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Research Article

Associations between *LPL* gene polymorphisms and coronary artery disease: evidence based on an updated and cumulative meta-analysis

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Lipoprotein lipase (LPL) is widely linked to lipid and lipoprotein metabolism, but its effects on coronary artery disease (CAD) are not clearly elucidated. The aim of the present study was to clarify the association between *LPL* gene polymorphisms and CAD susceptibility. The pooled odds ratio (OR) and 95% confidence interval (CI) were calculated to estimate the strength of the relationship between *LPL* gene polymorphisms and CAD risk. Comprehensive electronic databases, including PubMed, EMBASE, Web of Science, and the Cochrane Library, were systematically searched. A total of 45 records containing 80 eligible studies were analyzed. The results indicated an increased risk between the *LPL* D9N polymorphism and susceptibility to CAD in the dominant genetic model (AA + GA vs. GG: OR = 1.46, 95% CI = 1.14–1.87), whereas the *LPL* HindIII polymorphism showed a protective effect against CAD under all tested models (GG + GT vs. TT: OR = 0.85, 95% CI = 0.75–0.97; GG vs. TT + TG: OR = 0.62, 95% CI = 0.47–0.83; G vs. T: OR = 0.81, 95% CI = 0.71–0.92). No significant association was identified for the *LPL* N291S and PvuII polymorphisms. Stratification analysis by ethnicity suggested a significant correlation between the *LPL* S447X polymorphism and CAD susceptibility in Caucasians under the dominant and allele genetic models. In summary, our meta-analysis indicated that the *LPL* D9N polymorphism was associated with an increased risk of CAD, whereas the S447X and HindIII polymorphisms showed protective effects. There was no association observed between the N291S and PvuII polymorphisms and CAD risk.

Introduction

Coronary artery disease (CAD) is a complex multifactorial disease and a leading cause of morbidity and mortality worldwide [1]. Although genetic and environmental factors have been widely implicated in the mechanisms underlying the pathogenesis of CAD, these potential factors remain an area of active investigation [2]. Atherosclerosis is the underlying cause of CAD, which is primarily characterized by excessive lipid deposition in the endothelium of the vascular tree walls [3]. Individuals with aberrant lipid and lipoprotein metabolism, including elevated levels of triglyceride (TG), cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) and decreased levels of high-density lipoprotein cholesterol (HDL-C), are more inclined to the development of CAD [4]. Genome-wide association studies (GWAS) have also identified nearly 150 loci linked to plasma lipid traits, and some of these loci are associated with altered lipoprotein lipase (LPL) gene expression [5]. Furthermore, several studies have demonstrated a causal link between triglyceride-rich lipoproteins (TRLs) and CAD, with variants in several crucial genes involved in TRLs metabolism, including LPL and its regulators [2,6]. In the past decades, numerous studies have reported that the *LPL* gene variants directly affect abnormal lipid and lipoprotein metabolism and

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its influence on the risk of CAD [7-9]. However, the underlying mechanisms that mediate these effects remain poorly elucidated.

LPL is a glycoprotein containing 448 amino acids, which is synthesized and secreted by various tissues, such as adipose tissue, myocardium, and skeletal muscle [10]. As an important component in TRL metabolism, LPL binds to the capillary endothelium and primarily hydrolyzes TGs in circulating TRLs, chylomicrons (CM), and very-low-density lipoproteins (VLDL), providing fatty acids for the energy requirements of the heart and skeletal muscle and for storage [5,10].

The *LPL* gene maps to chromosome 8p22, and over 100 various mutations have been identified [11,12]. Several genetic variants in the *LPL* gene have been reported to be associated with CAD susceptibility [13-15]. However, the results were conflicted, and no general agreements existed between them. For example, the D9N (rs1801177, G to A mutations) and N291S (rs268, A to G mutations) polymorphisms, which both result in partial defects in LPL catalytic function, are reported to be associated with an increased risk of CAD [16-18]. Similarly, the HindIII (rs320, T to G mutations) and PvuII (rs285, C to T mutations) variant sites (located on introns 8 and 6 respectively), which are related to profound alterations in plasma lipids, also seemed to be associated with CAD [9,14]. However, other studies did not confirm these results [19-21]. Meanwhile, several gain-of-function *LPL* variants, such as the S447X (rs328) polymorphism, which lead to the transition of Serine (S) to a stop codon (X) at codon position 447, result in reduced TG levels and an overall favorable lipid profile [5]. In addition, certain studies demonstrated that carriers of the X447 allele are protected against CAD [22-24], while other studies drew the opposite conclusion [13,25].

To confirm the correlation existed between the *LPL* gene polymorphisms (HindIII, S447X, N291S, D9N, and PvuII) and CAD, we performed this meta-analysis by pooling all eligible studies to calculate the estimate of overall CAD risk.

Methods and materials

Literature search strategy

We performed the present study according to the MOOSE (Meta-analysis of Observational Studies in Epidemiology) guidelines for meta-analysis of observational studies (Supplementary Table S1) [26]. The literature search was performed by two authors (W.-Q.M. and Y.Z.). PubMed, Web of Science, EMBASE, and the Cochrane Library were systematically searched, and the time period for references searching was from the first available article to September 2017. The following search terms were applied: ("lipoprotein lipase" or "LPL" or "N291S" or "S477X" or "D9N" or "HindIII" or "PvuII") and ("genetic polymorphisms" or "mutation" or "variant" or "polymorphism") and ("coronary artery disease" or "coronary heart disease" or "atherosclerosis" or "acute coronary syndrome" or "angina" or "myocardial infarction"). Handsearching was also carried out to find potential relevant records.

Inclusion and exclusion criteria

The following criteria were applied for reference selection: (1) studies on the evaluation of the *LPL* gene polymorphisms (HindIII, S477X, D9N, N291S, and PvuII) and CAD susceptibility; (2) total CAD cases were documented by angiographic evidence of at least 50% stenosis of one major coronary vessel, myocardial infarction, angina, a history of prior angioplasty, or coronary artery bypass surgery; (3) the data in the reference were sufficient for the present estimation, such as the total number of cases and controls, distribution of genotypes or other relevant information; and (4) the language was limited to English. Studies were excluded if they met any of the following criteria: (1) non-English record; (2) abstracts, letters to the editor, reviews, case-only studies, meta-analysis, and animal studies; and (3) study with useless or insufficient data and multiple publications that reported the same or overlapping population information.

Data extraction

Data abstraction was independently performed by two investigators (W.-Q.M. and X.-Q.H.), and disagreements about study selection were discussed and resolved by a third investigator (N.-F.L.). The following information was extracted from each included article: author, publication date, ethnicity, total number of cases and controls, country, sources of controls, genotyping methods, genotype frequency in cases and controls, and Hardy–Weinberg equilibrium (HWE) in the controls.

Table 1 Summary of odds ratios (95% CI) in the analysis of the association between the *LPL* HindIII polymorphism and CAD susceptibility

Genetic model	Overall and subgroups	*N*	Test of association			Test of heterogeneity	
			OR	95% CI	*P*-value	$P_{Heterogeneity}$	I^2 (%)
GG + GT vs. TT	Overall	18	0.85	0.75,0.97	0.010	0.005	52%
	Asians	7	0.86	0.70,1.07	0.190	0.050	52%
	Caucasians	9	0.81	0.69,0.96	0.010	0.040	51%
	Large sample	6	0.94	0.85,1.05	0.300	0.320	15%
	Small sample	12	0.76	0.62,0.94	0.010	0.020	53%
GG vs. TT + TG	Overall	18	0.62	0.47,0.83	0.001	0.000	67%
	Asians	7	0.67	0.43,1.06	0.090	0.004	69%
	Caucasians	9	0.58	0.38,0.88	0.010	0.000	71%
	Large sample	6	0.82	0.60,1.12	0.220	0.030	60%
	Small sample	12	0.50	0.34,0.75	0.000	0.006	58%
G vs. T	Overall	18	0.81	0.71,0.92	0.001	0.000	72%
	Asians	7	0.82	0.65,1.05	0.110	0.000	77%
	Caucasians	9	0.78	0.66,0.92	0.003	0.001	69%
	Large sample	6	0.94	0.85,1.04	0.200	0.180	35%
	Small sample	12	0.73	0.59,0.89	0.002	0.000	70%

Abbreviations: CAD, coronary artery disease; CI, confidence interval; LPL, lipoprotein lipase; *N*, number of studies; OR, odds ratio; $P_{\text{-Value}}$, *P* value for association; $P_{\text{Heterogeneity}}$, *P* value for heterogeneity.

Quality assessment

The Newcastle–Ottawa scale (NOS) was applied in the quality assessment [27]. The validated quality assessment instrument was composed of the following three parameters of quality: selection, comparability, and exposure assessment. NOS scores ranged from zero to nine. Studies with an NOS score of five or greater were considered moderate to high quality studies, whereas those with an NOS score of less than five were considered low quality.

Statistics analysis

Pooled odds ratios (ORs) and 95% confidence intervals (CIs) were applied to estimate the strength of association between the *LPL* gene polymorphisms and CAD susceptibility. The dominant, recessive, and allele genetic models were applied to assess the correlation between the *LPL* HindIII, PvuII, and S477X gene polymorphisms and CAD risk. Only the dominant genetic model was applied for N291S and D9N, due to the low number of minor homozygotes. The Cochrane *Q*-test and index (I^2) were calculated to evaluate the heterogeneity within studies. *P*-value < 0.1 in the *Q*-test or $I^2 > 50\%$ indicated significant heterogeneity. According to the strength of heterogeneity among studies, the fixed- or random-effects model was applied to calculate the OR and the corresponding 95% CI. The *Z*-test was used to determine the significance of overall ORs. Subgroup analyses, which were based on ethnicity (Asians and Caucasians) and sample size (studies with more than 500 subjects were categorized as "large," and studies with less 500 subjects were categorized as "small"), were applied to detect sources of heterogeneity. In addition, the influence of sample sizes on the overall risk estimation was assessed by a cumulative meta-analysis [28]. A sensitivity analysis was performed to assess the stability of the individual studies. Possible publication bias was assessed using funnel plots and the Egger linear regression test. All calculations were performed and graphs were made with Review Manager v5.2 (The Cochrane Collaboration, Oxford, U.K.) and Stata 12.0 (Stata Corporation, College Station, Texas, U.S.A.).

Results

Selection and characteristics of studies

A total of 958 articles were acquired after initial searching. Among them, 712 duplicate articles were excluded, and 160 articles were excluded for ineligibility after screening the titles and abstracts. In addition, 41 articles were excluded because of insufficient data, reviews, meta-analyses, or conference abstracts. Finally, 45 articles containing 80 eligible studies were included in this meta-analysis [7-9,13-25,29-57]. The flow chart of the retrieved and excluded studies with specifications of reasons is summarized in Figure 1.

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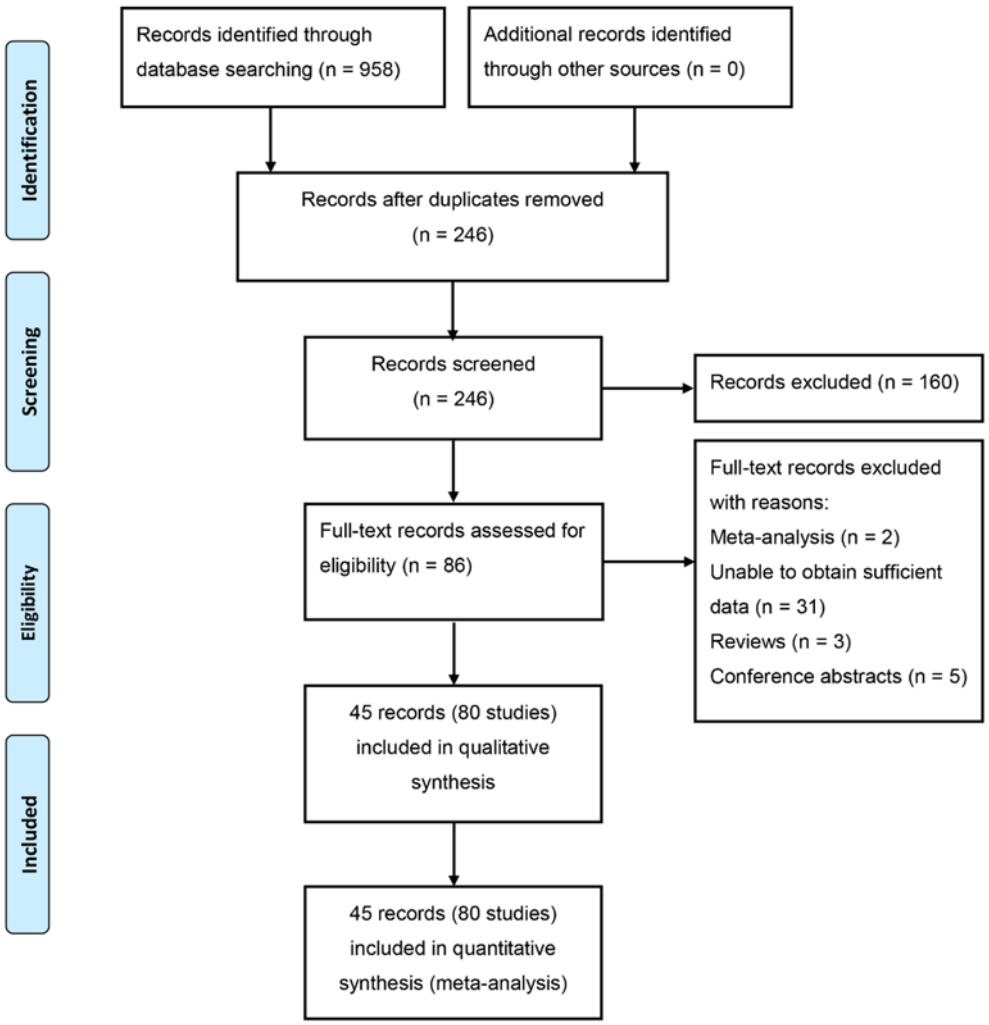

Figure 1. Flow diagram of the study selection process

The characteristics of the studies included in the meta-analysis are shown in Supplementary Table S2. Among 80 eligible studies, 18 studies, containing 5532 cases and 4813 controls, correlated the *LPL* HindIII polymorphism with susceptibility to CAD. Twenty-seven studies, involving 6959 cases and 9400 controls, focused on the relationship between the *LPL* S447X polymorphism and susceptibility to CAD. Eleven studies, including 9272 cases and 15,074 controls, focused on the relationship between the *LPL* N291S polymorphism and susceptibility to CAD. Eight studies, involving 2583 cases and 2525 controls, focused on the relationship between the *LPL* D9N polymorphism and susceptibility to CAD, and the remaining 16 studies, involving 7831 cases and 5966 controls, concerned the *LPL* PvuII polymorphism. The countries in which these studies occurred included the U.S.A., U.K., France, Brazil, China, Finland, and others. HWE had been applied for all polymorphisms in the controls. The quality of these enrolled studies was evaluated using the NOS quality scale (Supplementary Table S3).

