defect of ABCA1 and LCAT, there were a few interesting findings among them. The mRNA of SR-B1 increased in all the *abca1(−/−)lcat(+/+)*, *abca1(+/+)lcat(−/−)*, and *abca1(−/−)lcat(−/−)* genotypes, while mRNAs of apoA-I and apoE increased only in the *abca1(−/−)lcat(−/−)* and that of ABCG1 increased in the genotypes including *abca1(−/−)*. The apoE gene could be up-regulated through LXR activation [28] by the increase in hepatic cellular cholesterol. The SR-B1 gene expression could also be increased by LXRs [29]. Regulation of the apoA-I gene is more complicated, and various factors seem involved in its positive and negative regulation [30]. Bile acid-induced negative regulation may be decreased as cholesterol inflow from HDL is decreased in *abca1*/*lcat*-deficient conditions as a cause of increase in apoA-I mRNA [31]. The expression of ABCG1 seems specific to the ABCA1 deficiency but not responsive to the LCAT deficiency. Although an exact mechanism is unknown, *abcg1* may be up-regulated in compensation to the *abca1* defect rather than regulation by cellular cholesterol through LXRs. On the other hand, expressions of the genes of HMG-CoA reductase and SREBP2 were down-regulated in all the genotypes perhaps by the increase in hepatic cholesterol. In contrast, the expression of the ABCA1 gene was not changed even when hepatic cholesterol increased in *abca1(+/+)lcat(−/−)*. This may indicate the presence of a dual regulation of ABCA1 in hepatocytes by LXRs and SERBPs [27]. It is of interest that apoA-V mRNA was markedly decreased in either *abca1(−/−)* or *lcat(−/−)* condition. The apoA-V gene is known to be highly expressed in the liver and up-regulated by PPARα, RORα, LXR, and SREBP1c, suggesting that cellular lipid accumulation up-regulates this gene [24–26]. The results here are therefore apparently contradictory to these previous findings and quite unique, as increase in hepatic cholesterol is associated with a decrease in apoA-V mRNA. Regulation of the apoA-V gene expression should be further investigated.

Hepatic production of lipoproteins was estimated by measuring lipoprotein production by the hepatocytes in primary culture. HDL production was extremely low in the genotypes of *abca1(+/+)lcat(−/−)*, *abca1(−/−)lcat(+/+)*, *abca1(+/+)lcat(−/−)*, or *abca1(−/−)lcat(−/−)*. It is interesting that not only *abca1(−/−)* but also *lcat(−/−)* causes a decrease in hepatic HDL production despite that the expression of ABCA1 is not affected and that of apoA-I is even increased. Adding LCAT to the medium of the LCAT-deficient hepatocytes does not recover production of HDL and down-regulation of the LCAT gene by a specific siRNA does not induce reduction of HDL production in HepG2 cells (preliminary unpublished data). Therefore, this may not be caused directly by the decrease in LCAT production.

Vascular lipid deposition was not significantly induced in any of the genotypes even after cholesterol feeding for 2 weeks, in agreement with no cholesterol deposition in the peripheral tissues by deficiencies of ABCA1, LCAT, or both. These results are perhaps partially due to lack of HDL since it functions for both delivery and recovery of cholesterol to and from the extrahepatic tissues in mice. Cholesterol demand in the peripheral cells should be fulfilled by the local biosynthesis, and its removal must be mediated by various alternative pathways to HDL, such as plasma albumin, globulin, and others [6]. Erythrocytes can also be a substantial pool of cholesterol in blood and act as its transporter [3]. Even LDL acts as a secondary cholesterol transporter in its recovery pathway to the liver especially in the presence of CETP [32,33], but may play a more direct role in the case of HDL-deficient mouse that lacks CETP activity.

The overall results indicated that removal of cholesterol from the peripheral or extrahepatic tissues and its transport to the liver are carried out even without HDL in vivo. Cholesterol homeostasis is managed to maintain in mouse perhaps by various compensatory pathways [16]. This conclusion is consistent with the recent reports that body cholesterol homeostasis may largely be maintained even without ABCA1 [34] or LCAT [35]. The main production site of HDL is the liver in mouse, as the deficiency of the two major pathways of cell cholesterol release to HDL causes a significant increase in hepatic cholesterol, being consistent with our previous finding [16].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbalip.2009.08.009.

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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.

Clinical Perspective on p 62

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 torce-

trapib (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*," "TaqIB," "−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).

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 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

