we studied, for which both the lifelong effect of the genetic exposure and the shorter-term effect of the drug were consistent.

Although the precise functional alleles at the *CETP* locus have yet to be identified with certainty, the −629C>A (rs1800775) and I405V (rs5885) alleles are either likely to be functional themselves or to be in sufficiently strong linkage disequilibrium with functional variant(s) so as to be valid tools for this type of analysis. The −629C>A variant has been shown to alter binding of Sp transcription factors.[29] The Taq1B allele (rs708272) is intronic and less likely to be functional itself, but it is in strong linkage disequilibrium with several promoter polymorphisms (including the −629C>A variant), and as the present analyses show, it exhibits very strong association with multiple lipid traits. It is important to be clear, however, that for the analyses we have conducted, it is not necessary for functional alleles to have been delineated precisely provided that an effect of the alleles studied on the traits of interest can be demonstrated robustly.[30] Although there are likely to be other variants in and around the *CETP* gene that are also associated with CETP activity and lipids, some because they are causal and some because they are simply associated with causal SNPs by linkage disequilibrium, the use of a single SNP in this region does not compromise the analysis, provided it can be demonstrated that it provides a reliable index of CETP activity and differences in the lipid traits of interest (which we have demonstrated), and on the assumption that the SNP is in linkage disequilibrium with a causal SNP rather than causal itself, that the main analyses are grouped according to subjects of similar ancestry to ensure that the linkage disequilibrium relationships are consistent across studies. Moreover, SNPs at the CETP locus, including rs1800775 (−629C>A) and rs708272 (Taq1B) studied here, have emerged as among the strongest associated signals with HDL cholesterol in recent genome-wide association studies[25,31–33] (online-only Data Supplement Figure I).

Wider Implications of This Work

We used the principle that allelic variants in a gene encoding a specific drug target can be used to model the mechanism-based effect of modifying the same target pharmacologically. In the present analysis, this was applied to help distinguish the mechanism-based from off-target actions of a drug molecule in advanced development. However, further research should now address whether this principle could be exploited at earlier phases in the drug-development pathway to help, for example, with the validation of a promising new target or to assemble a panel of biomarkers of efficacy to test in clinical trials. The directional concordance of the effect of *HMGCR* SNPs in genetic studies and 3-hydroxy-3-methylglutaryl-coenzyme A reductase (statin) treatment on LDL cholesterol and CHD risk in clinical trials lends additional support to the potential utility of this approach. There is likely to be wide availability of genetic tools for this purpose, because the majority of drug targets are proteins, and regulatory genetic variants acting in *cis*, located within 100 kb of genes, appear to be a common feature of the human genome.[34]

Conclusions

In summary, a novel large-scale genetic approach has provided evidence that the hypertensive effect of torcetrapib is likely an off-target action. This provides reassurance that this particular adverse effect of torcetrapib is unlikely to be shared by other chemically dissimilar CETP inhibitors, but further drug development will be required to assess whether these other agents and the CETP inhibitor class of drugs in general are likely to be efficacious in the prevention of CHD events with an acceptable risk–benefit profile. Further research should investigate whether genetic studies could find use in drug-development programs as a new source of randomized evidence for drug-target validation in humans.

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Disclosures

Dr Hingorani is a member of the editorial board of the Drug and Therapeutics Bulletin, has provided nonremunerated advice to GlaxoSmithKline and London Genetics, and has received honoraria for speaking at educational meetings on cardiovascular risk that have been donated in whole or in part to charity. Dr Arca was on the Pfizer advisory board for torcetrapib.

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CLINICAL PERSPECTIVE

The inverse relationship between high-density lipoprotein cholesterol and risk of coronary heart disease suggests that therapeutic elevation of high-density lipoprotein cholesterol may provide an effective means of prevention of coronary heart disease. Pharmacological inhibition of cholesteryl ester transfer protein (CETP) leads to elevation in high-density lipoprotein cholesterol, but torcetrapib (the first-in-class CETP inhibitor) increased the risk of cardiovascular events in the ILLUMINATE trial (Investigation of Lipid Level Management to Understand Its Impact in Atherosclerotic Events), which may have resulted from an unexpected blood pressure–elevating effect of this agent. We used common genetic polymorphisms in the *CETP* gene to distinguish whether the hypertensive action of torcetrapib was mechanism based or off target, because a genetic study of these variants can be considered to be a type of natural randomized trial of a "clean" low-dose CETP inhibitor with no off-target actions. Common *CETP* gene polymorphisms and torcetrapib treatment had concordant effects on 8 lipid and lipoprotein markers, including high-density lipoprotein cholesterol, but *CETP* gene variants had no effect on blood pressure. The blood pressure–elevating effect of torcetrapib appears to be an off-target action that is unlikely to be shared by chemically dissimilar CETP inhibitors. Genetic studies could be used in drug-development programs as a new source of randomized evidence for drug-target validation in humans.

Correction

In the article "Separating the Mechanism-Based and Off-Target Actions of Cholesteryl Ester Transfer Protein Inhibitors With *CETP* Gene Polymorphisms" by Sofat et al, which appeared in the January 5/12, 2010 issue of the journal (*Circulation.* 2010;121;52–62), one affiliation for Folkert W. Asselbergs, MD, PhD was incorrect.

The incorrect portion of the affiliations on page 52 should now read, ". . . Department of Epidemiology and Biostatistics (A.I., J.C.M.W., C.M.v.D.), Erasmus MC, Rotterdam, the Netherlands; Boston University (M.P., R.S.V., R.B.D., J.O.), Department of Mathematics and School of Medicine, Boston, Mass; Department of Nutrition (F.W.A., T.Y.L.), Harvard School of Public Health, Boston, Mass; . . . "

The online version of the article has been corrected. The authors regret the error.

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(*Circulation*. 2010;121:e216.)

***Circulation* is available at http://circ.ahajournals.org**

Supplemental material:

Supplemental Methods

Search strategy and selection criteria; Genetic studies

Reference lists of articles identified from the primary search were additionally scanned for relevant articles, including previous meta-analyses and systematic reviews. For inclusion, genetic studies had to have more than 500 participants, involve unrelated subjects and be published as full length articles or letters in a peer reviewed journal. Authors of published studies were contacted (on at least 3 occasions) to obtain additional information on *CETP* genotypes and variables of interest, where unreported. The collaborative group for the genetic analysis was assembled by direct contact with principal investigators of any study known to the authors that involved more than 500 individuals that had previously reported at least one genetic finding, in any area, in a peer reviewed journal.

Twenty two studies were identified from the search and the published meta-analysis and seven were either reported after the previously published meta-analysis, or were contacted independently of the search as these studies were known to have published on genetic associations in lipids. Where data were duplicated in two publications, clarity was sought from the author, and limited tabular data were requested on the complete cohort. If there was no response, the larger of the data sets reported were included. Four unpublished cohorts were included.

