Human Genome Epidemiology (HuGE) Review

Seven Lipoprotein Lipase Gene Polymorphisms, Lipid Fractions, and Coronary Disease: A HuGE Association Review and Meta-Analysis

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Lipoprotein lipase (LPL) is a key enzyme in lipoprotein metabolism and a major candidate gene for coronary heart disease (CHD). The authors assessed associations between 7 *LPL* polymorphisms and lipid fractions and CHD risk in population-based cohort, case-control, and cross-sectional studies published by January 2007. Meta-analyses of 22,734 CHD cases and 50,177 controls in 89 association studies focused on the relations of the T-93G (rs1800590), D9N (rs1801177), G188E, N291S (rs268), *Pvu*II (rs285), *Hin*dIII (rs320), and S447X (rs328) polymorphisms to high density lipoprotein cholesterol, triglycerides, myocardial infarction, or coronary stenosis. Carriers of 9N or 291S had modestly adverse lipid profiles. Carriers of the less common allele of *Hin*dIII or of 447X had modestly advantageous profiles. The combined odds ratio for CHD among carriers was 1.33 (95% confidence interval (CI): 1.14, 1.56) for 9N, 1.07 (95% CI: 0.96, 1.20) for 291S, 0.89 (95% CI: 0.81, 0.98) for the less common *Hin*dIII allele, and 0.84 (95% CI: 0.75, 0.94) for 447X. For T-93G (odds ratio (OR) = 1.22, 95% CI: 0.98, 1.52) and *Pvu*II (OR = 0.96, 95% CI: 0.89, 1.04), there were null associations with lipid levels or CHD risk; information on G188E was limited (OR = 2.80, 95% CI: 0.88, 8.87). The study of *LPL* genotypes confirms the existence of close interrelations between high density lipoprotein cholesterol and triglyceride pathways. The influence of these genotypes on CHD risk warrants further investigation.

cholesterol, HDL; coronary disease; epidemiology; genetics; lipoprotein lipase; meta-analysis; myocardial infarction; triglycerides

Abbreviations: CHD, coronary heart disease; CI, confidence interval; HDL, high density lipoprotein; LPL, lipoprotein lipase.

BACKGROUND

Gene and gene variants

Lipoprotein lipase (LPL), an enzyme discovered in 1943 (1), plays a central role in lipid metabolism by hydrolyzing triglyceride-rich particles in muscle, adipose tissue, and macrophages, thereby generating free fatty acids and glycerol for energy utilization and storage (2, 3). LPL also plays a noncatalytic bridging role as a ligand in lipoprotein-cell surface interactions and receptor-mediated uptake of lipoproteins (4) with its ability to bind simultaneously to both lipoproteins and cell surface receptors. The *LPL* gene is located on chromosome 8p22 (OMIM [Online Mendelian Inheritance in Man] number 609708), spanning approximately 35 kilobases. It contains 10 exons and encodes a 448-amino acid mature protein (5).

Since the identification of the first mutations in the *LPL* gene (6), more than 100 mutations have been identified (reviewed in detail by Murthy et al. (7)). Dozens of rare mutations in the *LPL* gene have been associated with markedly reduced enzyme activity (7), whereas several relatively common variants have been associated with moderate changes in LPL catalytic function. The highly polymorphic *LPL* gene and its many single nucleotide polymorphisms in both the coding region and the noncoding region have been

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Table 1. Allele Frequencies Based on Combined Data From the Control Groups of Studies Identified for Inclusion in This Human Genome Epidemiology Review

Polymorphism (rs No. if Available)	Allele	Total				Whites			East Asians		
		Frequency, %	Range, %	No. of Studies	No. of Participants	Frequency, %	No. of Studies	No. of Participants	Frequency, %	No. of Studies	No. of Participants
T-93G (rs1800590)	G	1.6	0.8–6.8	7	10,395	1.5	5	9,112	NA	0	
D9N (rs1801177)	N	2.1	<0.1–8.0	21	18,518	1.5	16	15,494	NA	0	
G188E	E	0.03		3	8,595	0.03	2	8,445	NA	0	
N291S (rs268)	S	2.5	0.7–6.1	21	24,145	2.2	16	15,179	NA	0	
PvuII restriction enzyme site (rs285)	Absence of restriction site	46.3	32.5–55.1	18	8,807	48.5	11	1,928	38.9	3	351
HindIII restriction enzyme site (rs320)	Absence of restriction site	28.7	20.1–46.5	23	5,244	29.1	15	3,540	28.9	4	479
S447X (rs328)	X	9.9	5.6–21.1	26	20,233	10.3	18	16,612	12.2	3	391

Abbreviations: NA, not available; rs, reference SNP.

studied for associations with lipids, lipoproteins, and atherosclerosis. The majority of these mutations are rare, although they can appear at a relatively high prevalence in specific subpopulations (7).

Gene variant frequency

For this Human Genome Epidemiology review, we searched MEDLINE and EMBASE using the search terms specified in the Appendix for *LPL* gene polymorphisms that may be associated with coronary heart disease (CHD). We identified 7 that had been investigated in multiple studies. Characteristics of these polymorphisms are summarized in Web Figure 1 (posted on the *Journal*'s website (http://aje. oxfordjournals.org/)), along with their proposed effects on enzyme function, a protein model, and evidence of linkage disequilibrium between pairs of variants. We estimated allele frequencies from the control groups of all studies identified for inclusion in this review (Table 1). The 2 restriction site enzyme variants (*Pvu*II and *Hin*dIII) have high allele frequencies (46% and 29% respectively, for absence of the restriction site). The X allele of the S447X polymorphism has an approximate frequency of 10%, and the other variants (T-93G, D9N, and N291S) have frequencies less than 3%, with the E allele of the G188E variant having a frequency of only 0.03%, so that it does not qualify as a polymorphism.