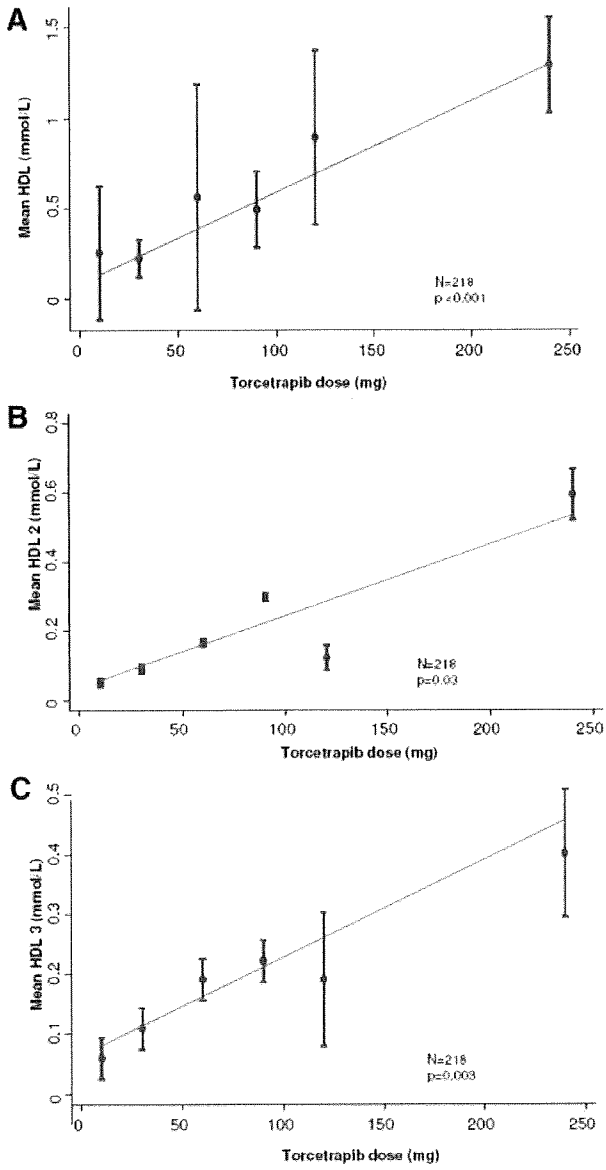


Figure 1. A through C, Relationship between torcetrapib dose and HDL cholesterol and HDL2 and HDL3 subfractions. *P* values refer to the results of a meta-regression, and N refers to the total number of individuals in the 3 dose-ranging studies that contributed to this analysis.

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 (TaqIB) polymorphism, and 2 studies (8535 participants) provided data only on the rs1800775 (−629C>A) polymorphism. Where studies provided data on both −629C>A and TaqIB, 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 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

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.

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 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

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.

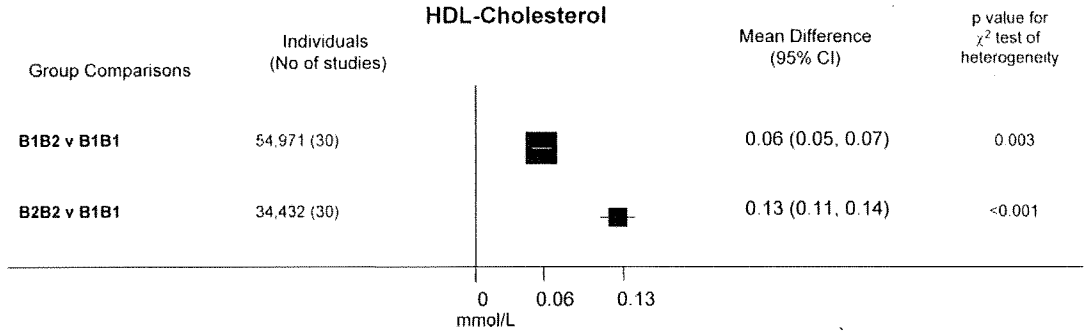


Figure 2. Effect of *CETP* genotype on HDL cholesterol in individuals of European ancestry. The B1B1 genotype is used as the reference group. The numbers refer to the total number of individuals that contribute to the comparisons shown.