Data Extraction

Randomised trials: Information was extracted on treatment regimen and comparator, pre- and post-treatment concentration of HDL-, LDL- and total cholesterol, triglycerides, apolipoproteins A-I (apoA-I) apolipoprotein B (apo-B), C-reactive protein (CRP), sodium, potassium, chloride, and bicarbonate, aldosterone, plasma creatinine and estimated glomerular filtration rate, systolic and diastolic blood pressure.

Genetic Studies: Information was obtained on study design, total number of participants and the number of individuals by genotype category, gender, ethnic origin and presence or absence of CHD at baseline. In addition, summary information on the following variables was obtained (where available) for each *CETP*-genotype group: CETP concentration, CETP activity, HDL-, LDL-, and total cholesterol, triglycerides, apoA-I and apoA-II, apoB, HDL sub-fractions 2 and 3, systolic and diastolic

blood pressure, blood glucose, CRP, urinary and plasma sodium, potassium and creatinine, body mass index, smoking status, age and treatment with anti-hypertensive medications or statins.

Statistical Analysis

For continuous traits, median values were assumed to be equal to mean values. If the standard deviation (SD) was not reported this was calculated from the standard error (SE) by multiplying the this by the square root of the sample size or from the inter-quartile range by dividing the width of the inter-quartile range by 1.349. For dose ranging studies, the SD was imputed from the largest study if unavailable. Where logged values were provided, these were back transformed and geometric means were used as means. Where cholesterol and triglycerides were reported in mg/ dl, values were converted to mmol/l by multiplying by 0.02586 and 0.01129 respectively. Similarly where glucose was reported as mg/dl values were converted to mmol/l by multiplying by 0.055, and creatinine was converted to µmol/L by multiplying by 88. We estimated using a MAF of 0.48, a sample size of 14,147 individuals was required to be able to detect a difference of 0.5 mmHg SBP, with a power of 0.8 at a significance of 0.05 (calculated using online genetic power calculator "Quanto" (S40)).

Simulation of observed gene vs expected torcetrapib dose equivalent effects: Once a linear dose response was confirmed for available variables, the summary effect with most data from dose finding or randomized trials (60mg) on HDL-C was used for simulation studies. The effect of 60 mg torcetrapib on HDL was called βd1(with standard error sd1), and for other i traits, similarly β_{di}(with standard error s_{di}). The simulation model then incorporated the variance in the effect estimates of the genotype and drug effects. This was done as follows: Firstly, a random draw (x_{d1}) of the 60mg drug effect on HDL cholesterol was taken from a normal distribution N($\beta_{d1,}s_{d1}$) and of the effect on each other trait (random draw x_{di} from N(β_{di},s_{di}), and of the gene effect on HDL cholesterol (draw x_{g1} from N($\beta_{g1,}s_{g1}$). The gene effect (x_{g1}) was then expressed as a torcetrapib dose equivalent, calculated as $dose_g=60\ x_{g1}/x_{d1}$, from which the expected effect of this dose on trait *i* was estimated as $e_{gi}=x_{di}x_{g1}/x_{d1,}$. Random draws were repeated 100000 times, generating a distribution of $dose_g$ from which its mean, 2.5th and 97.5th percentiles were used to estimate the mean (95% confidence interval) for the gene-dose equivalent. Similarly the mean of the distribution of expected effects on trait *i* was estimated (m_e_{gi}) as well as its standard error (s_{egi}) as (97.5th-2.5th percentiles)/(2x1.96). The observed gene

effect on trait i (β_{gi}) was compared to the expected effect of a comparable dose of torcetrapib (m_e_{gi}) using a Z- test, $z=(\beta_{gi}-m\_e_{gi})/\sqrt{(s_{gi}^2+ s_{egi}^2)}$.

Consistency between the CETP gene effects and equivalent torcetrapib dose

The 95% confidence intervals for the expected effect of a dose of torcetrapib comparable to the effect of genotype were obtained by simulation. To incorporate the uncertainty in the effect estimates, one hundred thousand replications were generated of the point estimates and standard errors of the 60 mg dose of torcetrapib. The values of the 2.5 and 97.5 centiles of the simulated distribution were uses as the 95% confidence intervals. The simulation process was conducted separately for individuals homozygous for the B2 allele and then repeated for heterozygous individuals.

Supplemental Results

Seven studies with 21, 353 individuals homozygous for the rs5882 (I405V) allele also had higher concentrations of HDL cholesterol (0.04 mmol/L; 0.00, 0.09), although the effect is less marked than that of rs7082872 (Taq1B). Similarly there was no evidence of a link between the I405V variant and blood pressure.

Supplemental Tables:

Table S1: Effect of torcetrapib (60 mg) and *CETP* genotype on other continuous and demographic variables.

Differences between continuous traits are those reported at the end of the randomised trial end unless otherwise indicated. Differences in demographic variables for RCTs were those recorded at baseline. ** only ILLUMINATE contributed to analysis.

Comparison	RCTs, Torcetrapib 60 mg (Number of Individuals)	Summary Mean Difference/ Odds Ratio (95% CI)	P value	Genetic Studies, B1B2 vs B1B1, no of studies (Individuals)	Summary Mean Difference/ Odds Ratio (95%CI)	P Value	Genetic Studies, B2B2 vs B1B1, no of studies (Individuals)	Summary Mean Difference/ Odds Ratio (95%CI)	P Value
Continuous traits									
C-reactive protein (mg/L)	2 (17,007)	0.02 (-0.04, 0.08)	0.52	13 (34,826)	0.03 (-0.07, 0.13)	0.60	13 (22,049)	0.16 (0.04, 0.29)	0.01
Glucose (mmol/L)	NA	NA	NA	11 (32,608)	0.00 (-0.02, 0.02)	0.95	11 (20,497)	0.03 (0.00, 0.06)	0.09
Demographic amd other variables									
Age	4 (17,911)	-0.48 (-1.29, 0.33)	0.25	19 (43,950)	0.00 (-0.20, 0.21)	0.98	19 (27,583)	0.18 (0.00, 0.35)	0.05
BMI (kg/m^2)	4 (17,911)	-0.06 (-0.22, 0.1)	0.46	19 (40,212)	-0.07 (0.15, 0.02)	0.12	19 (25,249)	-0.01 (-0.12, 0.10)	0.85
Treatment with statin	4 (17,911)	All treated with Atorvastatin	All treated with Atorvastatin	7 (20,600)	1.02 (0.10, 10.74)	0.99	7 (13,005)	0.92 (0.09, 9.79)	0.95
Treatment anti-hypertensive medication	3 (2,844)	0.98 (0.07, 13.3)	0.99	7 (20,077)	0.99 (0.83,1.17)	0.87	10 (15,154)	0.98 (0.80, 1.21)	0.85
Hypertension at baseline	4 (17,911)	1.04 (0.05, 22.5)	0.99	NA	NA	NA	NA	NA	NA
Current/ former vs never smoked	NA	NA	NA	9 (23,420)	0.92 (0.82, 1.03)	0.16	9 (14,649)	0.90 (0.78,1.04)	0.15