We did not observe strong evidence of variation in allele frequencies by ethnicity (Table 1). On the basis of data from up to 1,758 white participants, strong linkage disequilibrium was reported between S447X and *Hin*dIII ($D' \approx 0.9$), *Pvu*II ($D' \approx 0.9$), N291S ($D' \approx 0.9$), and D9N ($D' \approx -0.9$) and between T-93G and D9N ($D' \approx 0.9$); moderate linkage disequilibrium was reported between *Pvu*II and N291S ($D' \approx 0.7$) and between *Hin*dIII and *Pvu*II ($D' \approx 0.6$) (8–11). On the basis of data from up to 467 East Asian participants, strong linkage disequilibrium was reported for the relation of S447X with *Hin*dIII ($D' \approx 0.9$) and *Pvu*II ($D' \approx 0.9$) and for the relation of *Hin*dIII with *Pvu*II ($D' \approx 0.9$); moderate linkage disequilibrium of S447X with N291S ($D' \approx 0.7$) was reported (12–14).

Disease

CHD, including myocardial infarction, angina pectoris, and stenosis of the coronary arteries, is a leading cause of morbidity and mortality worldwide. It is the single most common cause of death in both the United States and the United Kingdom, accounting for approximately 1 in 5 deaths (15, 16). It is estimated that each year, about 700,000 Americans experience symptomatic first-ever myocardial infarction; a further 175,000 have "silent" myocardial infarction (i.e., without the normal indicators such as chest discomfort, shortness of breath, feeling dizzy or lightheaded, or numbness in 1 or both arms); and a further 500,000 have recurrent myocardial infarction (16). In the United Kingdom, CHD accounts for more than 208,000 deaths each year (15). Although the death rate from CHD fell by 33% in the United States from 1994 to 2004 (16) and by 24% in the United Kingdom over the past decade (15), the condition remains the leading killer worldwide, and its

burden is rising rapidly in low- and middle-income countries, particularly in South Asia.

Established risk factors for CHD include modifiable factors (such as smoking and high cholesterol and blood pressure levels) and nonmodifiable factors (such as family history, ethnicity, and age). In recent decades, there has been investigation of the contribution of genetic variation to CHD risk, with earlier studies having focused principally on candidate genes involved in biologically plausible pathways associated with lipids and hemostasis. For example, apolipoprotein E genotypes have been shown to be linearly associated with concentrations of low density lipoprotein cholesterol and CHD risk (17). More recently, with the completion of projects such as the Human Genome Project (18, 19) and the International HapMap Project (20, 21), genome-wide association studies have used assumption-free approaches to investigate loci with as-yet-unknown biologic relevance (22–28). One particular locus, 9p21.3, has been replicated consistently (22, 23, 25, 27). A recent meta-analysis of the rs1333049 single nucleotide polymorphism (as a proxy marker for 9p21.3) in 12,004 cases and 28,949 controls showed strong evidence of association (odds ratio = 1.24, 95% confidence interval (CI): 1.20, 1.29) (29).

LPL is a key enzyme in lipoprotein metabolism, and it has been studied in relation to CHD risk. Previous meta-analyses, the most recent of which was published in 2006, have reported associations of some common *LPL* variants with circulating concentrations of LPL, high density lipoprotein (HDL) cholesterol, and triglycerides, as well as, more tentatively, CHD risk (30–33). However, the amount of data available has increased more than 3-fold since the last comprehensive review was carried out in 1999 (33). Moreover, interpretation of previous reviews has been complicated by their combination of studies conducted in general populations and those involving families.

Objectives

Here we report results from a new meta-analysis of 89 association studies addressing the *LPL* gene, involving a total of 22,734 CHD cases and 50,177 controls. This analysis updates considerably the evidence available from previous meta-analyses (30–33). We focused on 7 polymorphisms (the T-93G, D9N, G188E, N291S, *Pvu*II, *Hin*dIII, and S447X variants (Web Figure 1)) and concentrations of HDL cholesterol and triglycerides as well as CHD outcomes (with initially separate consideration of myocardial infarction and coronary stenosis). The present report is restricted to population association studies (excluding family-based studies) and contains an investigation of potential sources of heterogeneity.

METHODS

Selection criteria and identification of studies

Eligible for inclusion were all population-based cohort, case-control, or cross-sectional studies reporting on associations of the 7 polymorphisms listed in Web Figure 1 with concentrations of HDL cholesterol or triglycerides or with risk of angiographic coronary stenosis (defined as at least

50% stenosis of 1 or more major coronary arteries) or myocardial infarction (defined by World Health Organization/MONICA [Monitoring of Trends and Determinants in Cardiovascular Disease] Study criteria). For lipid fractions, we used only data from apparently healthy controls (i.e., people without known coronary or other diseases or clinical lipid abnormalities).

We performed electronic searches, not limited to the English language, of MEDLINE, EMBASE, BIOSIS, the Science Citation Index, GDPInfo, and LocusLink, as described in the Appendix. The latest searches were undertaken on January 10 and 11, 2007. All relevant articles identified in the search were scanned on the basis of title, keywords, and abstract (where available) by one of us (I. T. or G. S. S.) and were rejected in the initial screening if the article clearly did not meet the inclusion criteria. Where a title/abstract could not be rejected with certainty, we obtained the full text of the article for evaluation. We also reviewed the reference lists of articles retrieved to identify relevant publications.