Association between the *LPL* HindIII polymorphism and susceptibility to CAD

In all study subjects, the results indicated a reduced risk of CAD susceptibility associated with the *LPL* HindIII polymorphism in all tested genetic models (GG + GT vs. TT: OR = 0.85, 95% CI = 0.75–0.97; GG vs. TT + TG: OR = 0.62, 95% CI = 0.47–0.83; G vs. T: OR = 0.81, 95% CI = 0.71–0.92) with some evidence of interstudy heterogeneity (Table 1; Figure 2). Stratification analysis by ethnicity and sample size indicated a significant association between the HindIII polymorphism and CAD susceptibility in Caucasians and small sample size under all tested models (Table 1; Supplementary Figures S1 and S2).

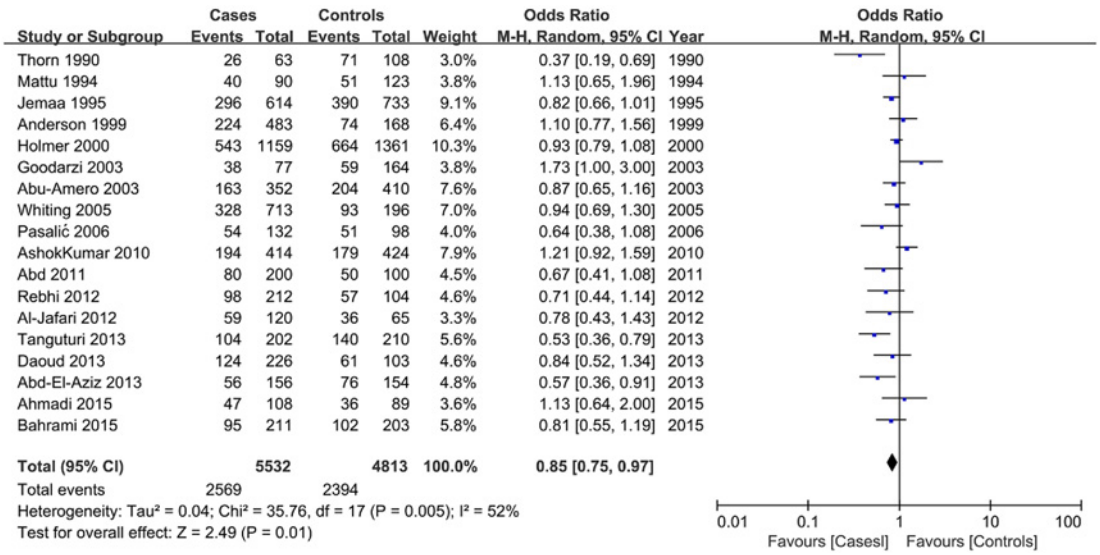

Figure 2. Forest plot of odds ratios for the association between the *LPL* HindIII polymorphism and CAD risk under dominant genetic model (GG + GT vs. TT)

Table 2 Summary of odds ratios (95% CI) in the analysis of the association between the *LPL* S447X polymorphism and CAD susceptibility

Genetic model	Overall and subgroups	*N*	Test of association			Test of heterogeneity	
			OR	95% CI	*P*-value	$P_{Heterogeneity}$	I^2 (%)
GG + GC vs. CC	Overall	27	0.87	0.73,1.03	0.100	0.000	68%
	Asians	8	1.08	0.71,1.65	0.730	0.000	84%
	Caucasians	16	0.77	0.64,0.93	0.008	0.007	52%
	Large sample	9	0.87	0.75,1.00	0.050	0.080	44%
	Small sample	18	0.87	0.62,1.22	0.430	0.000	74%
GG vs. GC + CC	Overall	19	1.00	0.60,1.68	1.000	0.040	40%
	Asians	6	0.90	0.30,2.69	0.850	0.002	74%
	Caucasians	11	0.78	0.45,1.35	0.370	0.730	0%
	Large sample	6	0.55	0.35,0.86	0.009	0.950	0%
	Small sample	13	1.59	0.81,3.11	0.180	0.200	24%
G vs. C	Overall	21	0.94	0.77,1.15	0.540	0.000	76%
	Asians	8	1.11	0.72,1.72	0.630	0.000	88%
	Caucasians	11	0.83	0.72,0.94	0.005	0.020	53%
	Large sample	6	0.87	0.74,1.02	0.080	0.080	50%
	Small sample	15	0.97	0.67,1.41	0.880	0.000	80%

Abbreviations: CAD, coronary artery disease; CI, confidence interval; LPL, lipoprotein lipase; *N*, number of studies; OR, odds ratio; $P_{\text{-Value}}$, *P* value for association; $P_{Heterogeneity}$, *P* value for heterogeneity.

Association between the *LPL* S477X polymorphism and susceptibility to CAD

No significant association was observed in any genetic model between the S477X polymorphism and CAD risk in the overall meta-analysis, and there was some evidence of interstudy heterogeneity (Table 2; Figure 3). The subgroup analysis stratified by ethnicity indicated that the S477X polymorphism was significantly associated with CAD risk for Caucasians, but not Asians, under the dominant and allele genetic models (GG + GC vs. CC: OR = 0.77, 95% CI = 0.64–0.93; GG vs. GC + CC: OR = 0.83, 95% CI = 0.72–0.94), with a reduction in interstudy heterogeneity (Table 2; Supplementary Figure S3). Stratification by sample size indicated that large sample size, but not small sample size, showed a reduced risk of CAD susceptibility associated with the S447X polymorphism under the recessive genetic model (GG vs. GC + CC: OR = 0.55, 95% CI = 0.35–0.86) (Table 2; Supplementary Figure S4).

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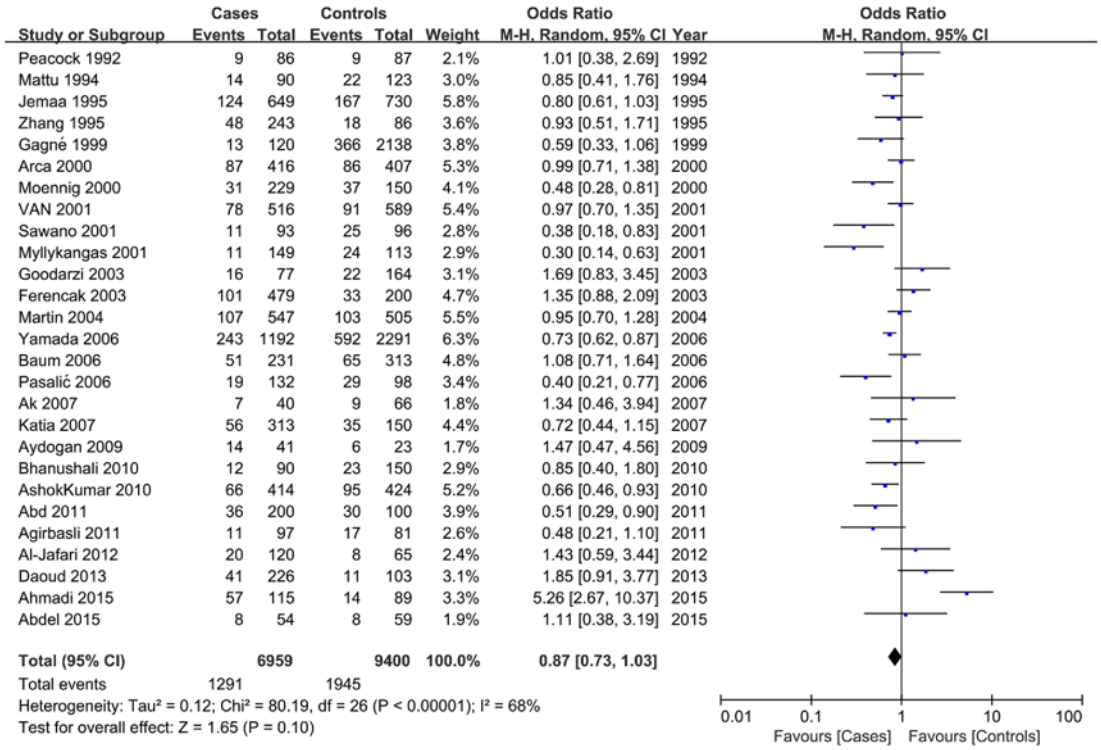

Figure 3. Forest plot of odds ratios for the association between the *LPL* S447X polymorphism and CAD risk under the dominant genetic model (GG + GC vs. CC)

Table 3 Summary of odds ratios (95% CI) in the analysis of the association between the *LPL* N291S and D9N polymorphisms and CAD susceptibility

Genetic model	Overall and subgroups	*N*	Test of association			Test of heterogeneity	
			OR	95% CI	*P*-value	$P_{Heterogeneity}$	I^2 (%)
N291S							
GG + GA vs. AA	Overall	11	1.11	0.97,1.28	0.130	0.420	3%
	Asians	1	1.54	0.89,2.68	0.120	N/A	N/A
	Caucasians	8	1.10	0.95,1.27	0.210	0.300	16%
	Large sample	7	1.13	0.98,1.30	0.100	0.320	15%
	Small sample	4	0.96	0.57,1.60	0.860	0.430	0%
D9N							
AA + GA vs. GG	Overall	8	1.46	1.14,1.87	0.002	0.360	9%
	Asians	1	0.41	0.05,3.73	0.430	N/A	N/A
	Caucasians	4	1.47	1.00,2.14	0.050	0.200	36%
	Large sample	4	1.49	1.03,2.15	0.040	0.180	38%
	Small sample	4	0.94	0.42,2.10	0.890	0.670	0%

Abbreviations: CAD, coronary artery disease; CI, confidence interval; LPL, lipoprotein lipase; *N*, number of studies; N/A, not applicable; OR, odds ratio; $P_{\text{-Value}}$, *P* value for association; $P_{Heterogeneity}$, *P* value for heterogeneity.