Table S2. Studies contributing to the CETP analysis

Study	Year of publication	Country	Gender male (%)	Mean age (years)	Total sample (n)	Study Description	Baseline coronary heart disease (CHD)	HWE χ^2 for main genotype included	CETP SNP(s) typed
*Published studies with blood pressure, **studies that did not respond to data request									
REGRESS[S30*]	1998	Netherlands	100	56	807	Cases of CHD from RCT	CHD	0.33	rs708272
Corella D [S11**]	1999	Spain	45	36.6	514	Cross sectional	No CHD	1.18	rs708272
Rekyavik [S13**]	2000	Iceland	100	71	1,134	Prospective cohort study - cases of CHD only	CHD	4.45	rs708272
Brousseau M [S9**]	2002	United States	100	64	833	Cross Sectional	CHD	3.71	rs708272
Atherogene [S6**]	2003	Germany	75	64.4	1,211	Prospective cohort of CHD	CHD	1.98	rs1800775
CARE[S12*]	2004	Canada, United States	86	59.5	3,205	Cases from RCT	CHD	1.78	rs708272
PHS [S24 **]	2005	United States	100	58.4	768	Prospective cohort	Mixed Population	1.01	rs708272
Marschang P [S31**]	2006	Austria	56	64.3	983	Prospective Cohort	CHD	0.06	rs708272
*Studies with unpublished data on blood pressure, *unpubublished studies									
EARS[S16+]	1999	Estonia, Belgium, Denmark, Finland, Germany, Greece, Italy, Portugal, Spain, Switzerland, United Kingdom	100	23	794	Cross sectional	No CHD	0.00	rs708272, rs5882
OPERA[S18,S19,S20*]	2000	Finland	49	51.4	524	Prospective cohort	Mixed Population	0.02	rs708272, rs158477
Framingham Offspring[S29*]	2000	United States	48.4	51.3	2,916	Prospective cohort	Mixed Population	1.84	rs708272
Arca M[S4*]	2001	Italy	65.4	58.7	798	Case control, additional group of population controls	Mixed Population	0.88	rs708272
ECTIM[S10,S15,S22*]	2002	France, United Kingdom	78	55.5	2,540	Case control	Mixed Population	1.27	rs708272, rs5882, rs1800775, G-971A
NPHS[S26*]	2002	United Kingdom	100	55.9	2,589	Prospective Cohort	No CHD	3.4	rs708272
WOSCOPS[S14*]	2003	United Kingdom	100	57	1,604	RCT	Mixed Population	0.28	rs708272
ACCESS[S25*]	2005	United states	60	60.3	2,106	RCT	Mixed Population	0.01	72 SNPs incuding rs708282, rs5882, rs1800775
Sorli[S27*]	2006	Spain	31	45.5	549	Cross sectional	Mixed Population	0.99	rs708272
PREVEND[S8*]	2006	Netherlands	50.5	49.4	8,166	Prospective cohort	Mixed Population	1.56	rs708272, rs1800775, rs5882
SAPHIR[S28*]	2008	Austria	68	52.7	1,503	Prospective Cohort	No CHD	0.31	rs708272
Busselton[S1*]	2007	Australia	45	49	1,574	Cross sectional	Mixed Population	1.05	rs708272, rs1800775, rs12149545
CUDAS[S1*]	2007	Australia	50	53	1,109	Cross sectional	Mixed Population	0.01	rs708272, rs1800775, rs12149546
CUPID[S1*]	2007	Australia	87	50	556	Cohort of patients presenting for coronary catheters	CHD	0.12	rs708272, rs1800775, rs12149547
Intermountain[S17*]	2007	United States	69	56	9,371	Cohort of patients presenting for coronary catheters	Mixed Population	1.22	32 SNPs incuding rs708282, rs5882, rs1800775
Health Professionals Study[S23*]	2007	United States	100	60	2,193	Prospective cohort study	No CHD	0.945	rs708272
Nurses Health Study[S5*]	2007	United States	0	57.4	1,291	Prospective cohort study	No CHD	1.3	rs708272
Rotterdam[S33*]	2007	Netherlands	41	68.8	6,421	Prospective cohort study	Mixed Population	0.48	rs5882, rs1800775
British Womens' Heart and Health Study[S21**]	Unpblished	United Kingdom	0	68.9	3,570	Prospective cohort study	Mixed Population	2.58	rs708272
EPIC-Norfolk[S7**]	Unpublished	United Kingdom	63	65.3	2,114	Prospective cohort study	No CHD	2.2	rs1800775
Whitehall II[S3**]	Unpublished	United Kingdom	73	49.8	5,049	Prospective cohort study	Mixed Population	0.61	rs708272
ELSA [S2**]	Unpublished	United Kingdom	46	63.6	5,541	Prospective cohort study	Mixed Population	1.35	rs708272
Erasmus[S32 ++]	Unpublished	Netherlands	40.7	53.12	873	Prospective cohort study	Mixed Population	0.024	rs5882

Table S3. Traits included from studies evaluating *CETP* Taq1B and -629C>A genetic variant in European descent individuals ("0" for not included and "1" for included)

Study	HDL-C	LDL-C	Total Cholesterol	Triglycerides	HDL2	HDL3	ApoA1	ApoB	SBP	DBP	PP	Glucose	BMI	CRP	Electrolytes and renal measures	Use of antihypertensive medication	Smoking status	Use of Statin
REGRESS	1	1	1	1	0	0	0	0	1	1	0	0	1	0	0	1	1	1
Corella D	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rekyavik	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brousseau	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	0	0	0
Atherogene	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0
CARE	1	1	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
PHS	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Marschang P	1	1	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
EARS	1	1	1	1	0	0	1	1	1	1	0	1	1	1	0	1	1	0
OPERA	1	1	1	1	0	0	0	0	1	1	0	0	1	0	1	0	0	0
Framingham	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	0	0	0
Arca M	1	1	1	1	0	0	0	0	1	1	0	0	0	0	1	0	0	0
ECTIM	1	1	1	1	0	0	1	1	1	1	0	0	1	0	0	1	1	0
NPHS II	1	1	1	1	0	0	1	1	1	1	1	1	1	1	0	0	1	0
WOSCOPS	1	1	1	1	0	0	1	1	1	1	0	1	1	1	1	1	1	1
ACCESS	1	1	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0
Sorli	1	1	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0
PREVEND	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1
SAPHIR	1	1	1	1	0	0	1	1	1	1	0	1	1	1	1	1	1	0
Busselton	1	1	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
CUDAS	1	1	1	1	0	0	0	0	1	1	0	1	1	1	0	0	0	1
CUPID	1	1	1	1	0	0	0	0	1	1	0	1	1	1	0	0	0	1
Intermountain	1	1	1	1	0	0	0	0	1	1	1	1	1	1	1	1	1	1
Health Professionals Study	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nurses Health Study	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BWHHS	1	1	1	1	0	0	0	0	1	1	1	1	1	1	1	1	1	1
EPIC	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	0
Rotterdam	1	0	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0
Whitehall II	1	1	1	1	0	0	1	1	1	1	1	1	1	1	0	0	1	0
ELSA	1	1	1	1	0	0	0	0	1	1	1	1	0	1	0	1	1	0