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

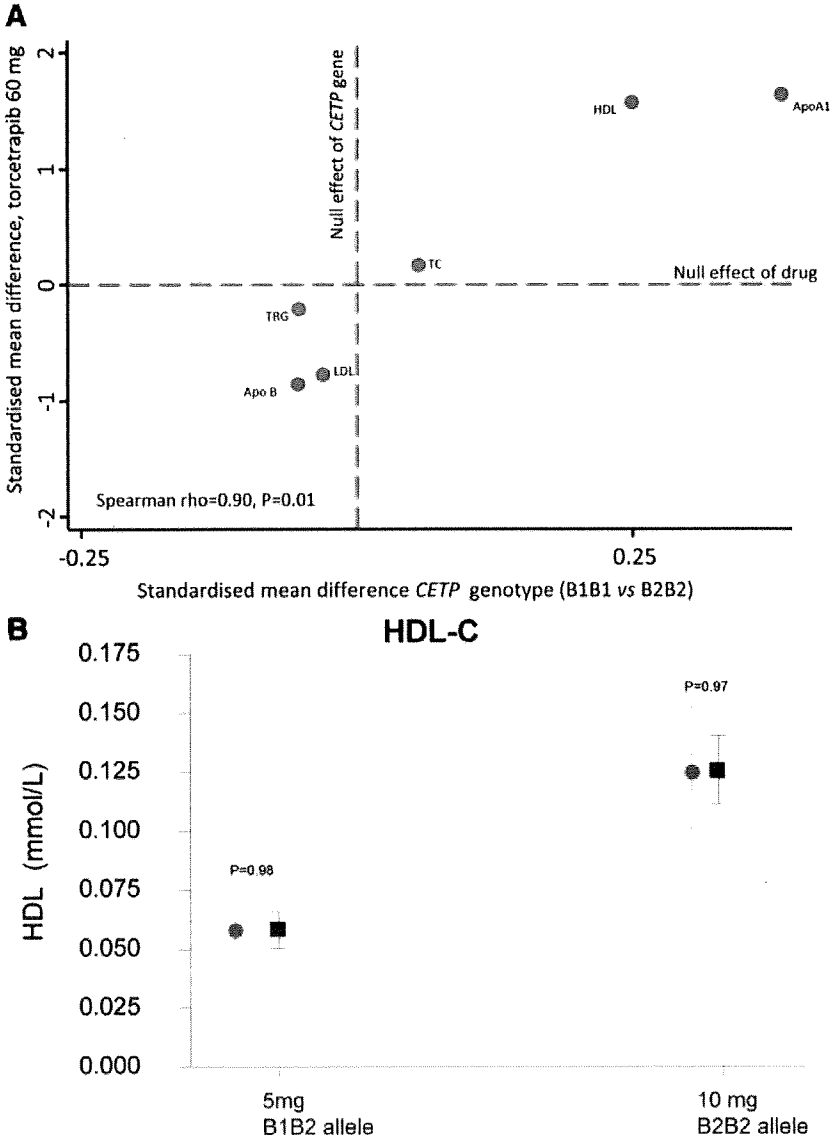


Figure 3. A, Effect of torcetrapib and *CETP* gene variants on 6 lipid traits evaluated in both genetic studies and randomized trials. B, Observed effects of the *CETP* gene and expected effects of a 5- and 10-mg dose of torcetrapib dose on HDL cholesterol.

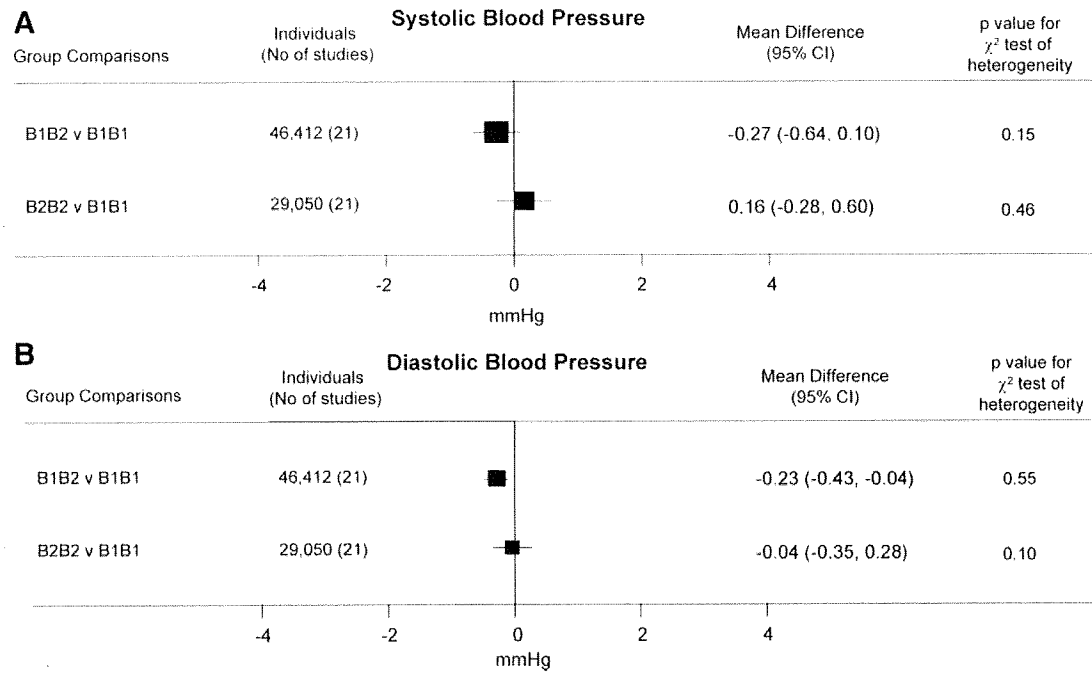


Figure 4. Effect of *CETP* genotype on systolic (A) and diastolic (B) blood pressure in populations of European descent. Weighted mean difference is given, with the B1B1 genotype used as the reference genotype. The numbers refer to the total number of individuals that contribute to the comparisons shown.

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).