Association between the *LPL* N291S and D9N gene polymorphisms and susceptibility to CAD

Because of the low number of minor homozygotes, only the dominant genetic model was applied to the N291S and D9N polymorphisms. An increased risk of CAD susceptibility was associated with the D9N polymorphism under the dominant genetic model (AA + GA vs. GG: OR = 1.46, 95% CI = 1.14–1.87) with low interstudy heterogeneity (Table 3; Figure 4). No significant association was observed between the N291S polymorphism and CAD risk (Table

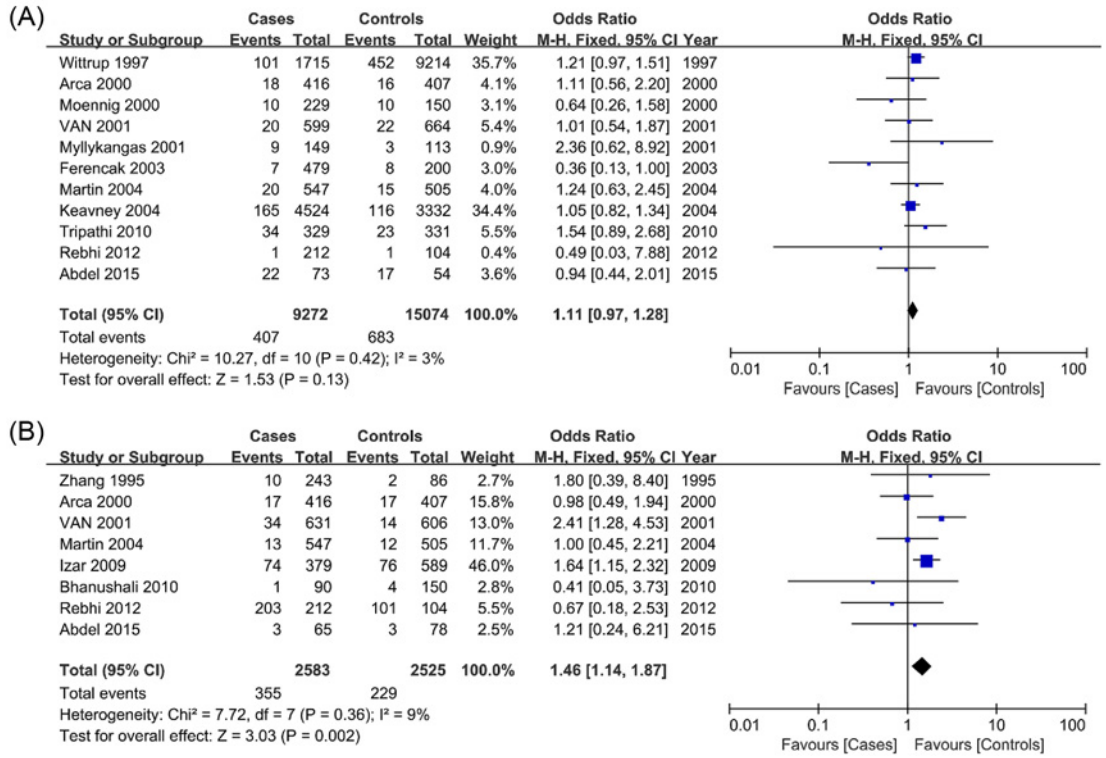

Figure 4. Forest plot of odds ratios for the association of polymorphisms in *LPL* N291S and D9N and susceptibility to CAD

(**A**) The N291S polymorphism under the dominant genetic model (GG + GA vs. AA). (**B**) The D9N polymorphism under the dominant genetic model (AA+GA vs. GG).

3; Figure 4). When we conducted subgroup analyses by ethnicity and sample size, the same significant association was observed in large sample size of the D9N polymorphism (Table 3; Supplementary Figure S5). However, no significant association was observed between CAD risk and the N291S polymorphism in the subgroup analysis (Table 3; Supplementary Figure S6).

Association between the *LPL* PvuII polymorphism and susceptibility to CAD

No significant associations were observed between the *LPL* PvuII polymorphism and CAD susceptibility in any genetic model (Table 4; Figure 5). This was also the case in the subgroup analysis (Table 4; Supplementary Figures S7 and S8).

Cumulative analysis

For the *LPL* HindIII and D9N polymorphisms, the cumulative meta-analysis showed that as publication year increased, the CI became increasingly narrower, and statistical significance was more common. The association between the *LPL* S447X polymorphism and CAD risk appeared to fluctuate with the number of studies accumulated. For the *LPL* N291S and PvuII polymorphisms, no significant association was observed with the number of studies accumulated (Figure 6).

Heterogeneity and sensitivity analysis

The heterogeneity within each study in each comparison is shown in Tables 1–4. The influence of each study on the overall meta-analysis was evaluated by deleting one study at a time. The results indicated that no individual study influenced the pooled OR significantly (Supplementary Figure S9).

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Table 4 Summary of odds ratios (95% CI) in the analysis of the association between the *LPL* PvuII polymorphism and CAD susceptibility

Genetic model	Overall and subgroups	*N*	Test of association			Test of heterogeneity	
			OR	95% CI	*P*-value	$P_{Heterogeneity}$	I^2 (%)
TT + CT vs. CC	Overall	16	1.00	0.92,1.08	0.920	0.450	0%
	Asians	4	1.09	0.90,1.33	0.360	0.660	0%
	Caucasians	11	0.99	0.91,1.08	0.810	0.480	0%
	Large sample	4	1.03	0.87,1.23	0.700	0.080	55%
	Small sample	12	0.92	0.78,1.09	0.320	0.790	0%
TT vs. CC + CT	Overall	16	0.90	0.78,1.04	0.150	0.100	32%
	Asians	4	0.95	0.74,1.22	0.670	0.670	0%
	Caucasians	11	0.85	0.69,1.05	0.120	0.030	51%
	Large sample	4	0.98	0.82,1.16	0.780	0.140	45%
	Small sample	12	0.82	0.68,1.00	0.040	0.290	16%
T vs. C	Overall	16	0.99	0.94,1.04	0.670	0.200	22%
	Asians	4	1.03	0.90,1.17	0.680	0.490	0%
	Caucasians	11	0.94	0.84,1.04	0.210	0.110	37%
	Large sample	4	1.01	0.89,1.14	0.900	0.040	65%
	Small sample	12	0.90	0.81,1.01	0.060	0.780	0%

Abbreviations: CAD, coronary artery disease; CI, confidence interval; LPL, lipoprotein lipase; *N*, number of studies; OR, odds ratio; $P_{\text{-Value}}$, *P* value for association; $P_{\text{Heterogeneity}}$, *P* value for heterogeneity.

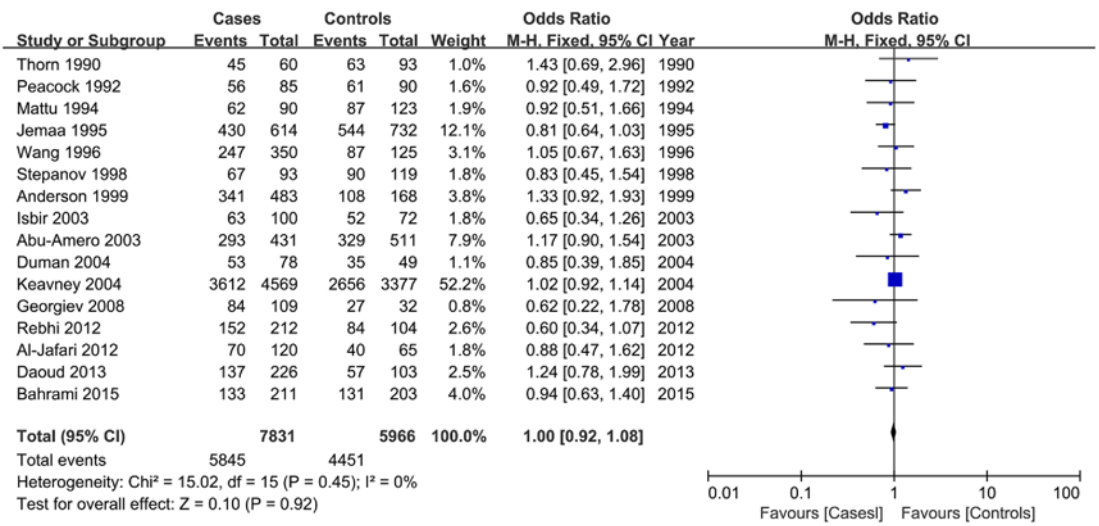

Figure 5. Forest plot of odds ratios for the association between the *LPL* PvuII polymorphism and CAD risk under the dominant model (TT + CT vs. CC)

Publication bias

The Funnel plot and Egger's regression test were applied to assess the publication bias of the included studies. The results indicated that the distribution of the included studies on the funnel plot appeared roughly symmetrical (Figure 7). The results of Egger's regression test are also presented under the dominant models (HindIII: $t = -1.23$, P=0.237; S477X: $t = -3.12$, P=0.005; N291S: $t = -0.96$, P=0.363; D9N: $t = -1.62$, P=0.157; PvuII: $t = -1.05$, P=0.311) (Supplementary Figure S10).

Discussion

Genetic variations in the *LPL* gene could influence lipid transport and metabolism and could consequently modulate an individual's susceptibility to atherosclerosis. However, it is difficult to draw a definite conclusion for whether LPL is a proatherosclerotic or antiatherosclerotic factor, since the effects of LPL partly depend on its locations and

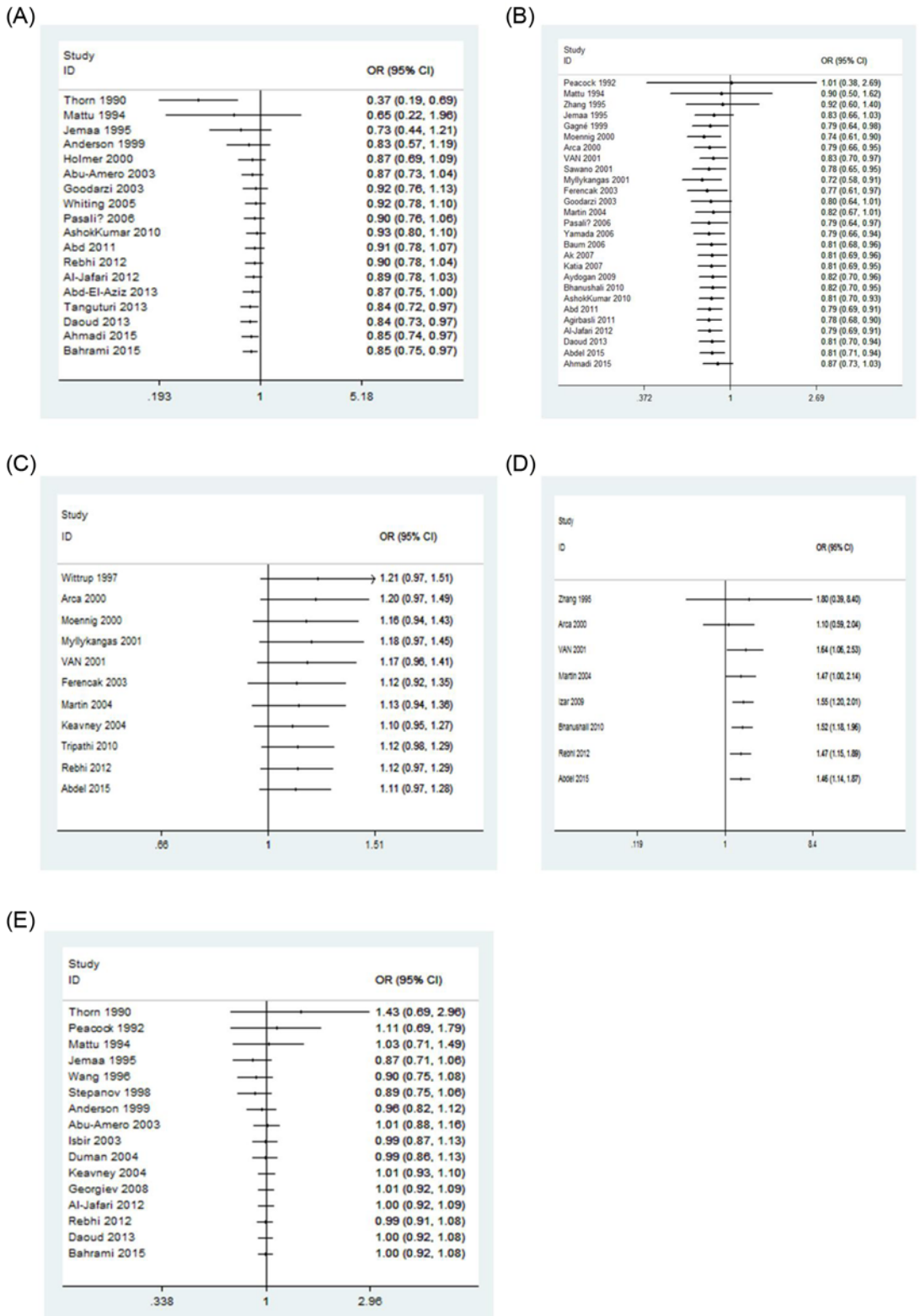

Figure 6. Forest plots of the cumulative odds ratio for the association between the *LPL* gene polymorphisms and CAD risk under the dominant genetic model

(**A**) HindIII polymorphism; (**B**) S447X polymorphism; (**C**) N291S polymorphism; (**D**) D9N polymorphism; (**E**) PvuII polymorphism.