Supplemental Figures and Figure Legends:

Figure S1 LD structure of the *CETP* gene. r^2 values are given from the ACCESS study. The main SNPs evaluated in this study were Taq1B (rs708272) and -629C>A (rs1800775) which are in LD (r^2=0.73). SNPs contributing to variance in HDL cholesterol identified from genome wide association scans are also shown (rs12596776, rs2217332, rs3764261, rs1800775, rs711752, rs1864163, rs7205804, s5880, rs5882, rs1800777, rs1566439). Data provided by J F Thompson, ACCESS study[16]

Figure S2a and S2b Standardised mean differences in lipid and lipoproteins between individuals homozygous for *CETP* variants in populations studies (a) and and those receiving torcetrpaib 60mg (b) daily as compared to placebo in clinical trials

Figures S3a-c Effect of *CETP* genotype on (a) CETP concentration, (b) CETP activity and (c) HDL cholesterol concentration. Forest plots indicate weighted mean difference and 95% confidence intervals. Results are stratified by ancestral origin, study size, and prevalent coronary heart disease, gender and polymorphism typed. (*the B1B1 genotype grouped is used as the reference group throughout)

Figure S4: Association between *CETP* genotype (B2B2 vs B1B1) and HDL-cholesterol level stratified by systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP) and LDL-Cholesterol. Data from 6 studies, (12,983 individuals)

Figure S5a-b Effect of *CETP* genotype on (a) systolic and (b) diastolic blood pressure in populations of European descent only. Forest plots show weighted mean difference and 95% confidence intervals. Results are stratified by study size, prevalent coronary heart disease, gender, polymorphism typed, and strata of LDL cholesterol. (The B1B1 genotype is used as the reference group, see text for details)