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

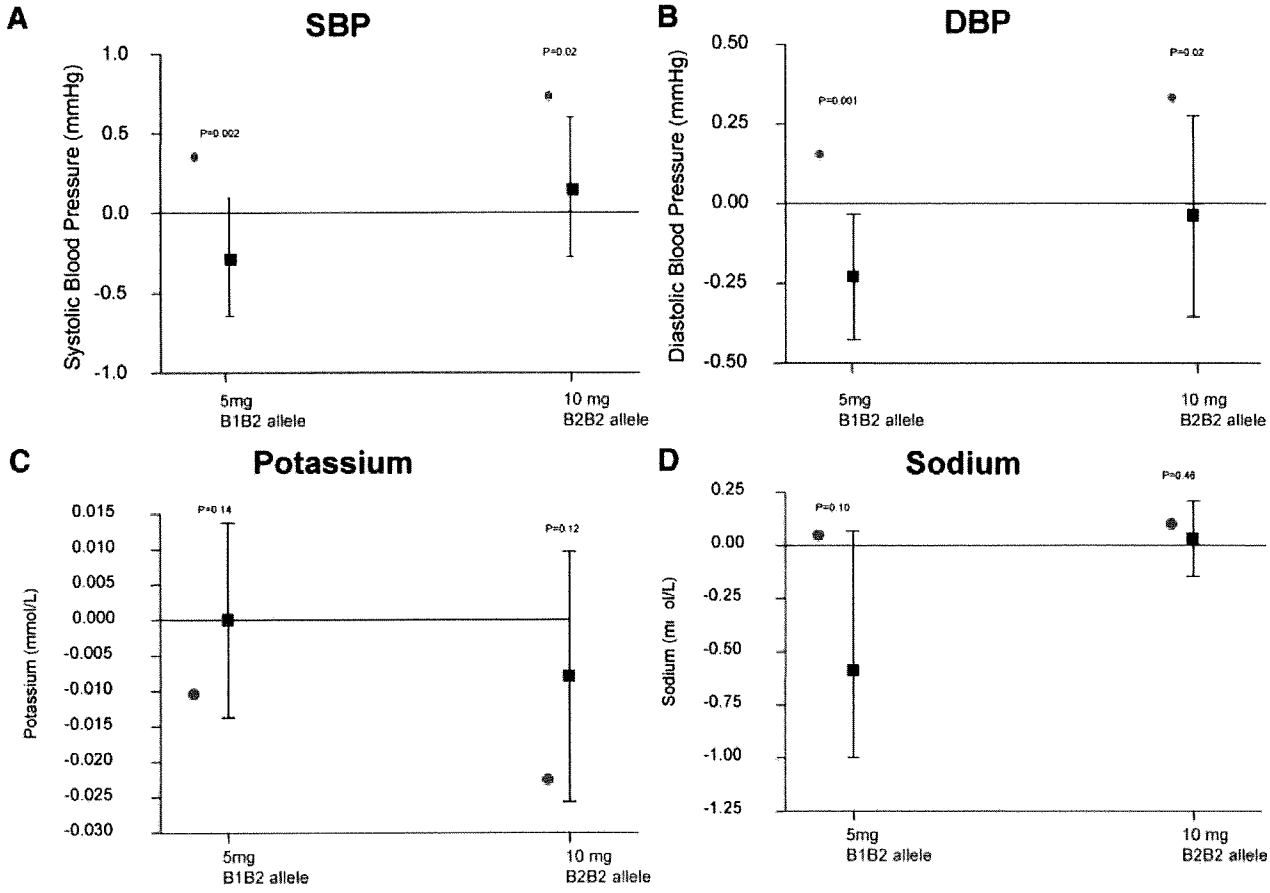
We found concordance in the effect of common variants in the *CETP* gene and pharmacological inhibition of CETP by torcetrapib on 8 continuous lipid and lipoprotein markers evaluated in both randomized trials and genetic studies (HDL cholesterol, HDL2, HDL3, LDL cholesterol, triglycerides, total cholesterol, 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



Abbreviations: ApoA- Apolipoprotein A1, ApoB- Apolipoprotein B, CRP- C-Reactive Protein, HDL-HDL cholesterol, LDL- LDL cholesterol, TC- Total Cholesterol, TRG- triglycerides.

■ Observed (Gene) ● Expected (Drug)

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).

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 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 TaqIB 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 (TaqIB) 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

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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.

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PEDIATRIC RESEARCH

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The Ratio of High-Molecular Weight Adiponectin and Total Adiponectin Differs in Preterm and Term Infants

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ABSTRACT: Adiponectin consists of three subspecies (high-, middle- and low-molecular weight adiponectin). Among these, high-molecular weight adiponectin (H-adn) is suggested to be an active form of this protein. To assess the relationship between H-adn and postnatal growth in preterm infants (PIs), serum H-adn and total adiponectin (T-adn) were measured in 46 PIs at birth and at corrected term, and 26 term infants (TI) at birth. T-adn and H-adn concentrations, and the ratio of H-adn to T-adn (H/T-adn) were significantly greater in TI and PI at corrected term than in PI at birth ($p < 0.001$). T-adn and H-adn concentrations in PI at corrected term were similar to those in TI, but H/T-adn in PI at corrected term was less than that in TI ($p < 0.02$). Stepwise multiple regression analysis revealed that the factors contributing to H/T-adn and serum concentrations of T- and H-adn in PI at corrected term were different from those in TI. These data suggest that quality of early postnatal growth in PIs is different from that in normally developed TI. Postnatal growth accompanying adipose tissue similar to TI may be important for PI to prevent future development of cardiovascular disease. (***Pediatr Res* 65: 580–583, 2009**)

Epidemiologic studies have demonstrated that low birth weight is associated with an increased risk for atherosclerotic cardiovascular disease (CVD) later in life (1–3); however, the mechanisms behind this relationship have yet to be fully elucidated. Adiponectin is one of several adipocytokines secreted by the adipocytes (4) and has numerous physiologic functions. Serum levels of adiponectin are inversely associated with insulin resistance, inflammatory markers, and CVD risk factors (5–7). Taken together, these data suggest that adiponectin has antidiabetic, antiinflammatory, and antiatherogenic functions. Adiponectin consists of three multimer species (high-, middle-, and low-molecular weight adiponectins: H-adn, M-adn, and L-adn) (8–11). Among these, H-adn is suggested to be an active form of this protein, having a higher bound affinity to surface of the cell membrane and remarkably high adenosine monophosphate kinase activation compared with M-adn and L-adn (10,11). Furthermore, the ratio of H-adn and total adiponectin (T-adn) (H/T-adn) are more significantly associated with insulin resistance than T-adn, thereby suggesting the usefulness of the H/T-adn in diagnosing insulin resistance (12).

Serum levels of adiponectin are inversely associated with body weight and body mass index (BMI) in adults and schoolchildren (4,13). In contrast, serum levels of adiponectin in neonates were positively associated with fetal birth weight (14), with their levels being much higher than those in adults and schoolchildren (2,4,7,13,14). The physiologic significance of these findings remains to be clarified, and no information exists regarding serum multimeric adiponectin concentrations in the fetal and neonatal period. Thus, in the present study, as a first step in clarifying the relationship between low birth weight and future CVD risk, we investigated the serum concentrations of multimeric adiponectin in neonates and the difference between premature and mature infants.