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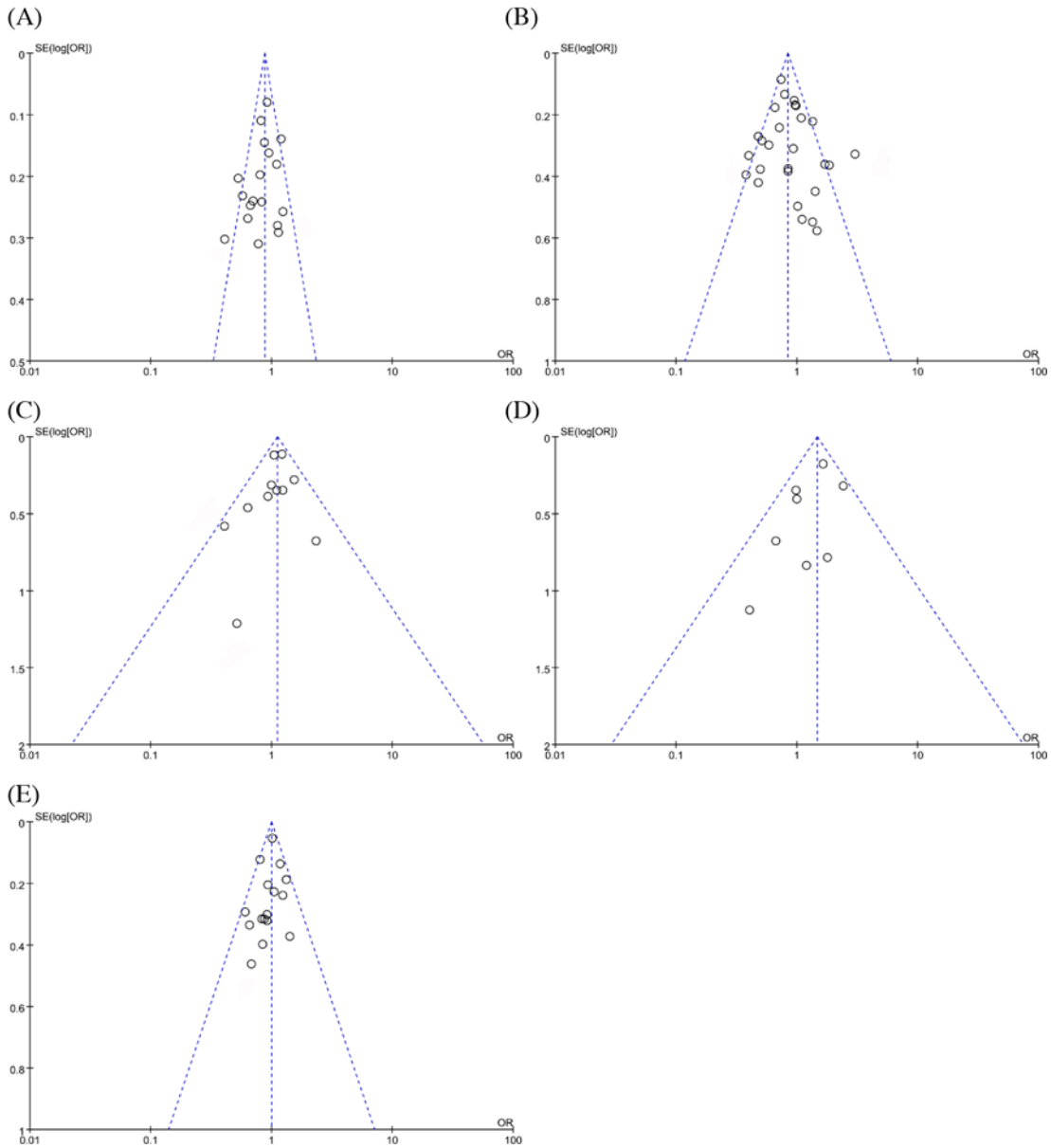

Figure 7. Funnel plot of publication bias for the association between *LPL* gene polymorphisms and susceptibility to CAD under dominant models

(**A**) Hind polymorphism; (**B**) S447X polymorphism; (**C**) N291S polymorphism; (**D**) D9N polymorphism; (**E**) PvuII polymorphism.

activity [58]. The enzyme, when expressed in adipose tissue, heart, and skeletal muscle, has been regarded as an antiatherosclerotic factor by reducing atherogenic lipoproteins or increasing HDL, whereas the effect of LPL on the biology of arterial wall seems to be atherogenic by accelerating lipid accumulation [58,59]. Several lines of evidence also suggest that LPL activity is higher in atherosclerotic arteries compared with normal arteries [10,60]. Increased plasma LPL activity could alter lipid traits, such as decreasing TG and increasing HDL levels, generating a profile associated with protection against atherosclerosis, while the down-regulation of *LPL* gene expression has been shown to play an opposite role [61,62].

Although numerous studies have investigated the correlation between LPL and CAD risk in the past several decades, no definite conclusions have been reached regarding gene polymorphisms. This meta-analysis has combined and reanalyzed individual participant data from 80 eligible studies of the effect of *LPL* gene polymorphisms on CAD incidence. In our study, all of the results revealed that three *LPL* gene variants (Hind III, S447X, and D9N)

were associated with CAD susceptibility. When Asians or Caucasians were analyzed independently, the heterogeneity of the population tended to be weaker, and the subgroup analysis indicated that S447X polymorphism decreased CAD risk in Caucasians. On the other hand, the stratified analysis by ethnicity for the S447X polymorphism was successfully applied to relieve the heterogeneity bias in the polymorphism analysis within Caucasians, suggesting that ethnicity may potentially be the source of the heterogeneity. In addition, it is worth noting that the *P* value of the Egger's regression test for the S447X polymorphism was less than 0.05, which indicated that publication bias likely existed; however, the funnel plot appeared roughly symmetrical, and the sensitivity analysis indicated the stability of the results. Consequently, future studies are warranted to validate our conclusion.

Although several relevant meta-analyses have been published, our study had certain specific advantages [63-65]. Compared with other studies, we incorporated more eligible articles, conducted quality assessment, and performed a comprehensive analysis, whereas previous studies primarily focused on the plasma levels of lipids and lipoproteins, or they only analyzed a single gene variant in the meta-analysis. Furthermore, in the present study, a cumulative meta-analysis was performed to assess the pattern of the evidence accumulated over time.

Several limitations in our study should also be addressed. First, some genetic models displayed high heterogeneity, although subgroup analysis was performed to detect the sources of this heterogeneity. Second, the ethnic distribution of included studies was primarily Asians and Caucasians. Racial bias may exist, and the conclusions may not be applicable to other races. Third, we searched and collected articles in English from four comprehensive electronic databases, including PubMed, Web of Science, EMBASE, and Cochrane database. Several publications related to this topic written in other languages might have been ignored. Thus, publication bias likely existed. However, the articles included in these four databases are more authoritative and more convenient for readers compared with the original literature.

In summary, this updated meta-analysis suggested that the *LPL* D9N polymorphism was associated with the increased risk of CAD, whereas the *LPL* HindIII and S447X polymorphisms showed protective effects against CAD. No associations were observed between the *LPL* N291S and PvuII polymorphisms and susceptibility to CAD.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Author Contribution

W.-Q.M. and N.-F.L. conceived and designed the study. X.-Q.H., Y.W., and N.-F.L. performed in data collection and management. Y.Z. performed in data analysis. W.-Q.M. and N.-F.L. wrote the paper. All the authors reviewed the manuscript.

Abbreviations

CAD, coronary artery disease; CM, chylomicrons; CI, confidence interval; GWAS, genome-wide association studies; HDL-C, high-density lipoprotein cholesterol; HWE, Hardy–Weinberg equilibrium; LDL-C, low-density lipoprotein cholesterol; LPL, lipoprotein lipase; NOS, Newcastle–Ottawa scale; OR, odds ratio; TC, cholesterol; TG, triglyceride; TRL, triglyceride-rich lipoprotein; VLDL, very-low-density lipoprotein.

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Associations between *LPL* gene polymorphisms and coronary artery disease: evidence based on an updated and cumulative meta-analysis

Short title: *LPL* gene polymorphisms and coronary artery disease

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Supplementary Information

Supplementary Table 1. MOOSE checklist for meta-analysis of observational studies.

Supplementary Table 2. Characteristics of the individual studies included in the meta-analysis.

Supplementary Table 3. Methodological quality of the selected studies according to the Newcastle-Ottawa Scale.

Supplementary Figure 1. Stratified analysis based on ethnicity for the association between the *LPL* HindIII polymorphism and CAD risk using dominant genetic model (GG+GT vs. TT).

Supplementary Figure 2. Stratified analysis based on sample size for the association between the *LPL* HindIII polymorphism and CAD risk using dominant genetic model (GG+GT vs. TT).

Supplementary Figure 3. Stratified analysis based on ethnicity for the association between the *LPL* S447X polymorphism and CAD risk using dominant genetic model (GG+GC vs. CC).

Supplementary Figure 4. Stratified analysis based on sample size for the association between the *LPL* S447X polymorphism and CAD risk using dominant genetic model (GG+GC vs. CC).

Supplementary Figure 5. Stratified analysis based on sample size for the association between the *LPL* D9N polymorphism and CAD risk using dominant genetic model (AA+GA vs. GG).

Supplementary Figure 6. Stratified analysis based on sample size for the association between the *LPL* N291S polymorphism and CAD risk using dominant genetic model (GG+GA vs. AA).

Supplementary Figure 7. Stratified analysis based on ethnicity for the association between the *LPL* PvuII polymorphism and CAD risk using dominant genetic model (TT+CT vs. CC).

Supplementary Figure 8. Stratified analysis based on sample size for the association between the *LPL* PvuII polymorphism and CAD risk using dominant genetic model (TT+CT vs. CC).

Supplementary Figure 9. Egger's regression test of publication bias for the association between the *LPL* gene polymorphisms and susceptibility to CAD. (a). HindIII polymorphism; (b). S447X polymorphism; (c). N291S polymorphism; (d). D9N polymorphism; (e). PvuII polymorphism.

Supplementary Figure 10. Sensitivity analysis on the correlation between the *LPL* gene polymorphisms and susceptibility to CAD. (a). sensitivity analysis for HindIII and CAD risk; (b). sensitivity analysis for S447X and CAD risk; (c). sensitivity analysis for N291S and CAD risk; (d). sensitivity analysis for D9N and CAD risk; (e). sensitivity analysis for PvuII and CAD risk;

Supplementary Table 1. MOOSE checklist for meta-analysis of observational studies.

Item No	Recommendation	Reported on Page No
Reporting of background should include		
1	Problem definition	2-3
2	Hypothesis statement	2-3
3	Description of study outcome(s)	2-3
4	Type of exposure or intervention used	2-3
5	Type of study designs used	2-3
6	Study population	2-3
Reporting of search strategy should include		
7	Qualifications of searchers (eg, librarians and investigators)	4
8	Search strategy, including time period included in the synthesis and key words	4
9	Effort to include all available studies, including contact with authors	4
10	Databases and registries searched	4
11	Search software used, name and version, including special features used (eg, explosion)	5
12	Use of hand searching (eg, reference lists of obtained articles)	4
13	List of citations located and those excluded, including justification	4-5
14	Method of addressing articles published in languages other than English	4
15	Method of handling abstracts and unpublished studies	5
16	Description of any contact with authors	No
Reporting of methods should include		
17	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	4-5
18	Rationale for the selection and coding of data (eg, sound clinical principles or convenience)	4-5
19	Documentation of how data were classified and coded (eg, multiple raters, blinding and interrater reliability)	4-6
20	Assessment of confounding (eg, comparability of cases and controls in studies where appropriate)	4-6
21	Assessment of study quality, including blinding of quality assessors, stratification or regression on possible predictors of study results	4-6
22	Assessment of heterogeneity	5-6
23	Description of statistical methods (eg, complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated	5-6
24	Provision of appropriate tables and graphics	5-6
Reporting of results should include		
25	Graphic summarizing individual study estimates and overall estimate	6-9
26	Table giving descriptive information for each study included	Table1
27	Results of sensitivity testing (eg, subgroup analysis)	6-14
28	Indication of statistical uncertainty of findings	6-9
Reporting of discussion should include		
29	Quantitative assessment of bias (eg, publication bias)	10-11
30	Justification for exclusion (eg, exclusion of non-English language citations)	10-11
31	Assessment of quality of included studies	14-16
Reporting of conclusions should include		
32	Consideration of alternative explanations for observed results	10-11
33	Generalization of the conclusions (ie, appropriate for the data presented and within the domain of the literature review)	10-11
34	Guidelines for future research	11
35	Disclosure of funding source	11

Supplementary Table 2. Characteristics of the individual studies included in the meta-analysis.