Figure S1

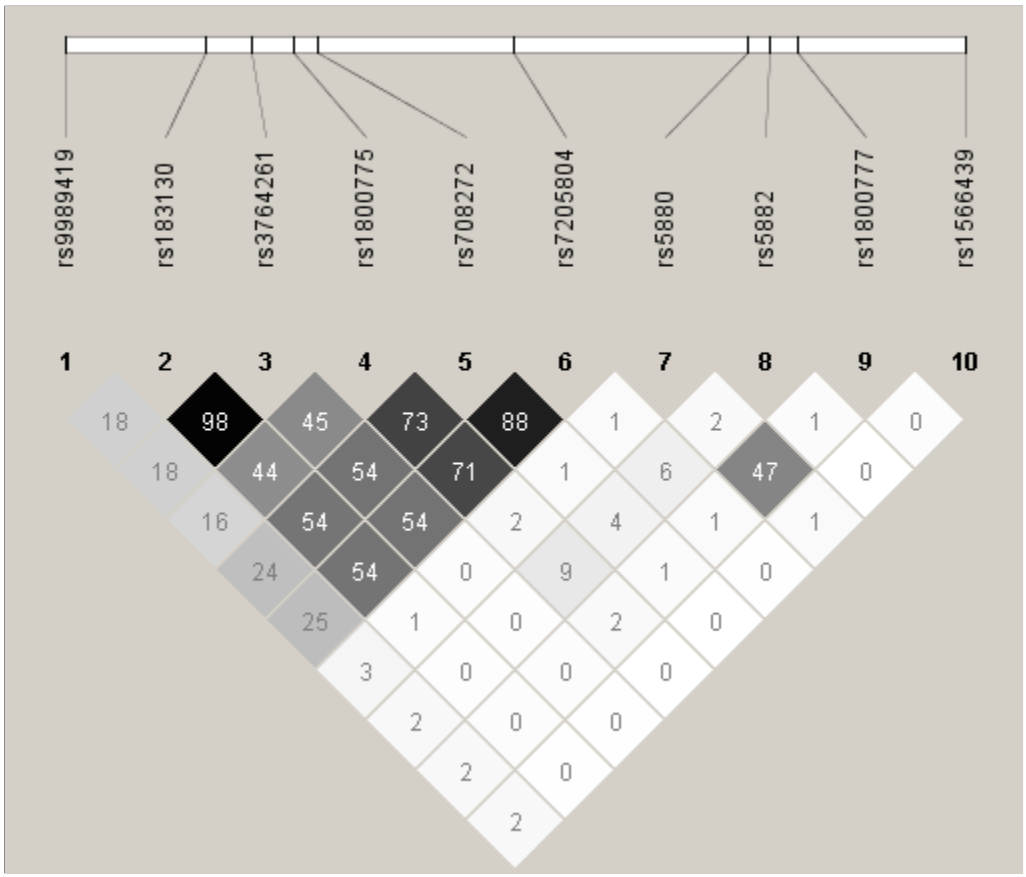


Figure S2a

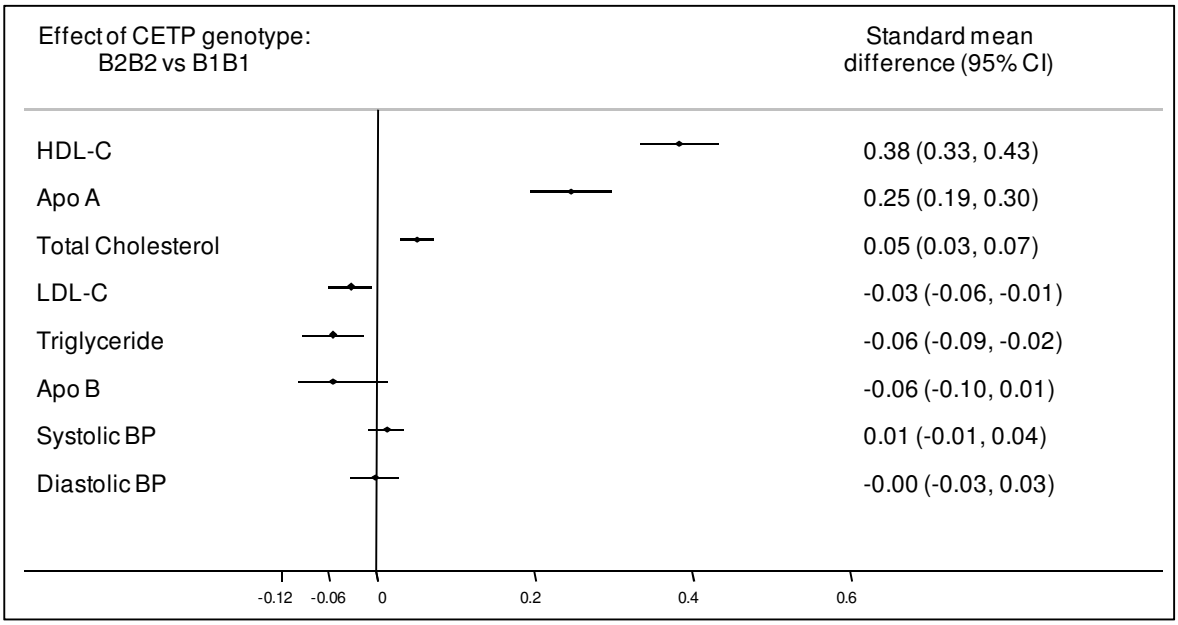


Figure S2b