Data collection

The following data were extracted independently by 2 investigators, using a piloted data extraction form (with any discrepancies being resolved by discussion and, when necessary, adjudicated by a third reviewer): genotype frequencies, by categorical disease outcome; mean values and standard deviations for lipid fractions, by genotype; mean age of cases; proportions of males and ethnic subgroups (defined as people of European continental ancestry, East Asians, or others); fasting status; genotyping and lipid assay methods; use of blinding of laboratory workers to participant case-control status; and linkage disequilibrium D' values among the 7 variants under study. For reports not printed in English, eligibility decisions and data extraction were done jointly by 1 author and a colleague fluent in the language of the report. We calculated allele frequencies from control groups only for each of the 7 variants, assuming Hardy-Weinberg equilibrium where appropriate. We compared key study characteristics such as location, time frame, authorship, and participant characteristics to determine the existence of multiple publications from the same study. In such situations, we extracted data from each report and included the most complete and up-to-date information in our analyses. Where important ambiguities remained or when data could not be extracted for inclusion in the meta-analysis, the investigators were contacted via letter and e-mail. We contacted all investigators who reported on CHD outcomes and investigators who reported on studies involving at least 250 participants for lipid fractions.

Data analysis

Primary analyses were conducted using a dominant inheritance model to maximize the number of studies included. Subsidiary codominant analyses used weighted logistic regression or weighted linear regression for studies in which this could be done, yielding either a per-allele odds ratio for CHD outcomes or a per-allele difference in mean lipid levels, respectively. Although triglyceride levels are typically skewed, we analyzed values on the original scale,

following the majority of reported findings, which would give appropriate confidence intervals for large sample sizes. Where mean values and standard deviations were given for log triglyceride levels, we converted these to approximate means and standard deviations for crude levels, assuming a log-normal distribution (34). For 3 studies (35–37) that included separate groups of nonoverlapping coronary stenosis cases and nonfatal myocardial infarction cases with a single (overlapping) control group, we analyzed the different case groups separately and then combined them into a single CHD group to avoid double-counting of individuals. Deviance from Hardy-Weinberg equilibrium was assessed for the controls of each study using the exact test if the genotype frequencies were low (38). Since tests for deviation from Hardy-Weinberg equilibrium are known to have low power, we calculated the fixation coefficient, interpreting an absolute value greater than 0.03 as an indication of serious departure from Hardy-Weinberg equilibrium (38, 39). We conducted meta-analyses of fixation coefficients to assess global evidence of departure from Hardy-Weinberg equilibrium across studies (40).

Consistency of the gene effect sizes across studies was assessed using a test for heterogeneity and the I^2 statistic, which describes the percentage of total variation in point estimates attributable to genuine variation rather than sampling error (41). Funnel plots and associated statistical tests (42, 43) were used to assess assumptions involved in meta-analysis and to explore the relation between precision and magnitude of association. To assess reporting biases, we compared CHD associations in published data with those in data we obtained by correspondence.

In our meta-analyses, we used a standard approach, weighting by precision and incorporating random effects to allow for the variation in true associations across studies. We included studies irrespective of any departure from Hardy-Weinberg equilibrium. We explored interstudy variation by prespecified subgrouping of studies according to sample size (<100, 100–499, ≥500), ethnicity (white, East Asian), source of controls (general population, hospital), design (retrospective, prospective), and blinding of genotyping to clinical outcome (yes, no, unknown). We conducted sensitivity analyses by repeating the meta-analyses assuming recessive, codominant, and unspecified (44) inheritance models and by performing fixed-effect meta-analyses. Results for lipid levels are expressed as percentage changes, obtained by applying absolute differences to mean levels in noncarriers, estimated as the overall weighted mean (1.33 mmol/L for HDL cholesterol, 1.53 mmol/L for triglycerides). All analyses were conducted using our own STATA programs, with meta-analyses performed using the "metan" subroutine (45).

RESULTS

Characteristics of the included studies

Literature searches yielded 6,202 reports, of which 104 were eligible studies (8–14, 35, 36, 46–139). Eighty-nine of these studies contributed to the present meta-analyses (8–14, 35, 36, 46–51, 67–139) (Web Tables 1 and 2 (http://aje.oxfordjournals.org/)); 15 studies (52–66) (comprising only

about 6% of potentially eligible CHD cases and only about 11% of potentially eligible controls) could not be included because of insufficient detail and lack of response to correspondence (Web Table 3 (http://aje.oxfordjournals.org/)). Investigators in 2 studies (108, 140) provided unpublished data. Study-level characteristics for the Stockholm Heart Epidemiology Program have previously been published (140).

Fifty contributing studies on CHD outcomes (Web Table 1), published between 1991 and 2006, were undertaken in a wide range of geographic settings, with 80% (18,103 of 22,734) of cases having white European continental ancestry, 3% (794 of 22,734) East Asian, and 17% (3,837 of 22,734) other ethnic origins (including Mexican-American and Turkish). Nine studies on CHD outcomes were prospective in design, including nested case-control studies (and therefore involved "internal" population controls), and 41 were retrospective, of which 21 involved population-based controls, 14 involved hospital-based controls, and 6 involved controls from other sources (e.g., workplaces). Of 22 (44%) studies that reported on blinding of laboratory workers to case-control status, 17 reported blinded assessments of genotypes and 5 reported unblinded assessments.

Investigators in 73 contributing studies reported on lipid fractions in articles published between 1989 and 2006 (Web Table 2), including 45 (62%) prospective studies and 28 (38%) case-control studies (with only noncases contributing to the lipid analyses). In 63 (86%) of these studies, investigators obtained blood samples after an overnight fast. Restriction fragment length polymorphism was the most common genotyping method. Twenty-one (42%) studies of CHD outcomes provided supplementary tabular data, as did 29 (40%) studies of lipid fractions.