First Author /Year	Country	Disease	Source of Controls	Gene-typing Methods	Sample Size	Genotype Distribution		P_{HWE}	NOS
					Cases/Controls	Cases	Controls		
Hind III						**TT/TG/GG**	**TT/TG/GG**		
Thorn 1990	UK	CAD	PB	PCR	63/108	37/23/3	37/51/20	0.743	7
Mattu 1994	Welsh	CAD	PB	PCR-RFLP	90/123	50/34/6	72/45/6	0.760	8
Jemaa 1995	France	CAD	PB	PCR	614/733	318/258/38	343/314/76	0.742	7
Anderson 1999	America	CAD	HB	PCR	483/168	259/194/30	94/52/22	>0.05	7
Holmer 2000	Germany	MI	PB	PCR	1159/1361	616/456/87	697/564/100	0.332	8
Abu-Amero 2003	Saudi Arabia	CAD	HB	PCR	352/410	189/138/25	206/173/31	0.518	6
Goodarzi 2003	USA	CAD	PB	PCR	77/164	39/33/5	105/52/7	0.861	7
Whiting 2005	America	CAD	HB	PCR	713/196	385/269/59	103/77/16	0.763	7
Pasalić 2006	Croatia	CAD	HB	PCR-RFLP	132/98	78/46/8	47/45/6	0.262	7
AshokKumar 2010	India	CAD	HB	PCR	414/424	220/168/26	245/158/21	0.486	7
Abd 2011	Egypt	MI	HB	PCR	200/100	120/70/10	50/36/14	0.834	7
Al-Jafari 2012	Saudi Arabia	CAD	HB	PCR	120/65	61/53/6	29/23/13	0.050	7
Rebhi 2012	Tunisia	CAD	HB	PCR–RFLP	212/104	114/83/15	47/39/18	0.569	7
Abd-El-Aziz 2013	Egypt	CAD	HB	PCR-RFLP	156/154	100/53/3	78/54/22	>0.05	7
Tanguturi 2013	India	MI	PB	PCR	202/210	98/72/32	70/68/72	>0.05	7
Daoud 2013	Saudi Arabia	CAD	HB	PCR-RFLP	226/103	102/81/43	42/35/26	>0.05	7
Ahmadi 2015	Iran	CAD	HB	PCR-RFLP	108/89	61/41/6	53/33/3	0.430	6
Bahrami 2015	Iran	MI	HB	PCR-RFLP	211/203	116/81/14	101/83/19	0.745	7
S477X						**CC/CG/GG**	**CC/CG/GG**		
Peacock 1992	Sweden	CAD	HB	PCR	86/87	77/9 [a]	78/9 [a]	>0.05	7
Mattu 1994	Welsh	CAD	PB	PCR-RFLP	90/123	76/14/0	101/21/1	0.936	8
Jemaa 1995	France	CAD	PB	PCR	649/730	525/118/6	563/154/13	0.514	7
Zhang 1995	Germany	CAD	HB	PCR	243/86	195/46/2	68/17/1	0.959	7
Gagné 1999	America	CAD	PB	PCR-RFLP	120/2138	107/13 [a]	1772/366 [a]	>0.05	7
Arca 2000	Italy	CAD	HB	PCR-RFLP	416/407	329/87 [a]	321/86 [a]	>0.05	6
Moennig 2000	Germany	CAD	PB	PCR	229/150	198/28/3	113/37/0	0.085	8
Sawano 2001	Japan	CAD	PB	PCR	93/96	82/10/1	71/23/2	0.932	8
VAN 2001	Australia.	CAD	PB	PCR	516/589	438/78 [a]	498/91 [a]	>0.05	7
Myllykangas 2001	Finland	CAD	HB	PCR	149/113	138/11 [a]	89/24 [a]	>0.05	7
Ferencak 2003	Croatia	CAD	HB	PCR	479/200	378/97/4	167/32/1	0.686	7

Goodarzi 2003	USA	CAD	PB	PCR	77/164	61/15/1	142/22/0	>0.05	7
Martin 2004	UK	MI	HB	PCR-RFLP	547/505	440/104/3	402/99/4	0.483	7
Baum 2006	China	MI	HB	PCR	231/313	180/51/0	248/64/1	0.137	6
Pasalić 2006	Croatia	CAD	HB	PCR-RFLP	132/98	113/19/0	69/28/1	0.312	7
Yamada 2006	Japan	MI	HB	PCR	1192/2291	949/231/12	1699/547/45	0.900	7
Ak 2007	Turkey	CAD	HB	PCR	40/66	33/7/0	57/8/1	0.275	6
Katia 2007	Brazil	CAD	PB	PCR-RFLP	313/150	257/47/9	115/34/1	0.110	7
Aydogan 2009	Turkey	CAD	PB	PCR-RFLP	41/23	27/4/10	17/4/2	0.058	8
AshokKumar 2010	India	CAD	HB	PCR	414/424	348/62/4	329/87/8	0.427	7
Bhanushali 2010	India	CAD	HB	PCR	90/150	78/11/1	127/21/2	0.306	7
Abd 2011	Egypt	MI	HB	PCR	200/100	164/32/4	70/26/4	0.431	7
Agirbasli 2011	Turkey	CAD	HB	PCR-RFLP	97/81	86/10/1	64/16/1	1.000	7
Al-Jafari 2012	Saudi Arabia	CAD	HB	PCR	120/65	100/20/0	57/8/0	0.597	7
Daoud 2013	Saudi Arabia	CAD	HB	PCR-RFLP	226/103	185/41/0	92/11/0	0.567	7
Ahmadi 2015	Iran	CAD	HB	PCR-RFLP	115/89	58/23/34	75/7/7	>0.05	6
Abdel 2015	Sudan	CAD	HB	PCR-RFLP	54/59	46/8 [a]	51/8 [a]	>0.05	5
N291S						**AA/AG/GG**	**AA/AG/GG**		
Wittrup 1997	Danish	IHD	PB	PCR	1715/9214	1614/101[b]	8762/452[b]	>0.05	8
Arca 2000	Italy	CAD	HB	PCR-RFLP	416/407	398/18/0	391/16/0	0.686	6
Moennig 2000	Germany	CAD	PB	PCR	229/150	219/10/0	140/10/0	0.673	8
VAN 2001	Australia.	CAD	PB	PCR	599/664	579/20 [b]	642/22 [b]	>0.05	7
Myllykangas 2001	Finland	CAD	HB	PCR	149/113	140/9 [b]	110/3 [b]	>0.05	7
Ferencak 2003	Croatia	CAD	HB	PCR	479/200	472/7/0	192/8/0	0.773	7
Martin 2004	UK	MI	HB	PCR-RFLP	547/505	527/20/0	490/15/0	0.242	7
Keavney 2004	UK	MI	PB	PCR	4524/3332	4359/162/3	3216/112/4	>0.05	7
Tripathi 2010	India	CAD	HB	PCR	329/331	295/34/0	308/23/0	0.513	6
Rebhi 2012	Tunisia	CAD	HB	PCR–RFLP	212/104	211/1/0	103/1/0	>0.05	7
Abdel 2015	Sudan	CAD	HB	PCR-RFLP	73/54	51/22 [b]	37/17 [b]	>0.05	5
D9N						**GG/GA/AA**	**GG/GA/AA**		
Zhang 1995	Germany	CAD	HB	PCR	243/86	233/10/0	84/2/0	0.913	7
Arca 2000	Italy	CAD	HB	PCR-RFLP	416/407	382/17/0	373/17/0	0.660	6
VAN 2001	Australia.	CAD	PB	PCR	631/606	597/34 [c]	592/14 [c]	>0.05	7
Martin 2004	UK	MI	HB	PCR-RFLP	547/505	534/13/0	493/12/0	0.787	7
Izar 2009	Brazil	MI	PB	PCR-RFLP	379/583	305/71/3	507/73/3	0.832	8
Bhanushali 2010	India	CAD	HB	PCR	90/150	89/1/0	146/4/0	0.869	7
Rebhi 2012	Tunisia	CAD	HB	PCR–RFLP	212/104	9/47/156	3/17/84	0.848	7
Abdel 2015	Sudan	CAD	HB	PCR-RFLP	65/78	62/3 [c]	75/3 [c]	>0.05	5
Pvull						**CC/CT/TT**	**CC/CT/TT**		
Thorn 1990	UK	CAD	PB	PCR	60/93	15/39/6	30/43/20	0.534	7

Peacock 1992	Sweden	CAD	HB	PCR	85/90	29/38/18	29/42/19	0.602	7
Mattu 1994	Welsh	CAD	PB	PCR-RFLP	90/123	28/42/20	36/64/23	0.561	8
Jemaa 1995	France	CAD	PB	PCR	614/732	184/302/128	188/357/187	0.506	7
Wang 1996	Australia	CAD	HB	PCR-RFLP	350/125	103/180/67	38/59/28	0.577	7
Stepanov 1998	Russia	CAD	PB	PCR-RFLP	93/119	26/52/15	29/57/33	0.655	7
Anderson 1999	America	CAD	HB	PCR	483/168	142/236/105	60/76/32	0.368	7
Abu-Amero 2003	Saudi Arabia	CAD	HB	PCR	431/511	138/225/68	182/248/81	0.819	6
Isbir 2003	Turkey	CAD	PB	PCR	100/72	37/49/14	20/40/12	0.289	7
Duman 2004	Turkey	CAD	HB	PCR	78/49	25/39/14	14/16/19	0.017	6
Keavney 2004	UK	MI	PB	PCR	4569/3377	957/2266/1346	721/1694/962	0.626	7
Georgiev 2008	Macedon	CAD	HB	PCR-RFLP	109/32	25/58/26	5/20/7	0.149	7
Al-Jafari 2012	Saudi Arabia	CAD	HB	PCR	120/65	50/52/18	25/28/12	0.408	7
Rebhi 2012	Tunisia	CAD	HB	PCR–RFLP	212/104	60/90/62	20/55/29	0.503	7
Daoud 2013	Saudi Arabia	CAD	HB	PCR-RFLP	226/103	89/102/35	46/44/13	0.627	7
Bahrami 2015	Iran	MI	HB	PCR-RFLP	211/203	78/101/32	72/93/38	0.414	7

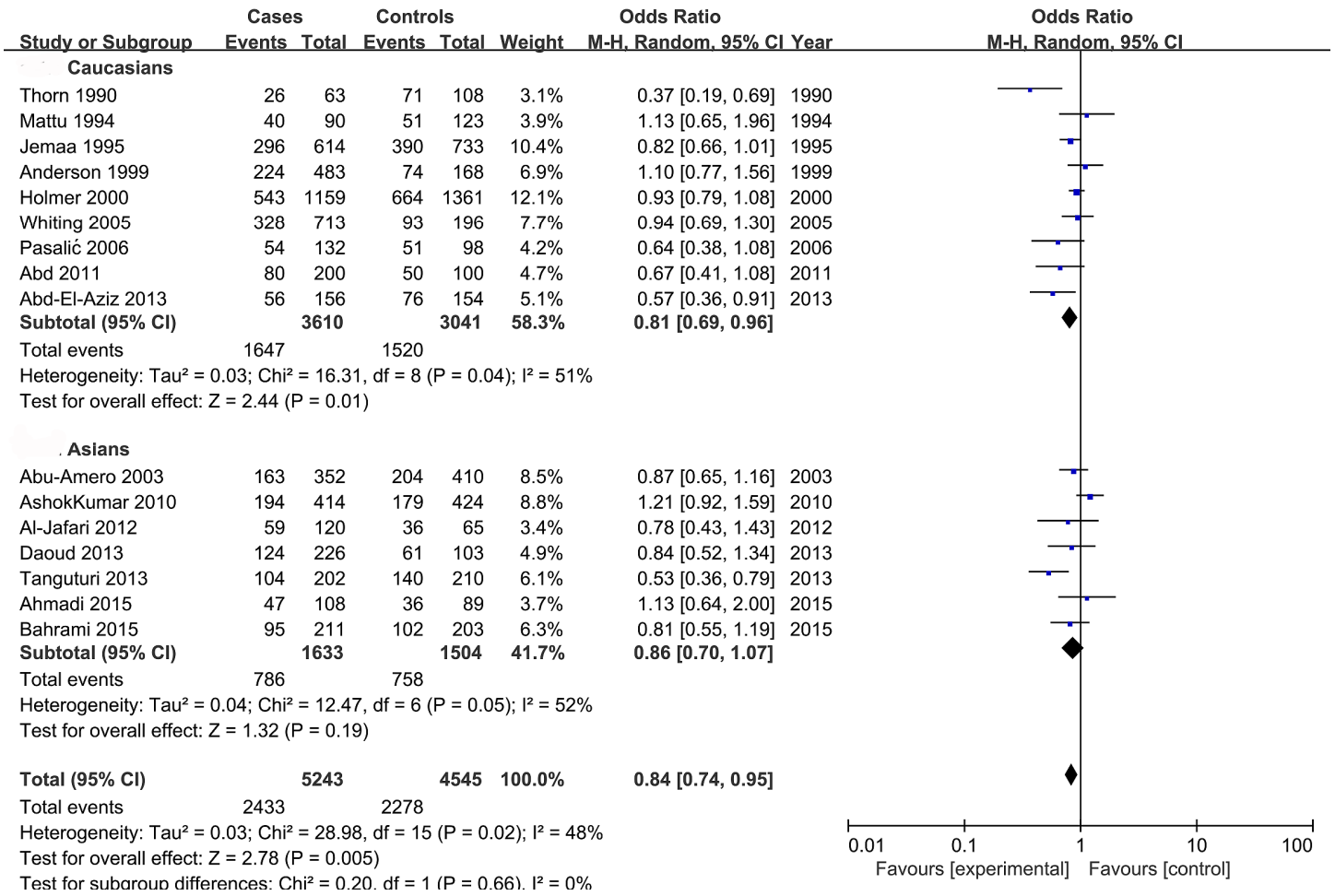
Abbreviations: CAD, coronary artery disease; MI, myocardial infarction; PCR, polymerase chain reaction; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; HWE, Hardy–Weinberg equilibrium for controls; NOS, Newcastle–Ottawa quality scale; IHD, ischemic heart disease; PB, population-based control; HB, hospital-based control. Note: a, CC vs. GC+GG; b, AA vs. AG+GG; c, GG vs. AG+AA.

Supplementary Table 3. Methodological quality of the selected studies according to the Newcastle-Ottawa Scale.