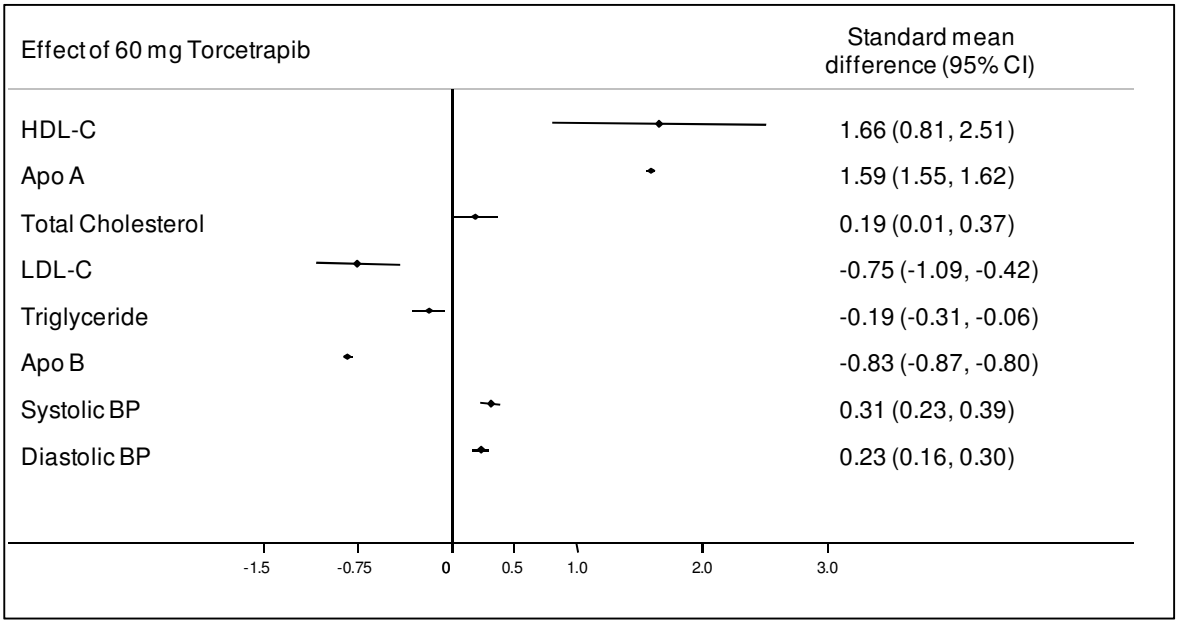


Figure S3a

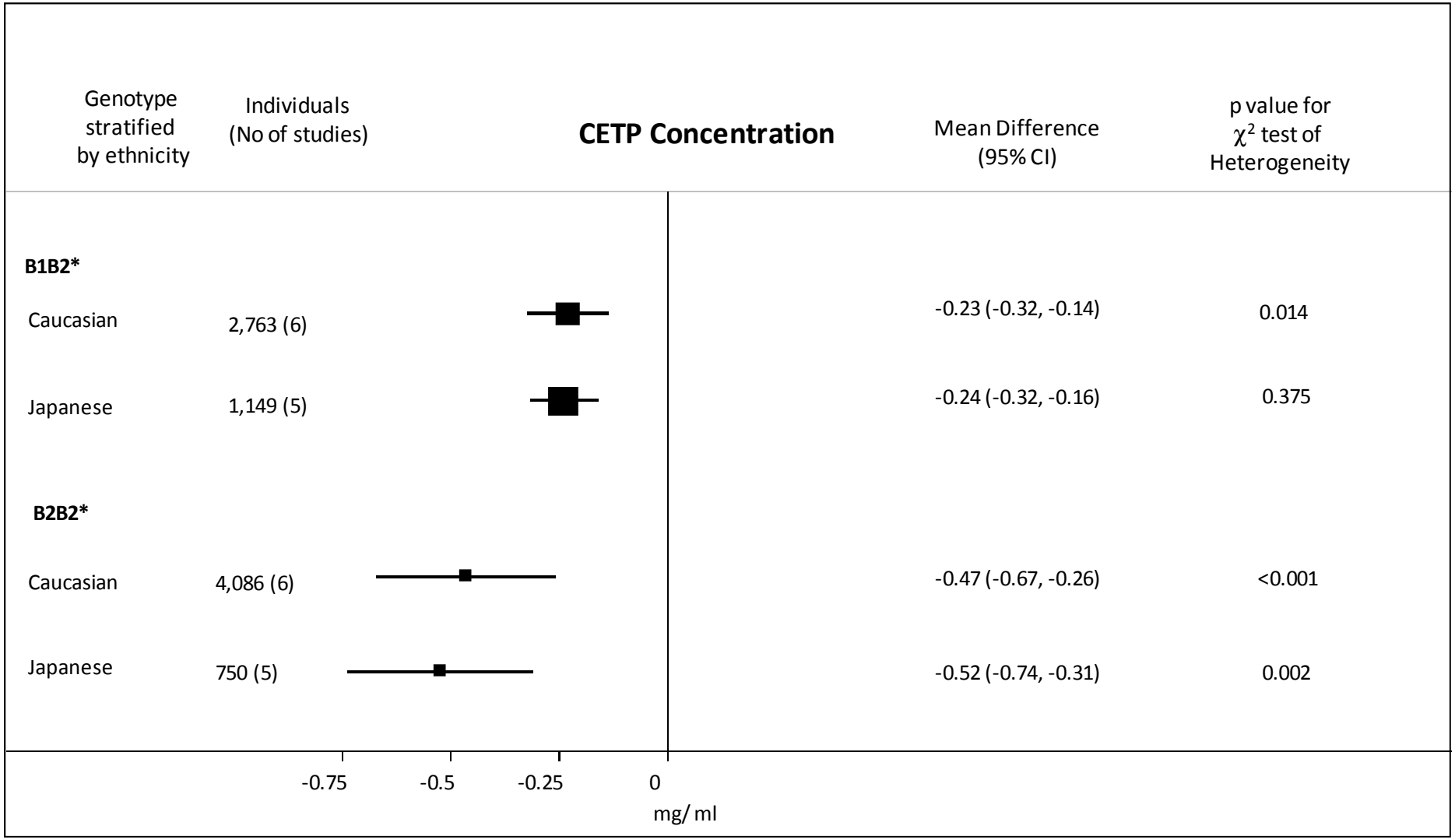


Figure S3b

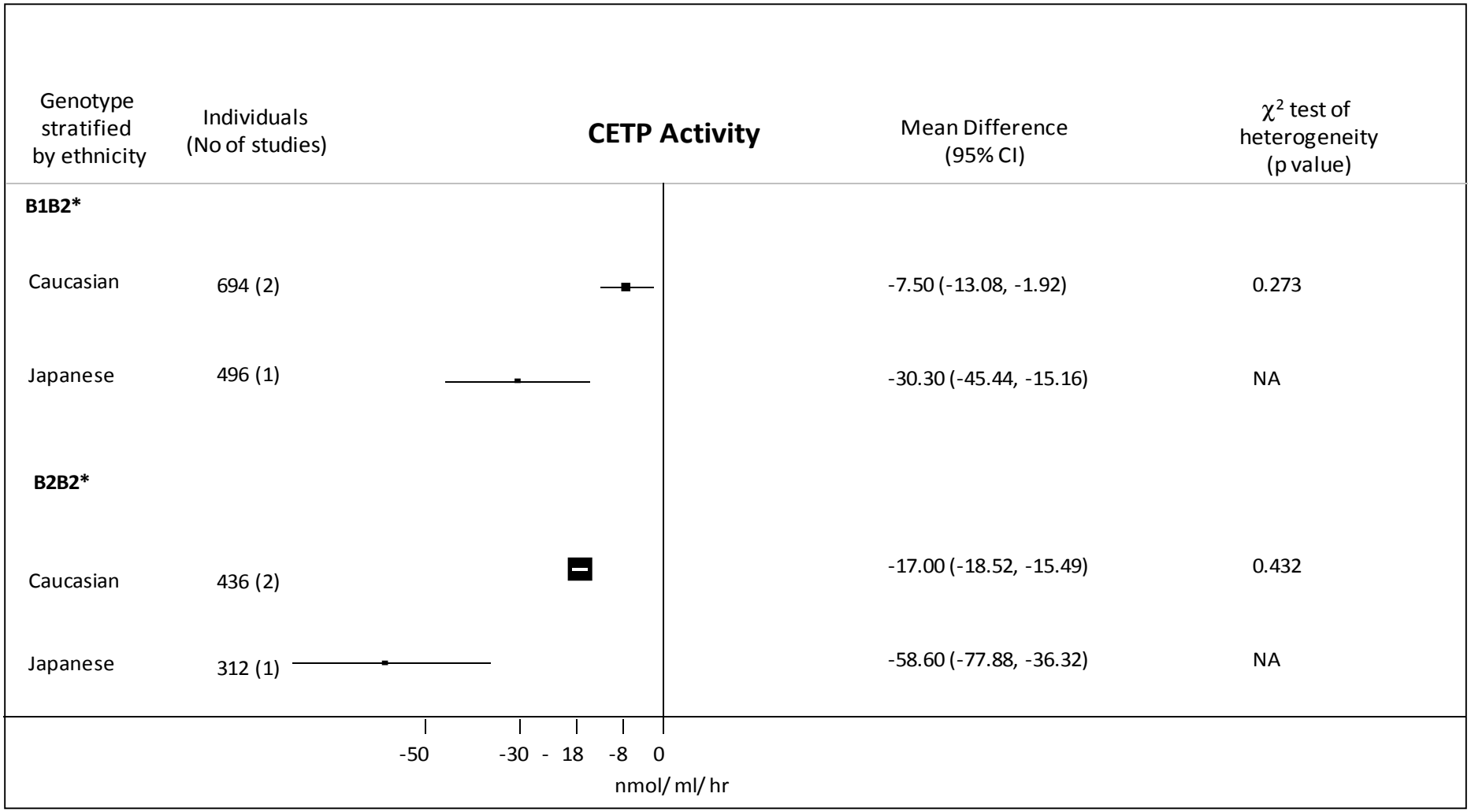