In total, 9 studies were found to deviate from Hardy-Weinberg equilibrium according to the exact test ($P < 0.05$) (for studies of CHD outcomes: Qian et al. (124) (*Hin*dIII), Thorn et al. (132) (*Pvu*II), Duman et al. (102) (*Pvu*II), Ferencak et al. (105) (D9N), and Keavney et al. (117) (N291S); for studies of HDL cholesterol: Zhang et al. (50) (*Hin*dIII) and Duman et al. (102) (*Pvu*II); for studies of triglycerides: Zhang et al. (50) (*Hin*dIII) and Duman et al. (102) (*Pvu*II)). Approximately 60% of the studies had fixation coefficients with absolute values larger than 0.03. However, for all polymorphisms, they seemed evenly distributed around 0 (results are presented in Web Table 4 (http://aje.oxfordjournals.org/) for those outcome groups with 3 or more studies by polymorphism). Pooled meta-analysis of the fixation coefficients for each polymorphism did not indicate substantial overall deviation from Hardy-Weinberg equilibrium in a common direction (data not shown). Estimation of the inheritance model from the data typically yielded estimates with confidence intervals that did not exclude any of the inheritance models. For 2 polymorphisms, however, the recessive model was not supported by the data (not shown): *Hin*dIII (data for CHD outcomes only) and S447X (CHD outcomes and HDL cholesterol data).

Associations with lipid fractions

Figure 1 summarizes associations of the variants under study with HDL cholesterol and triglyceride concentrations;
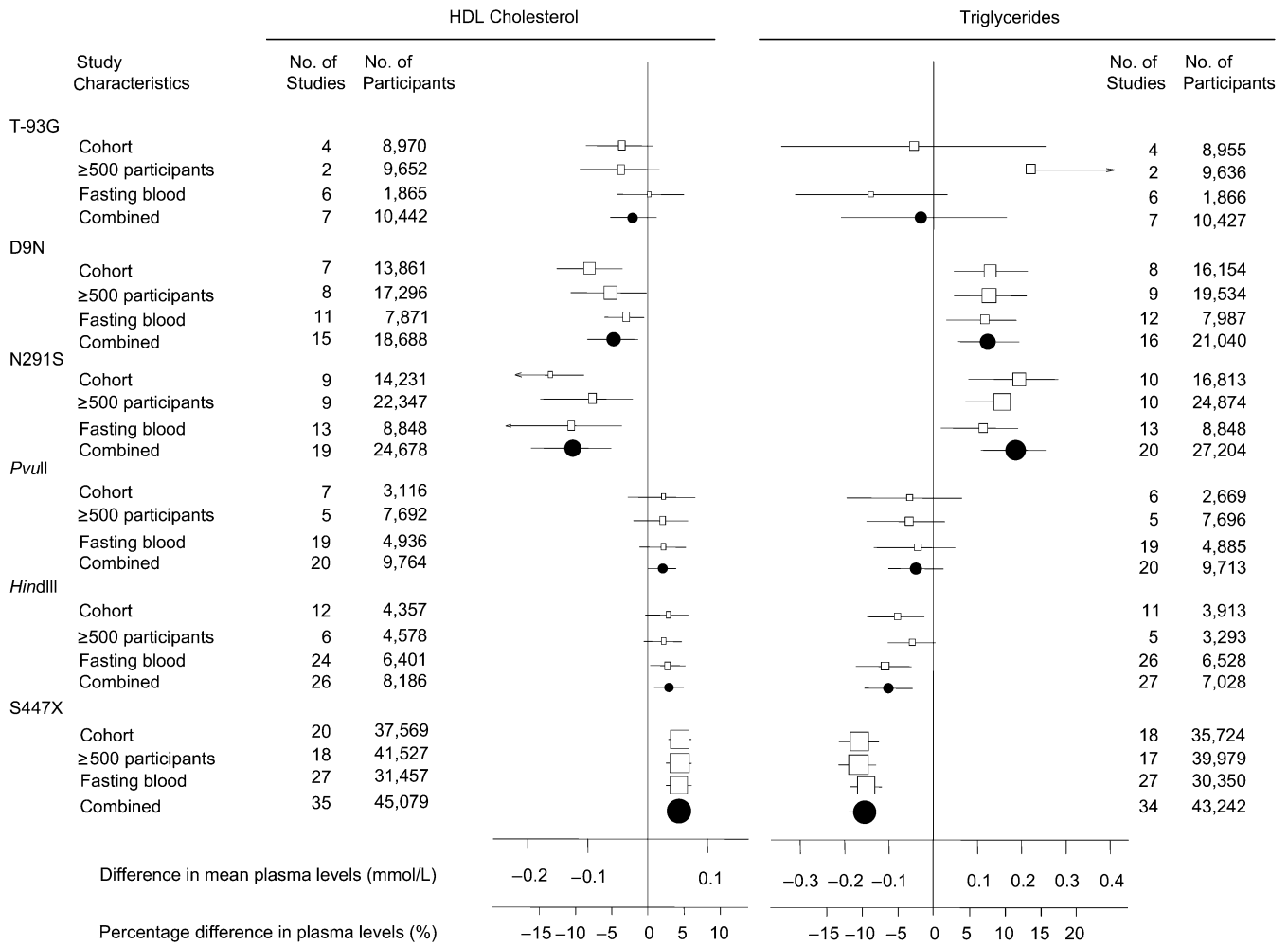
Figure 1. Associations between plasma high density lipoprotein (HDL) cholesterol and triglyceride levels and 6 polymorphisms of the lipoprotein lipase gene, grouped by various study characteristics using a dominant genetic model (carriers vs. noncarriers). Point estimates are shown as unfilled boxes for subgroup analyses and filled circles for the overall combined analyses. Horizontal lines, 95% confidence interval.