SUBJECTS AND METHODS

The subjects consisted of 72 newborn infants (46 preterm born between weeks 24 and 35 of gestation and 26 term and near-term infants born after week 36 of gestation from an uncomplicated pregnancy) who had been admitted to the Ryukyu University hospital, Japan. All term and near-term infants were appropriate for gestational age (AGA). In preterm infants (PIs), 37 infants were AGA and nine infants were small for gestational age (SGA). Preterm deliveries were induced because of threatened premature delivery (21 infants), nonreassuring fetal status (four infants), viral infection of mother (one infant), premature rupture of membrane (13 infants), placenta previa (four infants), hypertension of mother (two infants), and cervical cancer of mother (one infant). Among 46 mothers, two mothers had pregnancy-induced hypertension. All PIs were fed breast milk and infant formula. Serum sample collection and anthropometric measurements were performed at birth and at the corrected term of PIs. Written informed consent was obtained from the parents. The Ethics Committee of the Ryukyu University approved the study protocol.

Anthropometric measurements of subjects. Gestational age was confirmed by ultrasound before week 20 of gestation. The umbilical cord was cut, and the placental weight was measured with a calibrated scale and recorded. The infants were weighed immediately after birth, and birth length was measured using a measuring board. Heart rate (HR) and blood pressure in the lower limb were measured with an automated oscillometric device (MP-60, Phillips Medical Systems, Eindhoven, The Netherlands). Neonatal BMI and Ponderal index were calculated as weight (kg)/body length^2 (m^2) and weight (kg)/body length^3 (m^3), respectively.

Laboratory measurements. Umbilical vein samples were drawn and samples were stored at −40°C until assay. Serum adiponectin concentrations were measured by sandwich ELISA kits (Daiichi Pure Chemicals Co. Ltd., Tokyo, Japan) with a dynamic range of 0.075–4.8 ng/mL. Intraassay variations (CV) were 5.3% (T-Adn), 4.1% (M-adn + H-adn), and 3.3% (H-adn), as described previously (15).

Statistical evaluation. The differences between series of data were determined by Wilcoxon's rank sum test. Levels of T-adn, H-adn, and H/T-adn were markedly skewed. Thus, these parameters were normalized by log transformation. Pearson's correlation coefficient test was performed to assess the associations between data. To examine the relationship between adiponectin multimeric complexes and clinical data, a forward stepwise multiple regression analysis was performed. Values are reported as the mean ± SD. Significance was set at $p < 0.05$. All analyses were performed with JMP 5.1 (SAS Institute Inc., Cary, NC).

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Abbreviations: adn, adiponectin, CVD, cardiovascular disease, **DBP,** diastolic blood pressure, **H-adn,** high-molecular weight adiponectin, **H/T-adn,** the ratio of high-molecular weight adiponectin to total adiponectin, **HR,** heart rate, **PI,** preterm infant, **SBP,** systolic blood pressure, **T-adn,** total adiponectin, **TI,** term infants

Table 1. *Clinical and chemical data on preterm and term infants*

	Preterm infants				
	At birth	*p*	At corrected term	*p*	Term infants
Number of subjects (M/F)	46 (19/28)				26 (11/15)
Postmenstrual age (wk)	31.7 ± 2.9	<0.001	37.1 ± 1.1	ns	37.7 ± 1.2*
Placental weight (g)	371 ± 102				535 ± 138*
Body weight (g)	1609 ± 492	<0.001	2229 ± 374	<0.001	2908 ± 574*
Body length (cm)	40.9 ± 4.2	<0.001	43.8 ± 2.7	<0.001	47.3 ± 2.1*
BMI (kg/m^2)	9.3 ± 1.5	<0.001	11.6 ± 1.5	<0.001	12.9 ± 1.7*
Ponderal index (kg/m^3)	2.27 ± 0.29	<0.001	2.69 ± 0.42	ns	2.74 ± 0.34*
SBP (mm Hg)	61 ± 8	<0.001	69 ± 9	<0.001	62 ± 5
DBP (mm Hg)	35 ± 7	ns	36 ± 7	ns	36 ± 7
Heart rate	134 ± 12	<0.01	144 ± 12	<0.001	123 ± 17†
T-adn (μg/mL)	6.2 ± 3.3	<0.001	19.9 ± 13.5	ns	17.0 ± 10.1*
H-adn (μg/mL)	2.6 ± 2.0	<0.001	9.6 ± 5.7	ns	9.9 ± 6.0*
M-adn (μg/mL)	1.5 ± 1.0	<0.001	5.3 ± 5.2	ns	3.8 ± 3.5†
L-adn (μg/mL)	2.1 ± 1.2	<0.01	5.0 ± 6.7	ns	3.3 ± 2.1
H/T-adn	0.37 ± 0.16	<0.001	0.50 ± 0.12	<0.02	0.58 ± 0.11*

* $p < 0.001$, significantly different from preterm infants at birth.
† $p < 0.05$, significantly different from preterm infants at birth.
M, male; F, female; ns, not significant.

RESULTS

All data presented were not changed even after removing data of SGA infants. Therefore, we did not separate the data for SGA and AGA in analysis.