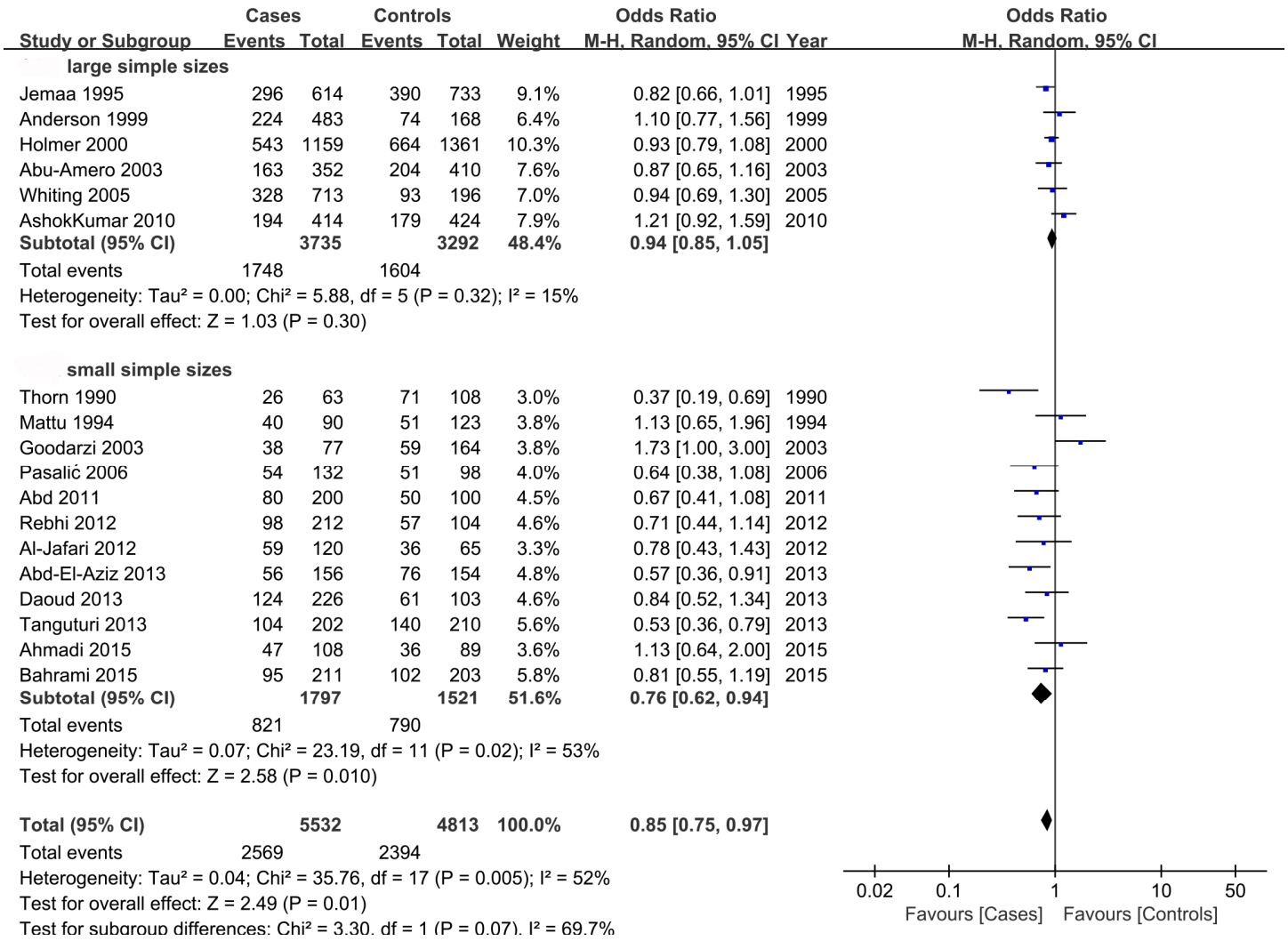
Study	Adequacy of Case Definition	Representativeness of the cases	Selection of controls	Definition of controls	Comparability of cases/controls	Ascertainment of exposure	Same method of ascertainment	Non-Response rate
Thorn 1990	★	★	★	★	★	★	★	N/A
Peacock 1992	★	★	N/A	★	★★	★	★	N/A
Mattu 1994	★	★	★	★	★★	★	★	N/A
Jemaa 1995	★	★	★	★	★	★	★	N/A
Zhang 1995	★	★	N/A	★	★★	★	★	N/A
Wang 1996	★	★	N/A	★	★★	★	★	N/A
Wittrup 1997	★	★	★	★	★★	★	★	N/A
Stepanov 1998	★	★	★	★	★	★	★	N/A
Anderson 1999	★	★	N/A	★	★★	★	★	N/A
Gagné 1999	★	★	★	★	★	★	★	N/A
Holmer 2000	★	★	★	★	★★	★	★	N/A
Arca 2000	★	★	N/A	★	★	★	★	N/A
Moennig 2000	★	★	★	★	★★	★	★	N/A
VAN 2001	★	★	★	★	★	★	★	N/A
Myllykangas 2001	★	★	N/A	★	★★	★	★	N/A
Sawano 2001	★	★	★	★	★★	★	★	N/A
Abu-Amero 2003	★	★	N/A	★	★	★	★	N/A
Goodarzi 2003	★	★	★	★	★	★	★	N/A
Ferencak 2003	★	★	N/A	★	★★	★	★	N/A
Isbir 2003	★	★	★	★	★	★	★	N/A
Martin 2004	★	★	N/A	★	★★	★	★	N/A
Keavney 2004	★	★	★	★	★	★	★	N/A
Duman 2004	★	★	N/A	★	★	★	★	N/A
Whiting 2005	★	★	N/A	★	★★	★	★	N/A
Baum 2006	★	★	N/A	★	★	★	★	N/A
Pasalić 2006	★	★	N/A	★	★★	★	★	N/A
Yamada 2006	★	★	N/A	★	★★	★	★	N/A
Ak 2007	★	★	N/A	★	★	★	★	N/A
Katia 2007	★	★	★	★	★	★	★	N/A
Georgiev 2008	★	★	N/A	★	★★	★	★	N/A
Izar 2009	★	★	★	★	★★	★	★	N/A
Aydogan 2009	★	★	★	★	★★	★	★	N/A
AshokKumar 2010	★	★	N/A	★	★★	★	★	N/A
Bhanushali 2010	★	★	N/A	★	★★	★	★	N/A
Tripathi 2010	★	★	N/A	★	★	★	★	N/A
Abd 2011	★	★	N/A	★	★★	★	★	N/A

Agirbasli 2011	★	★	N/A	★	★★	★	★	N/A
Al-Jafari 2012	★	★	N/A	★	★★	★	★	N/A
Rebhi 2012	★	★	N/A	★	★★	★	★	N/A
Abd-El-Aziz 2013	★	★	N/A	★	★★	★	★	N/A
Tanguturi 2013	★	★	★	★	★	★	★	N/A
Daoud 2013	★	★	N/A	★	★★	★	★	N/A
Ahmadi 2015	★	★	N/A	★	★	★	★	N/A
Abdel 2015	★	★	N/A	★	★	N/A	★	N/A
Bahrami 2015	★	★	N/A	★	★★	★	★	N/A

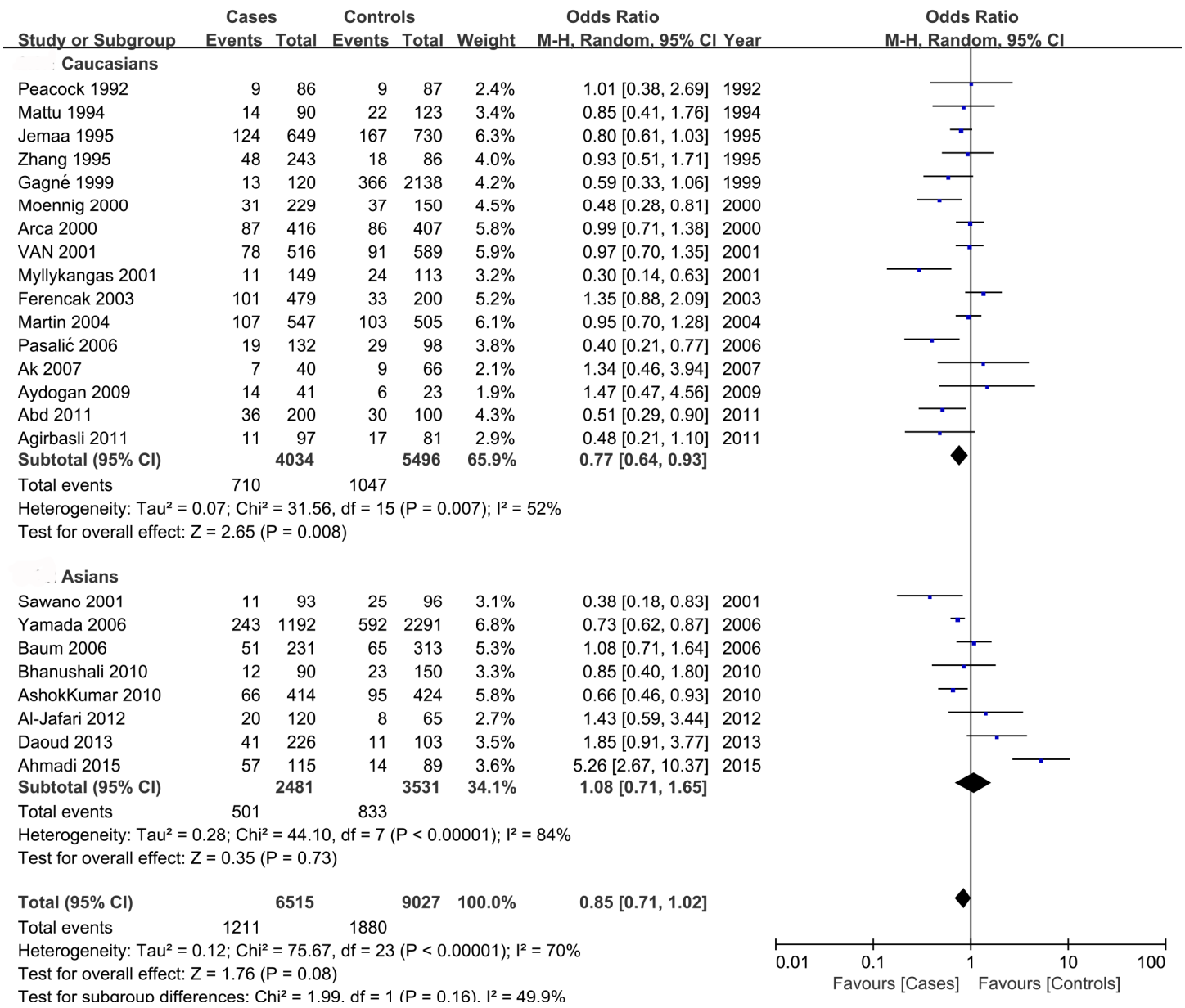
This table identifies ‘high’ quality choices with a ‘star’. A study can be awarded a maximum of 1 star for each numbered item within the Selection and Exposure categories. A maximum of 2 stars can be given for Comparability. ★, Yes; N/A, not applicable.



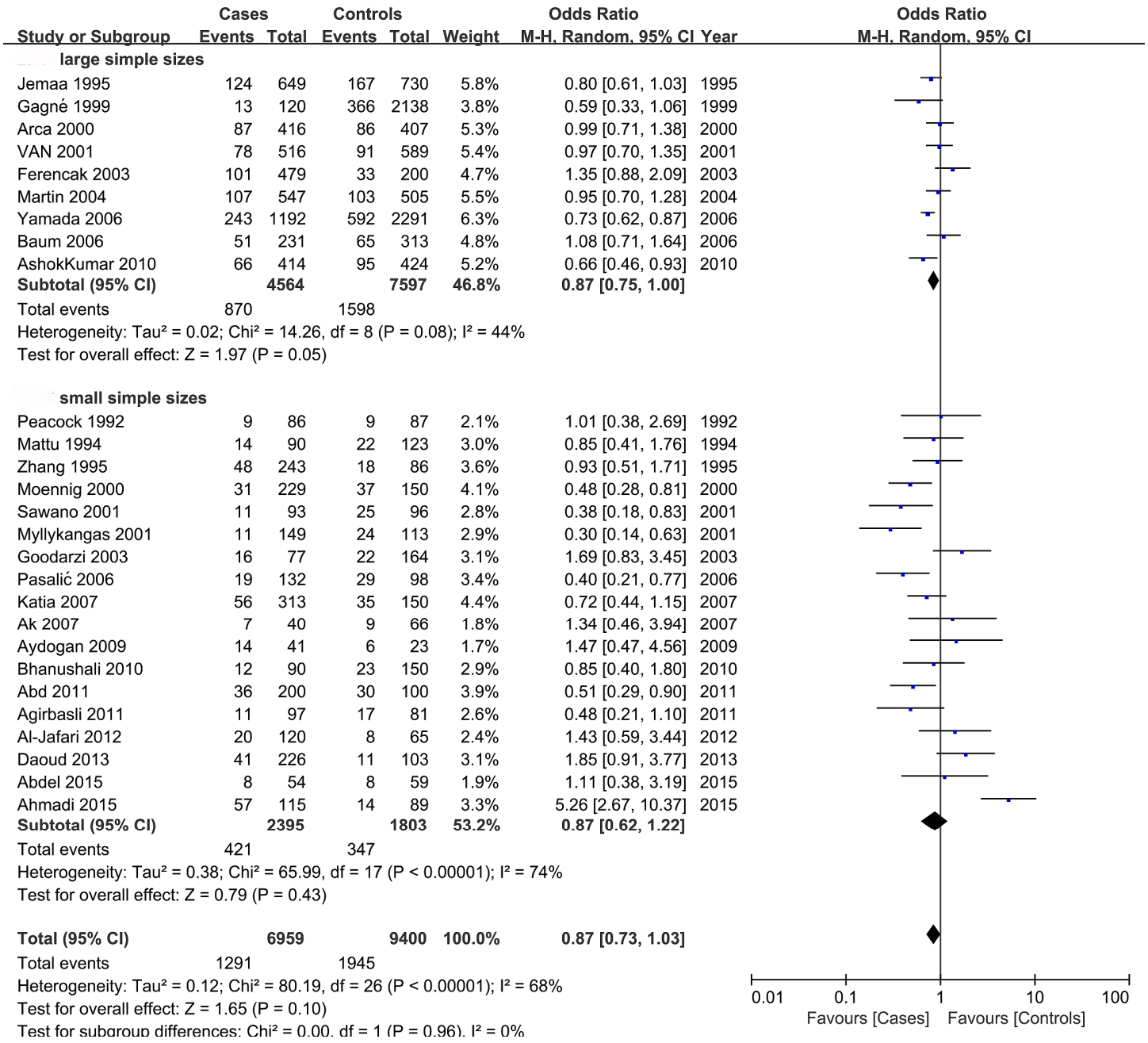
Supplementary Figure 1. Stratified analysis based on ethnicity for the association between the *LPL* HindIII polymorphism and CAD risk using dominant genetic model (GG+GT vs. TT).