results for each individual study are provided in Web Figures 2 and 3 (http://aje.oxfordjournals.org/). Based on data on up to 10,442 participants, carriers of the −93G polymorphism had 0.02 mmol/L (95% CI: −0.02, 0.06) lower HDL cholesterol levels and 0.02 mmol/L (95% CI: −0.17, 0.21) lower triglyceride levels than noncarriers. Based on data on up to 21,040 participants, carriers of the 9N polymorphism had 0.05 mmol/L (95% CI: 0.02, 0.09) lower HDL cholesterol levels and 0.14 mmol/L (95% CI: 0.08, 0.20) higher triglyceride levels than noncarriers. Based on data on up to 27,204 participants, carriers of the 291S polymorphism had 0.12 mmol/L (95% CI: 0.07, 0.18) lower HDL cholesterol levels and 0.19 mmol/L (95% CI: 0.12, 0.26) higher triglyceride levels than noncarriers. There was evidence of considerable heterogeneity in the HDL cholesterol findings ($I^2 = 83\%$, 95% CI: 75, 88; $P < 0.0001$). Based on data on up to 9,764 participants, carriers of the less common allele of the *Pvu*II polymorphism had 0.03 mmol/L (95% CI: 0.00, 0.05) higher HDL cholesterol levels and 0.04

mmol/L (95% CI: −0.02, 0.10) lower triglyceride levels than noncarriers. Based on data on up to 8,186 participants, carriers of the less common allele of the *Hin*dIII polymorphism had 0.04 mmol/L (95% CI: 0.02, 0.06) higher HDL cholesterol levels and 0.09 mmol/L (95% CI: 0.05, 0.14) lower triglyceride levels than noncarriers. Based on data on up to 45,079 participants, carriers of the 447X polymorphism had 0.05 mmol/L (95% CI: 0.04, 0.07) higher HDL cholesterol levels and 0.15 mmol/L (95% CI: 0.12, 0.19) lower triglyceride levels than noncarriers. We express these findings in terms of percentage differences in lipid fractions in Table 2.

Associations between each of these LPL variants and lipid fractions did not vary materially when analyses using recessive or codominant inheritance models were performed or when results were grouped by study design, size, or fasting status (Figure 1) or by ethnicity or source of controls (not shown). Only the funnel plot of *Hin*dIII and triglycer- ides suggested the possibility of an excess of smaller studies

Table 2. Comparison of Findings of the Present Meta-Analysis of Data From 89 Studies (Including 22,734 Cases of Coronary Heart Disease and 50,177 Controls) With Those Reported in Previous Meta-Analyses of 7 Polymorphisms in the Lipoprotein Lipase Gene, Obtained Using the Same Genetic Model (Carriers vs. Noncarriers)

Variant	Previous Review			Present Review		
	Study Details (Ref. No.)	% Difference or OR	95% CI	Study Details	% Difference or OR	95% CI
High Density Lipoprotein Cholesterol Levels[a]						
T-93G	Not done			7 studies; 10,442 participants	−2[c]	−5, 2
D9N	4 studies; 4,999 participants (33)	−6[b]	−9, −3	15 studies; 18,688 participants	−4	−7, −2
G188E	Not done[d]			Not done		
N291S	8 studies; 14,760 participants (33)	−9	−11, −7	19 studies; 24,678 participants	−9	−14, −5
*Pvu*II	Not done			20 studies; 9,764 participants	2	0, 4
*Hind*III	Not done			26 studies; 8,186 participants	3	2, 5
S447X	7 studies; 4,388 participants (33)	3	2, 5	35 studies; 45,079 participants	4	3, 5
Triglyceride Levels[a]						
T-93G	Not done			7 studies; 10,427 participants	−1[c]	−14, 11
D9N	5 studies; 6,496 participants (33)	20	9, 33	16 studies; 21,040 participants	9	5, 13
G188E	Not done[d]			Not done		
N291S	8 studies; 15,509 participants (33)	31	20, 43	20 studies; 27,204 participants	12	8, 17
*Pvu*II	Not done			20 studies; 9,713 participants	−3	−7, 1
*Hind*III	Not done			27 studies; 7,028 participants	−6	−9, −3
S447X	8 studies; 4,567 participants (33)	−8	−11, −4	34 studies; 43,242 participants	−10	−12, −8
Coronary Heart Disease[e]						
T-93G	Not done			7 studies; 5,045 cases, 10,395 controls	1.22[f]	0.98, 1.52
D9N	3 studies; 979 cases, 981 controls (33)	1.4	0.8, 2.4	20 studies; 9,792 cases, 18,469 controls	1.33	1.14, 1.56
G188E	1 study; 948 cases, 9,259 controls (33)	4.9	1.2, 19.6	2 studies; 2,374 cases, 8,445 controls	2.80	0.88, 8.87
N291S	4 studies; 2,252 cases, 10,235 controls (33)	1.2	0.9, 1.5	21 studies; 13,883 cases, 24,145 controls	1.07	0.96, 1.20
*Pvu*II	6 studies; 2,618 sample size (30)	0.90	0.80, 1.01	18 studies; 8,407 cases, 8,807 controls	0.96	0.89, 1.04
*Hind*III	6 studies; 2,259 sample size (30)	0.84	0.73, 0.96	23 studies; 6,226 cases, 5,244 controls	0.89	0.81, 0.98
S447X	5 studies; 1,576 cases, 1,862 controls (33)	0.8	0.7, 1.0	26 studies; 11,046 cases, 20,223 controls	0.84	0.75, 0.94

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] Percent differences are given for high density lipoprotein cholesterol and triglyceride levels.