Characteristics of the subjects. As shown in Table 1, postmenstrual age of PI was significantly younger than that of term infant (TI). Postmenstrual age at corrected term of PI was similar to that of TI. Placental weight was significantly lighter in PI than that in TI. Body sizes (weight and length) were much smaller in PI at birth than those in TI. Body sizes were significantly larger in PI at corrected term than those at birth but still significantly smaller than those in TI. Systolic blood pressure (SBP) of PI at birth was similar to that of TI. SBP of PI at corrected term was significantly higher than those of PI at birth and TI. No differences in diastolic blood pressure (DBP) were found among the three groups. HR was significantly greater in PI at corrected term than in PI at birth and in TI. HR of TI was significantly less than that of PI at birth.

Serum multimeric adiponectin levels. Concentrations of adiponectin in cord serum were similar to those in serum of neonates at birth; therefore, we compared adiponectin levels of cord serum and serum levels of PI at corrected term (14). As shown in Table 1, serum concentrations of T-adn, H-adn, M-adn, and L-adn in PI at birth were significantly less than those in TI. Those of PI at corrected term were similar to those of TI. In PI at corrected term, all of these parameters were significantly higher than those of PI at birth. The ratio of H-adn to T-adn (H/T-adn) of PI at birth was significantly less than those of PI at corrected term and TI. H/T-adn of PI at corrected term was significantly less than that of TI.

Tables 2–4 show the univariate correlations between T-adn, H-adn, H/T-adn, and other parameters in PI at birth and at corrected term ($n = 46$) and those in TI. In PI at birth (Table 2), serum concentrations of T-adn and H-adn were positively correlated with postmenstrual age, placental weight, body weight, body length, BMI, and Ponderal index ($r = 0.31$–0.79, $p = 0.036$–0.000). H/T-adn was positively correlated with postmenstrual age, body weight, body length, BMI, and Ponderal index ($r = 0.36$–0.48, $p = 0.018$–0.001). No significant correlation was found among T-adn, H-adn, H/T-adn, and other parameters. In PI at corrected term (Table 3), serum concentrations of T-adn were positively correlated with body weight and SBP ($r = 0.32$–0.34, $p = 0.03$–0.021). Serum concentration of H-adn was positively correlated with postmenstrual age, placental weight, body weight, and body

Table 2. *Univariate correlations between T-adn, H-adn, H/T-adn, and clinical variables of preterm infants at birth* (n = *46*)

	T-adn		H-adn		H/T-adn	
Independent variables	*r*	*p*	*r*	*p*	*r*	*p*
Postmenstrual age (wk)	**0.75**	**0.000**	**0.75**	**0.000**	**0.48**	**0.001**
Placental weight (g)	0.59	0.000	0.52	0.001	0.29	0.080
Body weight (g)	**0.79**	**0.000**	**0.73**	**0.000**	**0.44**	**0.003**
Body length (cm)	**0.75**	**0.000**	**0.66**	**0.000**	**0.39**	**0.009**
BMI (kg/m^2)	**0.77**	**0.000**	**0.77**	**0.000**	**0.48**	**0.001**
Ponderal index (kg/m^3)	**0.31**	**0.036**	**0.47**	**0.001**	**0.36**	**0.018**
SBP (mm Hg)	0.21	0.196	0.23	0.175	0.13	0.444
DBP (mm Hg)	−0.01	0.953	0.04	0.814	−0.02	0.886
Heart rate	−0.02	0.898	−0.09	0.590	−0.02	0.889

Bold type indicates significant correlations.

Table 3. *Univariate correlations between T-adn, H-adn, H/T-adn, and clinical variables of preterm infants at corrected term* (n = *46*)

	T-adn		H-adn		H/T-adn	
Independent variables	*r*	*p*	*r*	*p*	*r*	*P*
Postmenstrual age (wk)	0.14	0.371	**0.30**	**0.047**	**0.38**	**0.010**
Placental weight (g)	0.29	0.069	**0.38**	**0.017**	0.24	0.138
Body weight (g)	**0.34**	**0.021**	**0.41**	**0.005**	0.19	0.209
Body length (cm)	0.26	0.085	**0.36**	**0.014**	0.26	0.086
BMI (kg/m^2)	0.14	0.340	0.09	0.570	−0.12	0.416
Ponderal index (kg/m^3)	0.08	0.609	0.04	0.782	−0.08	0.619
SBP (mm Hg)	**0.32**	**0.030**	0.18	0.241	**−0.30**	**0.040**
DBP (mm Hg)	0.13	0.379	0.06	0.684	−0.15	0.311
Heart rate	0.29	0.051	0.06	0.685	**−0.50**	**0.000**

Bold type indicates significant correlations.

Table 4. *Univariate correlations between T-adn, H-adn, H/T-adn, and clinical variables of term infants* (n = 26)

	T-adn		H-adn		H/T-adn	
Independent variables	*r*	*p*	*r*	*p*	*r*	*p*
Postmenstrual age (wk)	0.26	0.195	0.37	0.060	**0.39**	**0.047**
Placental weight (g)	0.12	0.567	0.04	0.863	−0.23	0.273
Body weight (g)	**0.39**	**0.049**	**0.40**	**0.045**	0.10	0.625
Body length (cm)	0.18	0.383	0.17	0.409	0.01	0.962
BMI (kg/m^2)	**0.43**	**0.027**	**0.46**	**0.017**	0.18	0.369
Ponderal index (kg/m^3)	0.31	0.119	0.36	0.069	0.21	0.296
SBP (mm Hg)	**0.39**	**0.047**	**0.44**	**0.023**	0.24	0.236
DBP (mm Hg)	**0.45**	**0.022**	**0.52**	**0.007**	0.31	0.125
Heart rate	0.09	0.674	0.07	0.736	−0.03	0.871

Bold type indicates significant correlations.