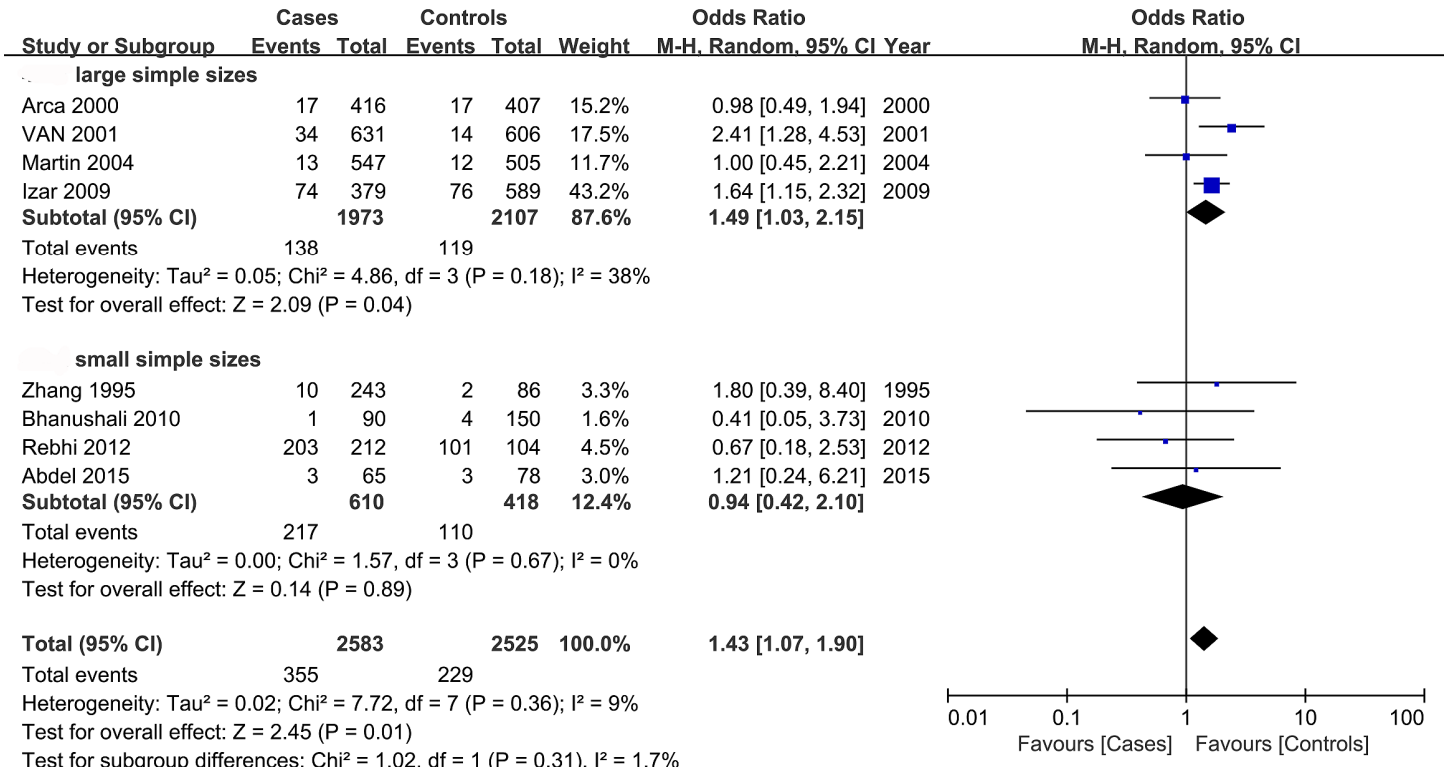
Supplementary Figure 2. Stratified analysis based on sample size for the association between the *LPL* HindIII polymorphism and CAD risk using dominant genetic model (GG+GT vs. TT).



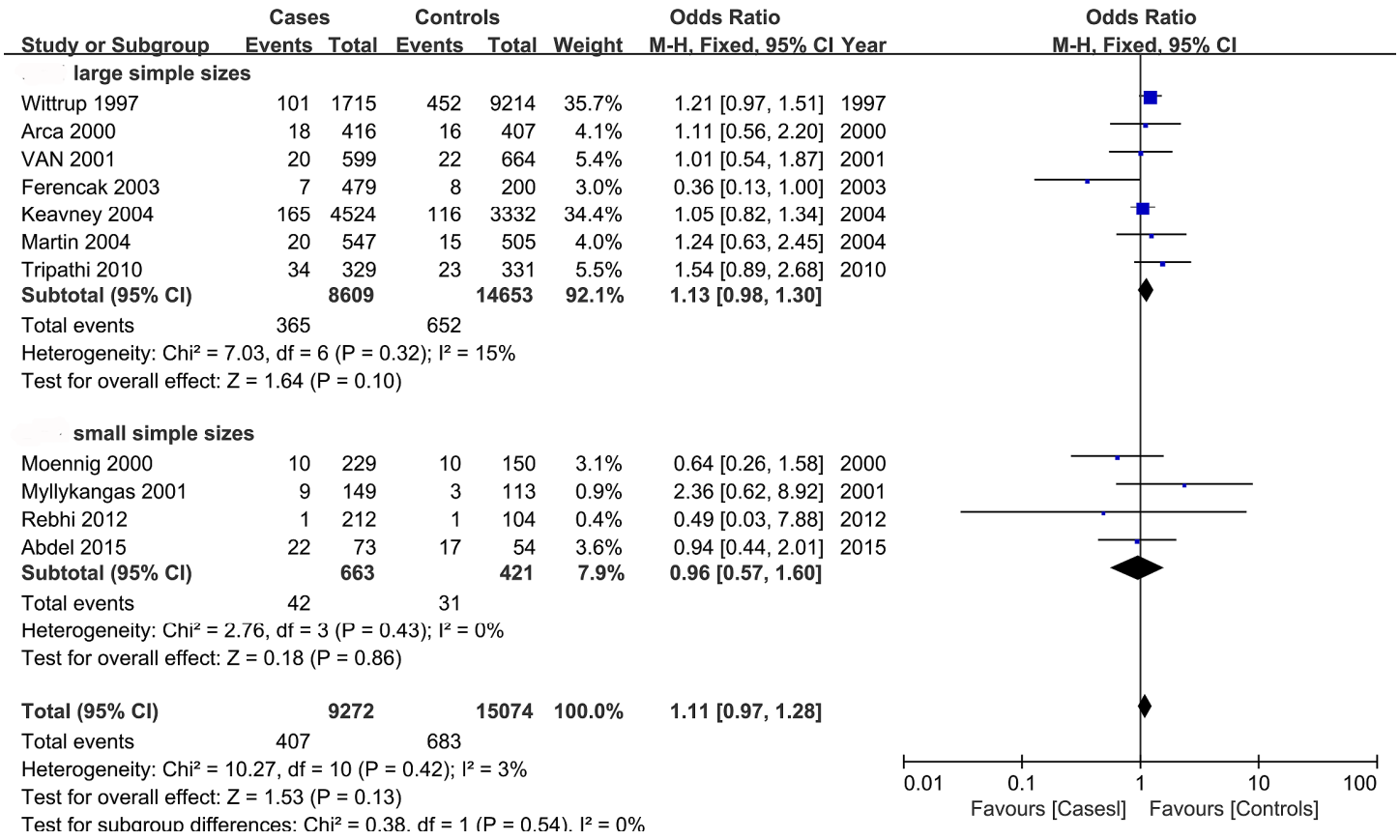
Supplementary Figure 3. Stratified analysis based on ethnicity for the association between the *LPL* S447X polymorphism and CAD risk using dominant genetic model (GG+GC vs. CC).



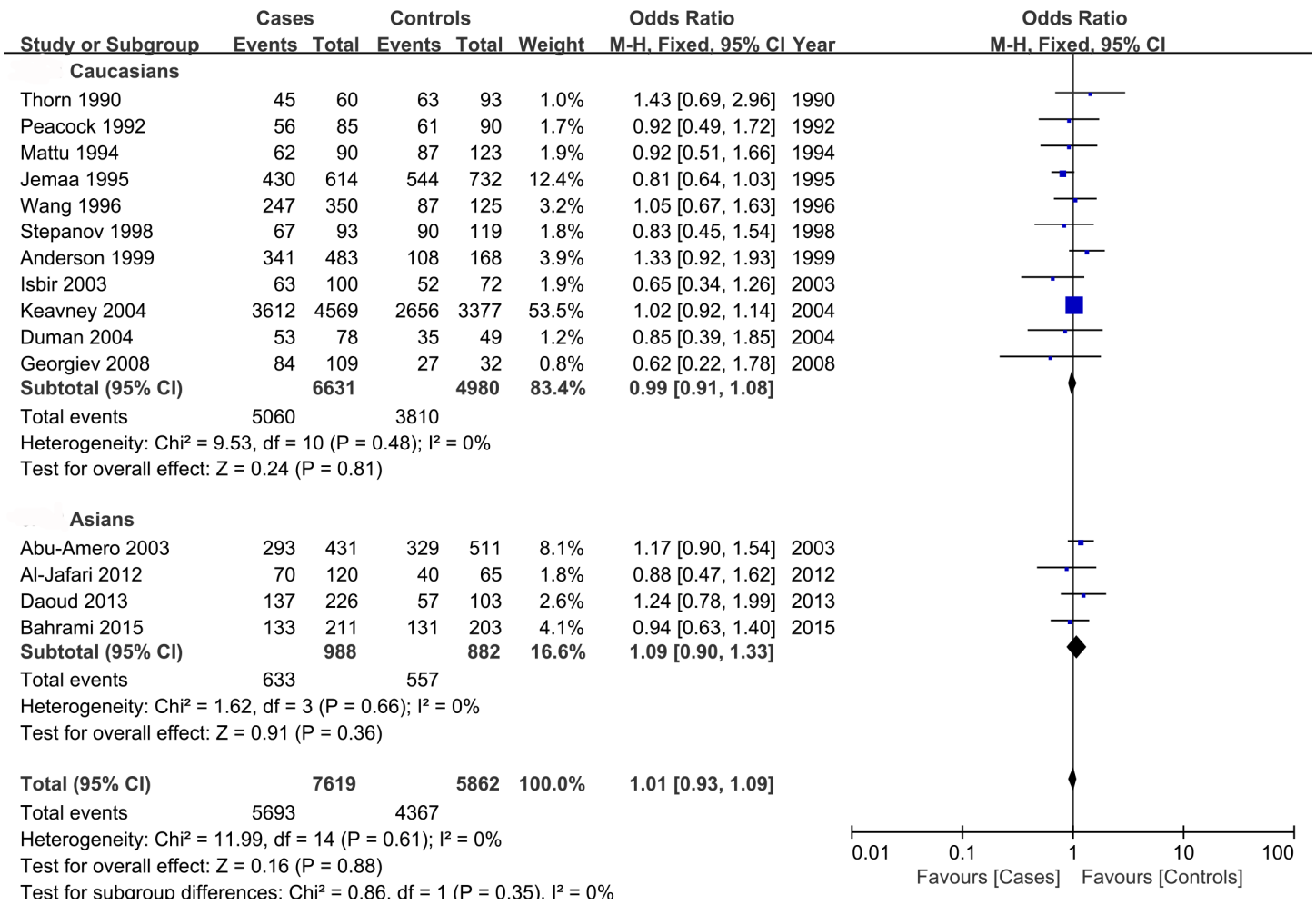
Supplementary Figure 4. Stratified analysis based on sample size for the association between the *LPL* S447X polymorphism and CAD risk using dominant genetic model (GG+GC vs. CC).