Figure S3c

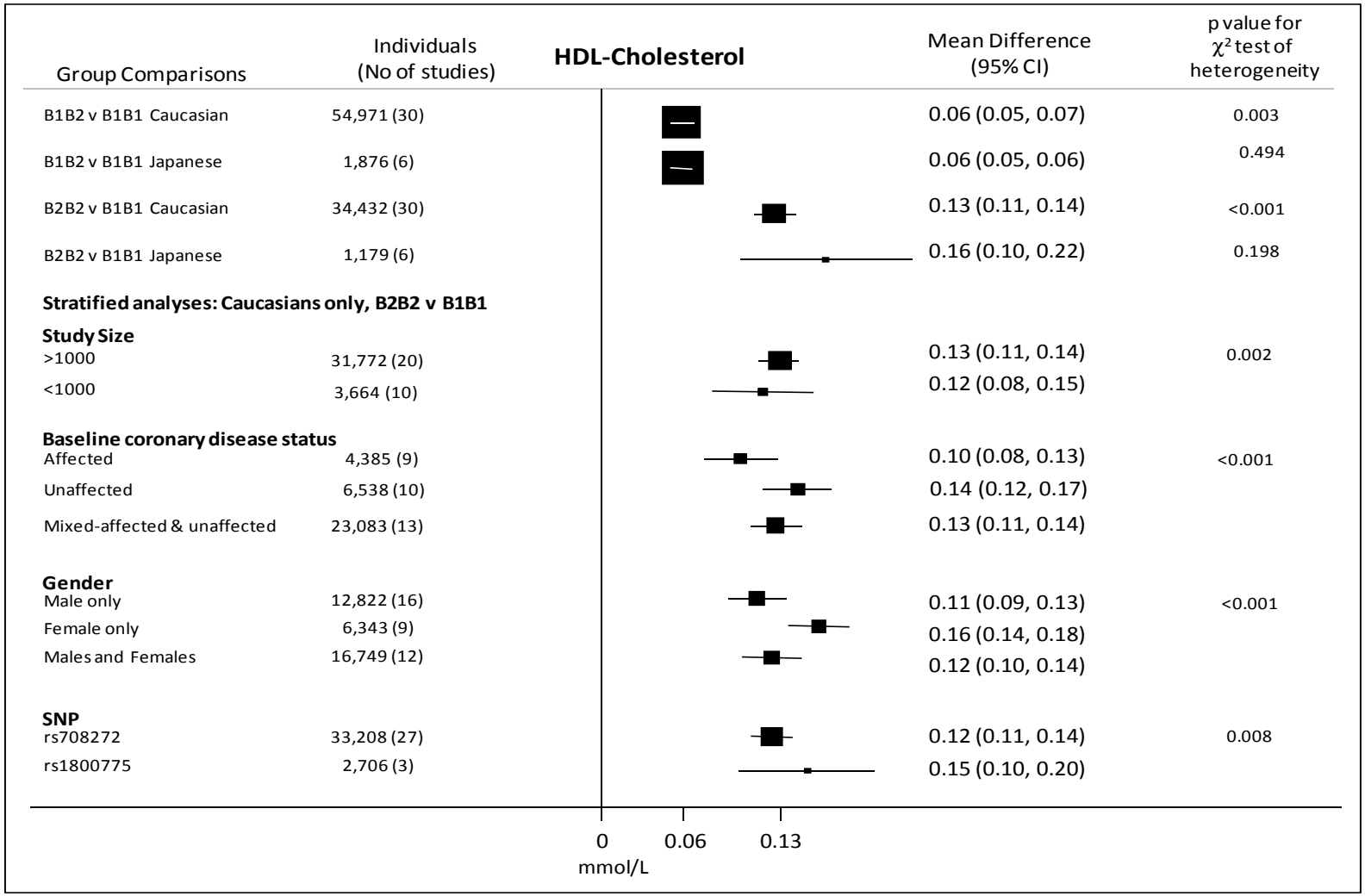


Figure S4

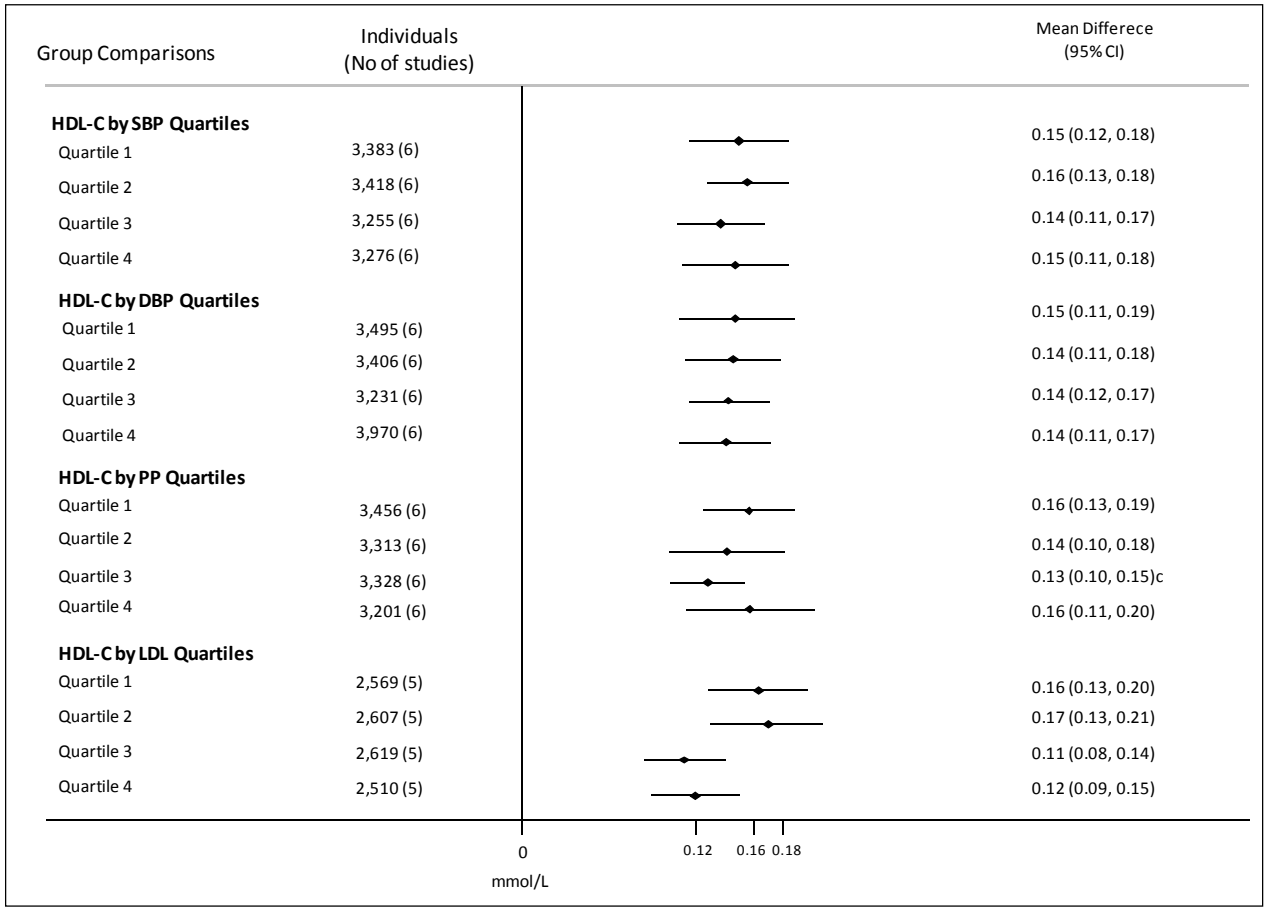


Figure S5a