[b] Because the mean difference in high density lipoprotein cholesterol levels between carriers and noncarriers in the previous review was reported as absolute difference in mmol/L (rather than as percentage difference), the percentage difference has been calculated using the mean level in noncarriers from the present meta-analysis.

[c] By application of estimated mean difference to weighted mean value across noncarrier groups, confidence intervals reflect uncertainty in absolute difference in means.

[d] The G188E variant has been previously reviewed (30, 31, 33), but those reviews included familial studies and so have not been included here.

[e] Odds ratios are given for coronary heart disease.

[f] A random-effects meta-analysis will tend to have wider confidence intervals despite the larger set of cases and controls. Previous meta-analyses used fixed-effect models (30) or unclear models (33), whereas our analysis uses random-effects models. This explains why our intervals are not necessarily narrower despite large increases in data.

with striking results (data available upon request), but even for these analyses, findings in the larger studies (which may be less prone to selective reporting) were very similar to the overall results.

Associations with CHD risk

The odds ratios for myocardial infarction and coronary stenosis were similar within each of the 7 variants studied

Table 3. Relations Between 7 Lipoprotein Lipase Polymorphisms and Coronary Heart Disease Outcomes in a Meta-Analysis of Data From 89 Studies (Including 22,734 Cases and 50,177 Controls), Obtained Using a Dominant Genetic Model (Carriers vs. Noncarriers)

Variant	All CHD						Nonfatal Myocardial Infarction						Coronary Stenosis					
	No. of Studies[b]	No. of Cases	No. of Controls	OR	95% CI	I^2, %[a]	No. of Studies	No. of Cases	No. of Controls	OR	95% CI	I^2, %	No. of Studies	No. of Cases	No. of Controls	OR	95% CI	I^2, %
T-93G	7	5,045	10,395	1.22	0.98, 1.52[c]	9.5	3	2,360	9,576	1.27	0.91, 1.78	35.4	5	2,685	8,807	1.22	0.88, 1.71	17.3
D9N	20	9,792	18,469	1.33	1.14, 1.56[d]	17.2	10	4,829	12,524	1.22	1.01, 1.48	20.2	9	4,618	10,652	1.42	1.15, 1.76	0.5
G188E	2	2,374	8,445	2.80	0.88, 8.87	0.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
N291S	21	13,883	24,145	1.07	0.96, 1.20[d]	4.6	10	9,153	17,758	1.03	0.91, 1.16	0.0	11	4,397	10,383	1.05	0.87, 1.28	0.0
*Pvu*II	18	8,407	8,807	0.96	0.89, 1.04	2.6	5	5,474	6,891	0.95	0.83, 1.09	19.9	13	2,933	1,916	0.97	0.85, 1.11	0.0
*Hin*dIII	23	6,226	5,244	0.89	0.81, 0.98	15.7	9	2,809	3,091	0.88	0.79, 0.98	5.9	15	3,376	2,085	0.91	0.77, 1.07	43.1
S447X	26	11,046	20,223	0.84	0.75, 0.94[d]	49.8*	11	6,033	16,031	0.87	0.75, 1.00	49.2	16	4,851	11,229	0.83	0.70, 0.98	51.0

Abbreviations: CHD, coronary heart disease; CI, confidence interval; NA, not available; OR, odds ratio.

* $P < 0.01$ (P for heterogeneity).

[a] I^2 is a measure of the extent to which between-study variation is not due to chance alone, on a scale from 0% to 100%.

[b] Because studies such as the Copenhagen City Heart Study (37) contained multiple case groups that were combined in the overall analysis and because of the inclusion of studies with CHD outcomes other than myocardial infarction or coronary stenosis (death by CHD), the number of studies is not always the sum of the 2 clinical subgroups.

[c] The Copenhagen City Heart Study (37) was included in both CHD subgroup analyses, but only the nonoverlapping cases were combined for the total CHD analysis because both case groups shared the same overlapping control group (to avoid double counting of the control group).

[d] In addition to the Copenhagen City Heart Study (37), the total CHD outcome also included data from an unpublished study (8) investigating death by CHD.

(Table 3), encouraging combined analyses of the 2 CHD outcomes. Using a dominant genetic model, the combined odds ratios for total CHD were 1.22 (95% CI: 0.98, 1.52) for carriers of −93G (5,045 cases, 10,395 controls), 1.33 (95% CI: 1.14, 1.56) for carriers of 9N (9,792 cases, 18,469 controls), 2.80 (95% CI: 0.88, 8.87) for carriers of 188E (2,374 cases, 8,445 controls), 1.07 (95% CI: 0.96, 1.20) for carriers of 291S (13,883 cases, 24,145 controls), 0.96 (95% CI: 0.89, 1.04) for carriers of the less common allele of *Pvu*II (8,407 cases, 8,807 controls), 0.89 (95% CI: 0.81, 0.98) for carriers of the less common allele of *Hin*dIII (6,226 cases, 5,244 controls), and 0.84 (95% CI: 0.75, 0.94) for carriers of 447X (11,046 cases, 20,223 controls). There was little evidence of heterogeneity among the available studies, with the exception being studies of S447X ($I^2 = 50\%$, 95% CI: 11, 67; $P = 0.003$). Findings did not change materially when different inheritance models were assumed or when studies were grouped by genotyping conditions, type of controls used, or study size (Figure 2) or by ethnicity or study design (not shown). We did not observe visual or statistically significant asymmetry in funnel plots, and published data were not consistently nearer to (or further away from) the null than data obtained via correspondence. Web Figures 4–9 (http://aje.oxfordjournals.org/) plot the available data for each variant.