Table 5. *Stepwise multiple regression models for predicting T-adn, H-adn, and H/T-adn*

	Selected independent parameters	r^2	*p*
Preterm infants at birth (*n* = 46)			
T-adn	Body weight	0.62	0.000
H-adn	BMI	0.59	0.000
H/T-adn	Postmenstrual age	0.23	0.001
Preterm infants at corrected term (*n* = 46)			
T-adn	SBP, placental weight	0.22	0.011
H-adn	Body weight	0.17	0.005
H/T-adn	HR, body weight	0.34	0.000
Term infants at birth (*n* = 26)			
T-adn	DBP	0.20	0.022
H-adn	DBP	0.27	0.007
H/T-adn	Postmenstrual age, placental weight	0.33	0.011

length (r = 0.30–0.41, p = 0.047–0.005). H/T-adn was positively correlated with postmenstrual age (r = 0.38, p = 0.010). SBP and HR showed significant negative correlations with H/T-adn (r = −0.30 to −0.50, p = 0.04–0.000). No significant correlation was found among T-adn, H-adn, H/T-adn, and other parameters. In TI (Table 4), serum concentration of T-adn was positively correlated with body weight, BMI, SBP, and DBP (r = 0.39–0.45, p = 0.049–0.022), as well as H-adn was positively correlated with body weight, BMI, SBP, and DBP (r = 0.40–0.52, p = 0.045–0.007). H/T-adn was positively correlated with postmenstrual age (r = 0.39, p = 0.047). No significant correlation was found among T-adn, H-adn, H/T-adn, and other parameters.

Because each of the above parameters can potentially contribute directly to the regulation of serum multimeric adiponectin levels, we performed stepwise multiple regression analysis with T-adn, H-adn, and H/T-adn as the dependent variables and postmenstrual age, placental weight, body weight, body length, BMI, SBP, DBP, and HR as the independent variables (Table 5). In PI at birth, the predictors of T-adn, H-adn, and H/T-adn were body weight, BMI, and postmenstrual age, respectively. In PI at corrected term, the major predictors of T-adn were SBP and placental weight. The predictor of H-adn was body weight. HR and body weight were selected as the predictors of H/T-adn. In TI at birth, the predictor of T- and H-adn was DBP. Postmenstrual age and placental weight were selected as the predictors of H/T-adn.

DISCUSSION

In the present study, we showed that i) the serum concentrations of T-adn and H-adn in PI at corrected term were three times higher than those in PI at birth, whereas serum concentrations of T-adn and H-adn of PI at corrected term were similar to those of TI; ii) the H/T-adn of TI and PI at corrected term were significantly greater than that of PI at birth, but that of PI at corrected term was still significantly less than that of TI; and iii) the factors contributing to serum concentrations of T- and H-adn differed among the three groups.

A recent study reported that concentrations of adiponectin in fetal circulation shows a 20-fold increase between week 24 of gestation and term, and serum concentrations of adiponectin were positively associated with body weight in PIs (16). In addition, Kotani et al. (14) reported that the cord serum adiponectin concentrations in full-term neonates are higher than serum adiponectin concentrations in adults (22.4 μg/mL *versus* 8.2 μg/mL) and are correlated positively with fat mass-related parameters (birth weight, BMI, and leptin concentration). In the present study, serum concentrations of T- and H-adn increased by 2.5–3-fold between 32 and 38 wk of gestation, and their levels were also higher than those reported in schoolchildren (2,7,17). These data suggest that fetal development during late pregnancy contributes to serum concentrations of T- and H-adn, and that the relationship between adiposity and adiponectin levels in neonates may differ from that in adults and children. Interestingly, Inami et al. (18) recently reported a positive association between serum concentrations of adiponectin and body weight in TIs at birth, but no association was found at 1 mo of age. To date, some roles of adipocytokines such as IL-6 and TNF-α have been highlighted in the inverse correlation between adiponectin and body weight in adults (19). It has been shown that IL-6 and TNF-α reduce the secretion of adiponectin from adipocytes (20). Some types of inflammation in adipose tissue might contribute to the inverse correlation between adiponectin and adiposity. The rapid increase in fetal body weight during late pregnancy and the perinatal period is a physiologic development; thus, it is reasonable to consider that an increase in adipose tissue at this stage may not be accompanied by hypertrophy of adipocytes and the inflammation in adipose tissue. This may also cause the higher levels of adiponectin and positive correlations between adiponectin and body weight observed during the perinatal period. With respect to the higher concentrations of adiponectin in PI at corrected term and TI, Kim et al. (21) reported that serum levels of adiponectin are a starvation signal released by adipocytes, and that normal-sized adipocytes do not induce insulin resistance, even in obese status. It is well known that development of adipose tissue is greatest in perinatal period (22). A recent report of Pinar et al. (23) showed that the high concentrations of H-adn found in the fetus were associated with higher insulin sensitivity. Thus, high levels of adiponectin in the perinatal period may reflect rapid growth during this period.