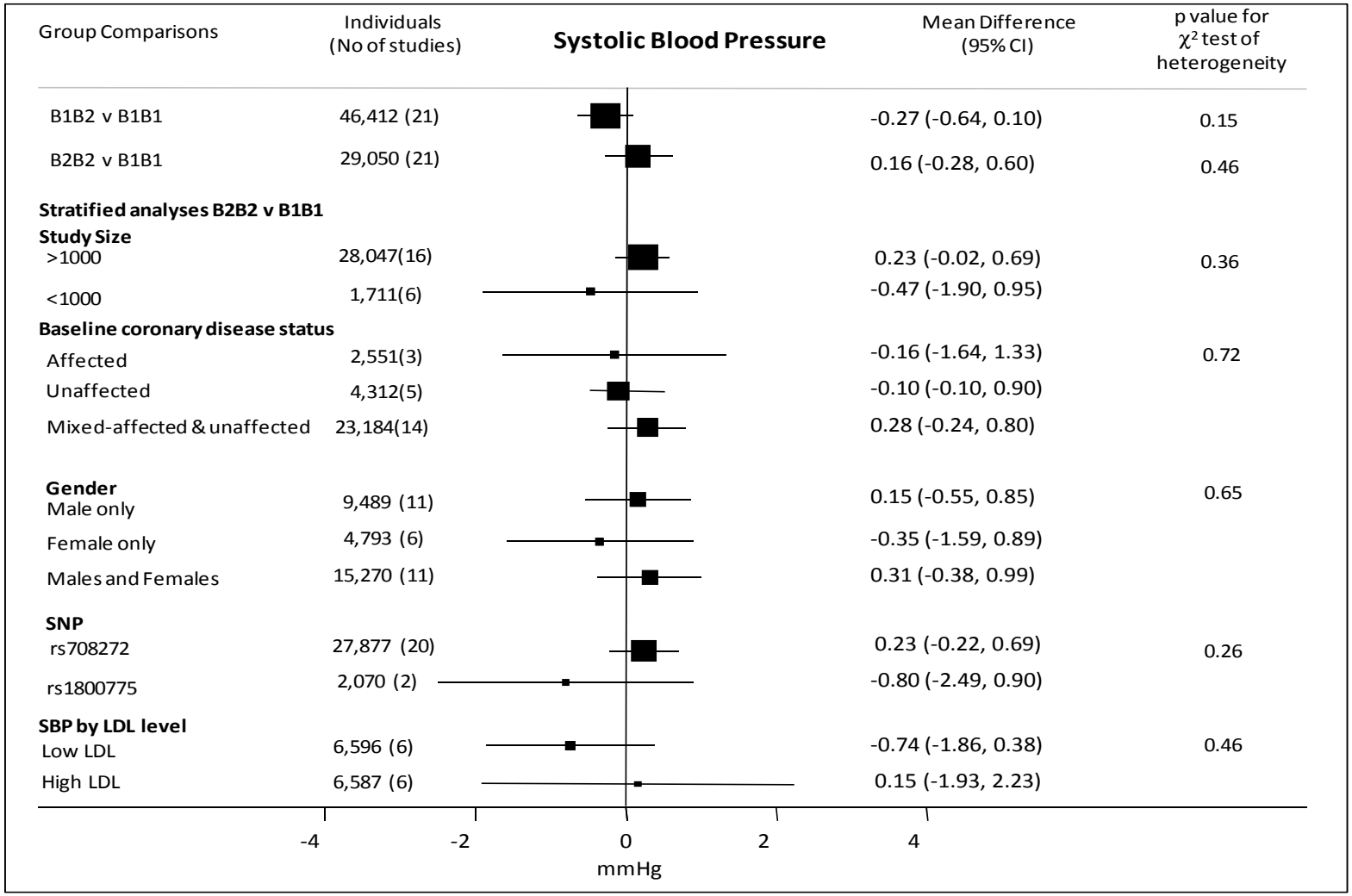
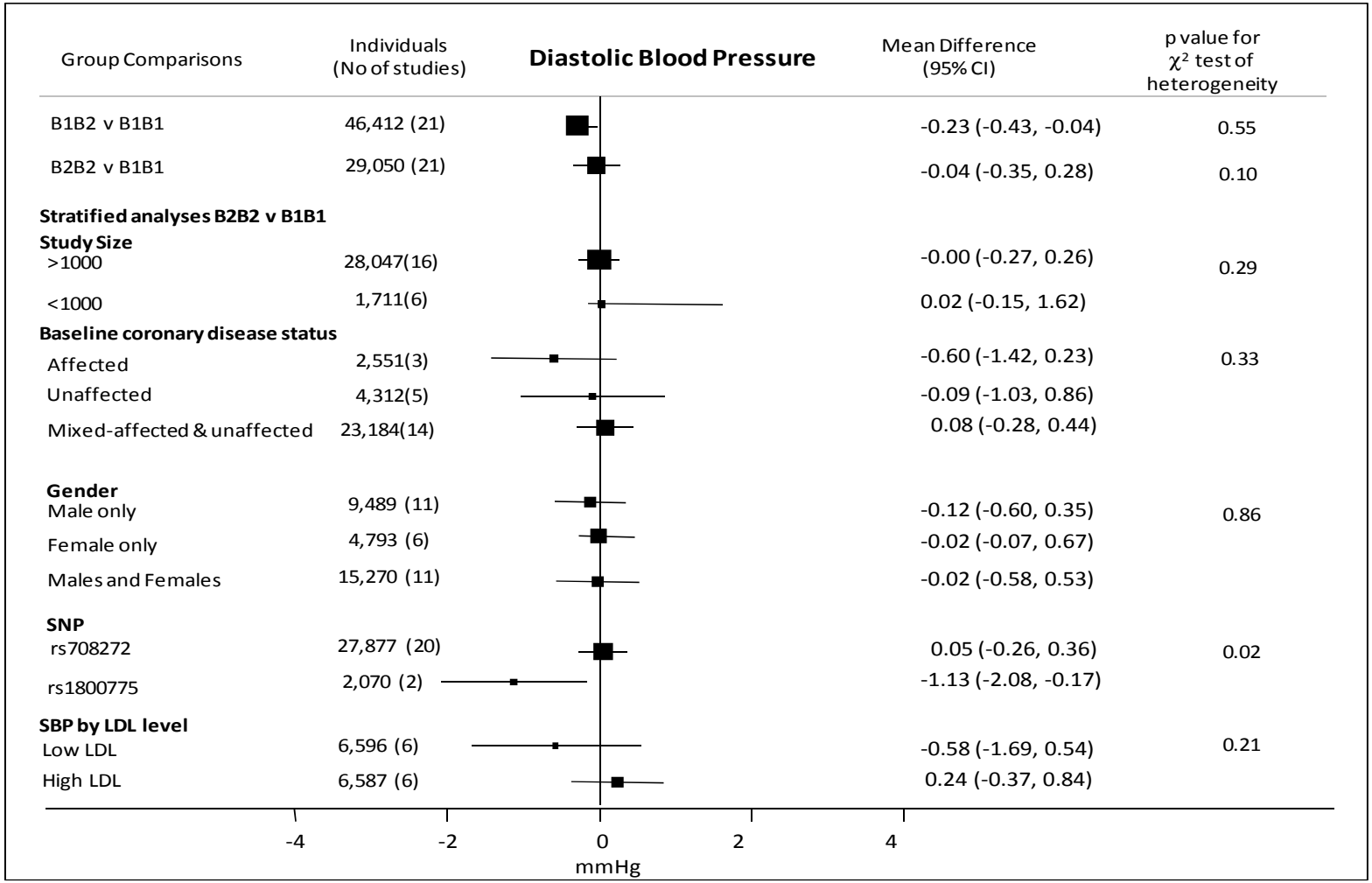


Figure S5b



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