DISCUSSION

Main findings

We have presented new data and meta-analysis results on the associations between 7 *LPL* polymorphisms and HDL cholesterol, triglycerides, and CHD risk in 22,734 cases and 50,177 controls from 89 studies, counting every study's cases and controls only once. Four variants (i.e., 9N, 291S, *Hin*dIII, and 447X) were each found to have modest and opposing effects on HDL cholesterol and triglyceride concentrations, suggestive of close interrelations between pathways regulating these lipid fractions. Our findings, based on 3 times as many data as in earlier reviews (30, 31, 33), indicate that the percentage increases in triglyceride levels in carriers of the 9N or 291S alleles are approximately 10% for each, rather than the 20%–30% increases previously reported (Table 2). In addition, the direction of association between CHD risk and carrier status for each of 9N, 291S, *Hin*dIII, and 447X was consistent with their corresponding lipid effects; however, as noted below, further data are needed to enhance precision in this regard. Proposed guidelines for assessing the strength of evidence from gene-disease association studies (141) would designate the findings as showing ''moderate'' evidence for S447X, D9N, and *Hin*dIII and ''weak'' evidence for the other 4 variants (Web Table 5 (http://aje.oxfordjournals.org/)).

Limitations

Although available data on each of 6 polymorphisms in this review (i.e., all those but G188E) comprised at least 5,000 CHD cases and at least 5,000 controls, there was still insufficient power to reliably assess relative risk reductions
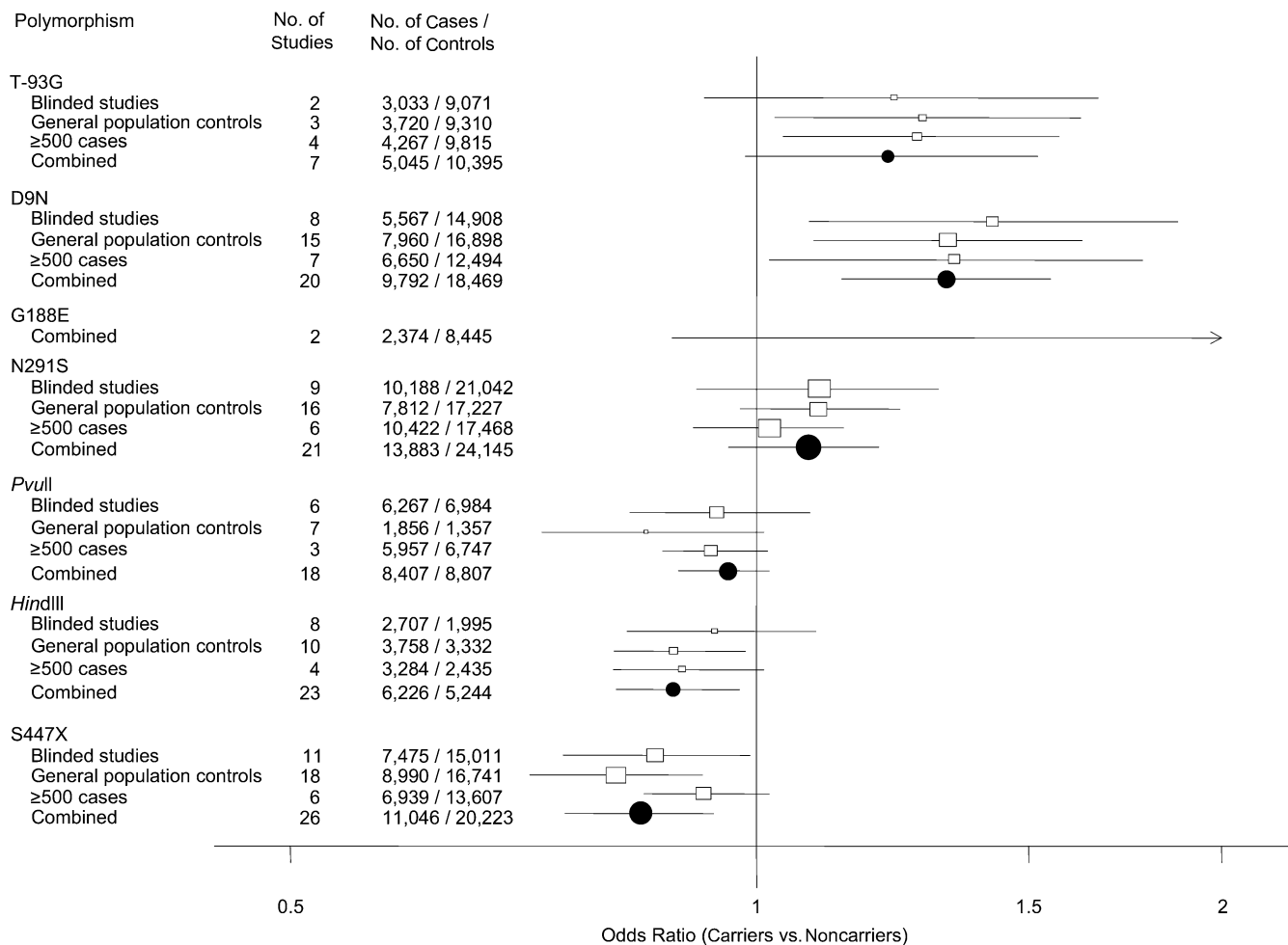
Figure 2. Meta-analyses of studies of coronary heart disease and 7 polymorphisms of the lipoprotein lipase gene, grouped by various study characteristics using a dominant genetic model (carriers vs. noncarriers). No filled circle is shown for G188E because the point estimate is off the scale. Point estimates are shown as unfilled boxes for subgroup analyses and filled circles for the overall combined analysis. Horizontal lines, 95% confidence interval.

of approximately 10%–20% in carriers of these *LPL* alleles, particularly for the less common polymorphisms studied. Without data on individual participants, we were unable to conduct more detailed analyses (e.g., studies of haplotypes or investigation of any joint effects of gene-gene or gene-lipid factors), direct assessment in these populations of the impact of changes in lipid concentrations on CHD risk, or "Mendelian randomization" analyses (142). Nonetheless, previous prospective studies of HDL cholesterol and triglycerides suggest that even the modest changes observed in these lipid concentrations related to *LPL* variants should be associated with an approximately 10% change in the risk of CHD, if the lipid markers have causal effects (143).