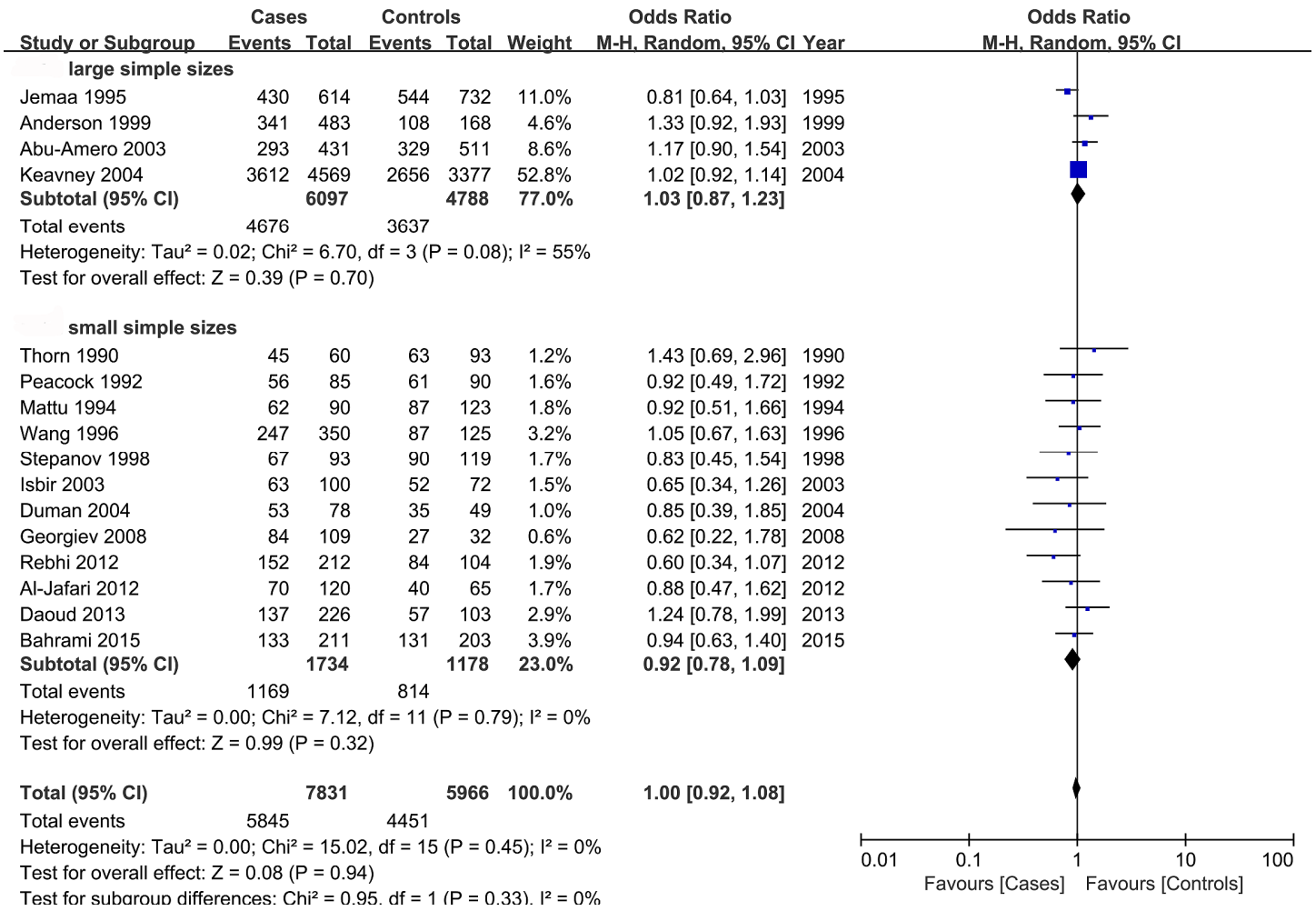
Supplementary Figure 5. Stratified analysis based on sample size for the association between the *LPL* D9N polymorphism and CAD risk using dominant genetic model (AA+GA vs. GG).



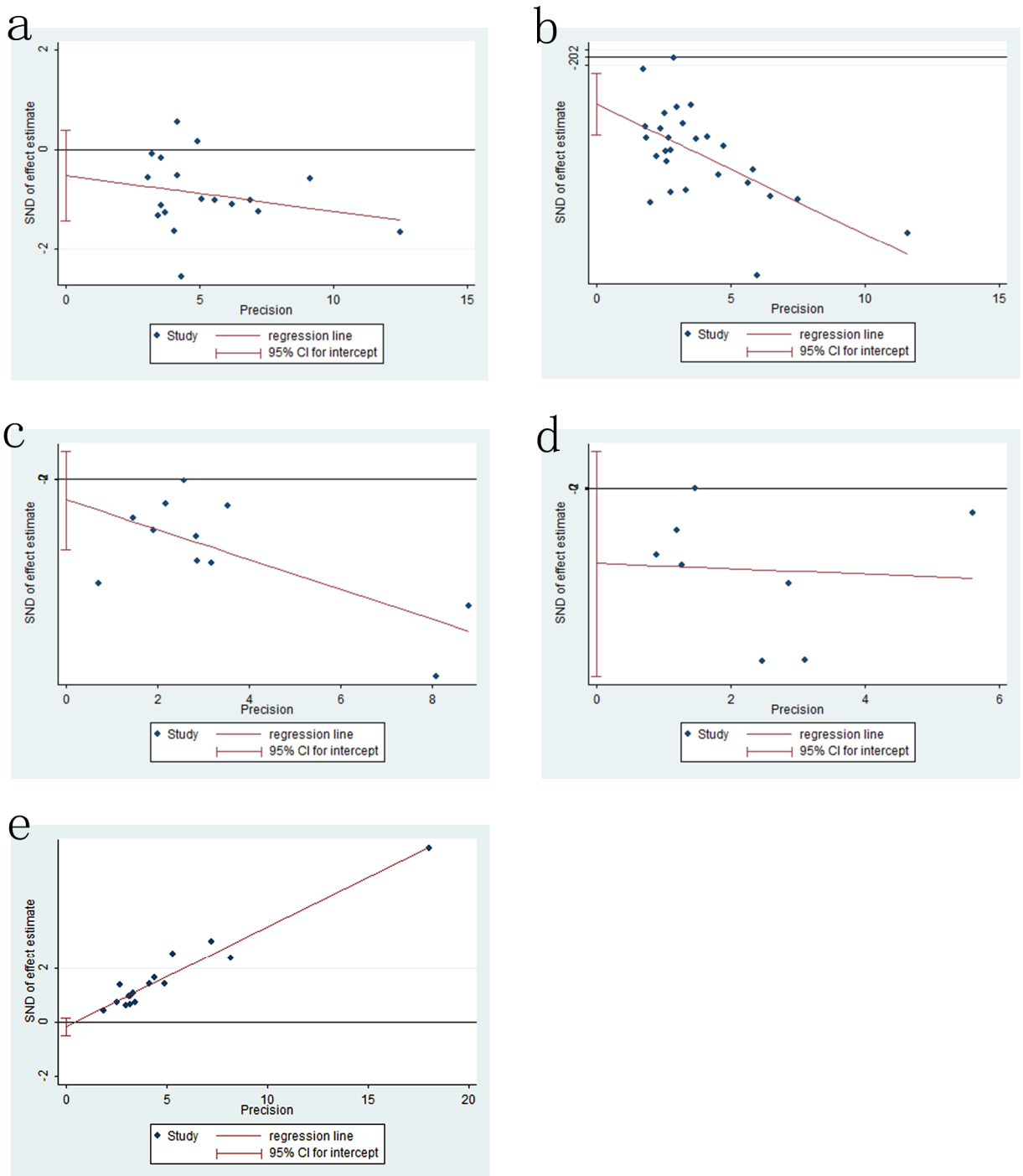
Supplementary Figure 6. Stratified analysis based on sample size for the association between the *LPL* N291S polymorphism and CAD risk using dominant genetic model (GG+GA vs. AA).



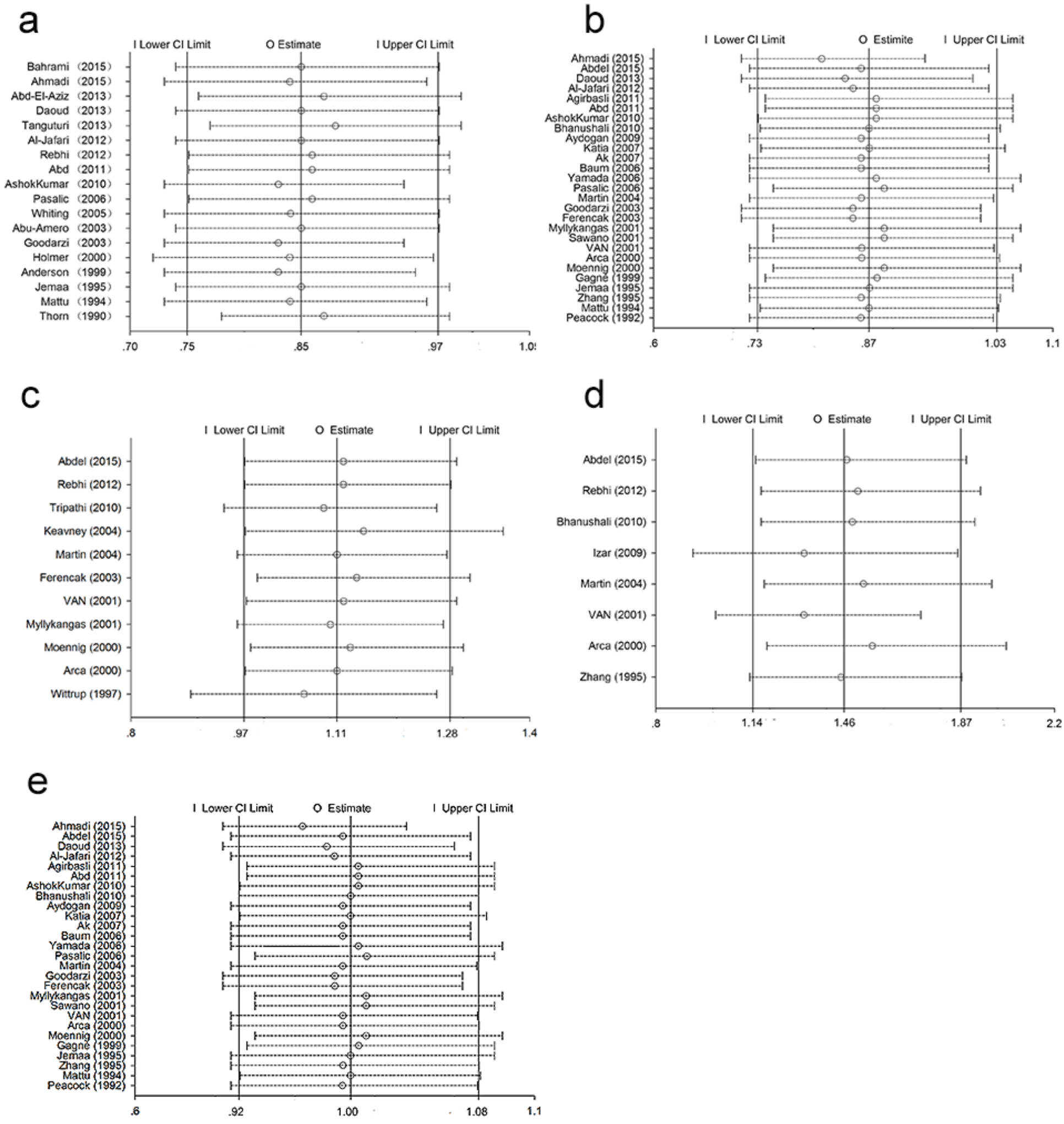
Supplementary Figure 7. Stratified analysis based on ethnicity for the association between the *LPL* PvuII polymorphism and CAD risk using dominant genetic model (TT+CT vs. CC).



Supplementary Figure 8. Stratified analysis based on sample size for the association between the *LPL* PvuII polymorphism and CAD risk using dominant genetic model (TT+CT vs. CC).



Supplementary Figure 9. Egger's regression test of publication bias for the association between the *LPL* gene polymorphisms and susceptibility to CAD. (a). HindIII polymorphism; (b). S447X polymorphism; (c). N291S polymorphism; (d). D9N polymorphism; (e). PvuII polymorphism.



Supplementary Figure 10. Sensitivity analysis on the correlation between *LPL* gene polymorphisms and susceptibility to CAD.

(a). sensitivity analysis for HindIII and CAD risk; (b). sensitivity analysis for S447X and CAD risk; (c). sensitivity analysis for N291S and CAD risk; (d). Sensitivity analysis for D9N and CAD risk; (e). sensitivity analysis for PvuII and CAD risk;