As shown in Table 2, T-adn, H-adn, and H/T-adn were significantly associated with adiposity-related parameters (body weight, BMI, and Ponderal index) in PI at birth. However, these associations were weakened in PI at corrected term and TI (Tables 3 and 4). Different from PI at birth, hemodynamic

parameters (blood pressure and/ or HR) were significantly associated with T-adn and H/T-adn in PI at corrected term, and with T-adn and H-adn in TI (Tables 3–5). It is of interest that expression of adiponectin in fetus was pronounced in vascular endothelium, thereby suggesting that in addition to adipose tissues, vascular endothelial cells may contribute to fetal adiponectin levels (23). It is plausible that hemodynamic parameters affect the status of vascular endothelium and lead to significant correlation of these to adiponectin in PI at corrected term and TI. Taken together, our data suggest that vascular endothelium might contribute to serum concentrations of T-adn and H-adn during late pregnancy. However, in PI at corrected term, hemodynamic parameters were not associated with H-adn. SBP and HR were inversely associated with H/T-adn. These results indicate that in PI at corrected term, ex-utero factors may influence adipose tissue development and contribution of vascular endothelium to adiponectin status. Placental weight was significantly correlated with T-adn and H-adn in PI at birth (Table 2). Serum concentrations of H-adn in PI at corrected term were still positively associated with placental weight (Table 3). A recent report showed that the adiponectin concentration in umbilical cord serum is positively associated with the weight ratio of fetus to placenta (24). In contrast, Pinar et al. proved the absence of endogenous placental adiponectin and showed its exclusive production by fetal tissues. All of these findings indicate that effect of placental weight on adiponectin status may be indirect effects; however, further studies are needed to clarify this.

Wang et al. (25) showed that ectopic fat accumulation occurred even in nonobese mice, which showed hyperinsulinemia, insulin resistance, and hypertrophy of adipocytes because of subnormal adipocyte storage capacity, whereas Ruderman et al. (26) reported metabolically obese, normal-weight patients. These previous studies, combined with the report by Kim et al. (21) strongly suggest that in neonates, normal development of adipose tissue is important to prevent future development of cardiometabolic diseases such as diabetes, hypertension, and dyslipidemia. Subnormal development of adipose tissue in neonates may reduce the number of adipocytes. If our notion is valid, the limited number of adipocytes in subjects with low birth weight may induce hypertrophy of adipocytes more readily than in subjects with normal birth weight. As a result, these subjects may be at high risk for cardiometabolic diseases in the future, even if normal weight is maintained. Ibanez et al. (27) recently reported that children born small for gestational age tend to be viscerally adipose with hypoadiponectinemia, even if they are not overweight. This may support our hypothesis.

In conclusion, our present data suggest that postnatal growth accompanying adiposity similar to TI may be important for premature and/or low birth weight infant to prevent future development of cardiometabolic diseases. Monitoring of T- and H-adn could be useful to evaluate the maturation of adipose tissue in PIs.

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Case Report

An 11-Year-Old Boy with Familial Hypercholesterolemia Showing Multiple Xanthomas and Advanced Atherosclerosis, Who Responded to Lipid-Lowering Therapy Using Statin

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Familial hypercholesterolemia (FH) is characterized by a high level of LDL-cholesterol (LDL-C) and a high prevalence of atherosclerotic coronary heart disease; however, hypercholesterolemia is usually the only clinical finding in children with heterozygous FH in their first decade of life. We report a case of FH in an 11-year-old boy who presented with multiple xanthomas at both elbows, thickened Achilles tendons, and hyperplasia of the intima-media complex of the carotid artery. Echocardiogram revealed partial calcification of the aortic and mitral valves, but no stenosis of the coronary arteries was detected on 3D-computed tomography. The activity of LDL receptors was reduced to 32% by lymphocyte assay. The family history showed vertical transmission of hypercholesterolemia from father to son, thereby suggesting dominant inheritance. After 12 months of treatment with statin and resin, his LDL-C decreased from 446 to 220 mg/dL, thickening of the Achilles tendons decreased from 16–18 mm to 13 mm, and hyperplasia of the intima-media complex decreased from 1.3 mm to 0.7 mm. These findings suggest that our patient had heterozygous FH. However, based on his advanced atherosclerosis, we cannot exclude the possibility that our patient may be accompanying dyslipidemia due to causes in addition to heterozygous FH.

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Key words; Familial hypercholesterolemia, Drug therapy, Xanthoma, Atherosclerosis

Introduction

Familial hypercholesterolemia (FH) is one of the most common inherited metabolism errors, and is inherited as an autosomal dominant trait[1]. Homozygote and heterozygote frequencies are estimated to be 1 in 1 million and 1 in 500 in the general population, respectively[2]. The clinical diagnosis of homozygous FH (homo FH) is not difficult for a pediatrician because affected children have cutaneous xanthomas and juvenile atherosclerosis in addition to hypercholesterolemia. In contrast, hypercholesterolemia is usually the only clinical finding in children with heterozygous FH (hetero FH) in their first decade of life[3]. Pathologically, atherosclerotic changes in the coronary arteries originate during childhood, and the extent of atherosclerotic lesions correlates positively with plasma LDL-cholesterol (LDL-C) levels and negatively with plasma HDL-cholesterol (HDL-C) levels, even in children and young adults[4]. These data suggest that the development of atherosclerosis might be accelerated in children with hetero FH, even if clinical symptoms are not observed. Here, we report a possible case of hetero FH in an 11-year-old boy with advanced atherosclerosis.

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Case Presentation

An 11-year-old boy was referred to our hospital from a regional hospital and presented with thickened Achilles tendons, multiple xanthomas on both elbows, and high levels of total cholesterol (530 mg/dL). He had attended a regional hospital for removal of multiple xanthomas on both elbows. The father, who is hetero FH, recognized the xanthomas and thickening of Achilles tendons apparent in his son at the age of 8 years as being similar to his own case. The patient did