We did not find evidence of appreciable biases affecting the results of the studies. Subgrouping of studies by characteristics such as design, source of controls, laboratory methods, and use of blinding did not reveal any major differences; and case definitions were comparable across studies, so the likelihood of spectrum bias is low. However,

this does not mean that the studies were free from internal biases. A more substantial potential problem is reporting bias, in the form of either selective reporting of findings within the identified studies or the omission of whole studies with less striking findings. Although we reduced the scope of publication bias through collation of unreported data by correspondence with authors (and we noted that findings were similar in previously unreported studies and in published studies), it remains impossible to exclude entirely the likelihood of reporting biases in literature-based meta-analyses (144–146). Nevertheless, at least for the lipid outcomes studied, any material effect seems unlikely given the scale and consistency of the findings observed in the larger studies.

Biology

The observed associations between *LPL* variants and concentrations of HDL cholesterol and triglycerides may help correlate genetic epidemiology with current understanding

of the LPL enzyme's structure and function. The modestly adverse lipid profile in carriers of 9N or 291S is consistent with reduced enzyme activity owing to amino acid substitutions in the N-terminal domain of LPL (see Web Figure 1), the part of the enzyme that is important for catalytic function (147). In particular, the 291S variant (i.e., the Asn291Ser substitution) is located in a heparin-binding cluster and may therefore affect the interaction of LPL with the cell wall glycosaminoglycans, whereas the 9N variant (the Asp9Asn substitution) is situated near a glycosylation site that may influence overall catalytic activity and secretion (148). By contrast, the modestly advantageous lipid profile in carriers of the 447X variant (the Ser447Ter substitution) is consistent with the increased receptor binding affinity that this 2-amino acid truncation confers on the C-terminal domain (see Web Figure 1), the part of the enzyme important for the LPL-mediated uptake of lipoproteins by receptors on the cell surface (149–151). This change could explain the altered lipid profile seen in carriers of 447X, as well as in carriers of the intronic *Hin*dIII variant, which is in nearly complete linkage disequilibrium with 447X.

Potential public health impact and other implications of results

Each of several *LPL* variants, known to be in linkage disequilibrium, has modest effects on HDL cholesterol and triglyceride concentrations, as well as modest associations with CHD risk in directions consistent with their respective lipid effects. At present, the potential public health impact is limited, given the small magnitude of these associations. Studies involving at least 10,000 cases and a similar number of controls with concomitant information on genetic and lipid markers would be required to enhance precision appreciably in relation to CHD and to facilitate informative Mendelian randomization analyses. Such studies would also permit investigation of any interactions among *LPL* (and other genetic) variants and assessment of haplotype-based associations.

CONCLUSION

The modest and opposing effects of each of 4 *LPL* variants on HDL cholesterol and triglyceride concentrations underscore the close interrelations between these lipid pathways. Although the associations of these *LPL* genotypes with CHD risk each pointed in directions consistent with their respective lipid effects, larger genetic studies will be required to judge the relevance of these lipid markers to CHD.

ACKNOWLEDGMENTS

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APPENDIX

Search Strategy Used in the Updated Meta-Analysis

The MEDLINE search strategy using PubMed for assessing the association between lipoprotein lipase (*LPL*) polymorphisms and coronary heart disease was the following: (lipoprotein lipase* OR lipoprotein lipase[MeSH] OR LPL* OR lipid*) AND (gene* OR polymorphi* OR genetic* OR mutation* OR allel* OR genotyp*) AND (coronary stenosis* OR coronary stenosis[MeSH] OR coronary artery disease* OR coronary artery disease[MeSH] OR CAD* OR coronary arteriosclerosis* OR coronary arteriosclerosis[MeSH] OR myocardial infarction* OR myocardial infarction[MeSH] OR MI OR coronary heart disease* OR coronary heart disease[MeSH] OR ischemic heart disease* OR ischemic heart disease[MeSH] OR myocardial ischemia* OR myocardial ischemia[MeSH]).

The search strategy for assessing the associations between *LPL* polymorphisms and measures of lipid metabolism was the following: (lipoprotein lipase* OR lipoprotein lipase[MeSH] OR LPL* OR lipid*) AND (gene* OR polymorphi* OR genetic* OR mutation* OR allel* OR genotyp*) AND (triglyceride* OR triglycerides[MeSH] OR TG OR high density lipoprotein OR high density lipoprotein[MeSH] OR HDL OR intermediate density lipoprotein OR intermediate density lipoprotein[MeSH] OR IDL OR low density lipoprotein OR low density lipoprotein[MeSH] OR LDL OR very low density lipoprotein OR very low density lipoprotein[MeSH] OR VLDL OR cholesterol OR cholesterol[MeSH] OR hyperlipidemia OR hyperlipidemia[MeSH] OR hypertriglyceridaemia OR hypertriglyceridaemia[MeSH]).

Searches of other databases translated these strategies term by term, using equivalent thesaurus terms as appropriate.