Title: Genetic variants influencing circulating lipid levels and risk of coronary artery disease.

Authors: Waterworth DM, Ricketts SL, Song K, Chen L, Zhao JH, Ripatti S, Aulchenko YS, Zhang W, Yuan X, Lim N, Luan J, Ashford S, Wheeler E, Young EH, Hadley D, Thompson JR, Braund PS, Johnson T, Struchalin M, Surakka I, Luben R, Khaw KT, Rodwell SA, Loos RJ, Boekholdt SM, Inouye M, Deloukas P, Elliott P, Schlessinger D, Sanna S, Scuteri A, Jackson A, Mohlke KL, Tuomilehto J, Roberts R, Stewart A, Kesäniemi YA, Mahley RW, Grundy SM, Wellcome Trust Case Control Consortium., McArdle W, Cardon L, Waeber G, Vollenweider P, Chambers JC, Boehnke M, Abecasis GR, Salomaa V, Järvelin MR, Ruokonen A, Barroso I, Epstein SE, Hakonarson HH, Rader DJ, Reilly MP, Witteman JC, Hall AS, Samani NJ, Strachan DP, Barter P, van Duijn CM, Kooner JS, Peltonen L, Wareham NJ, McPherson R, Mooser V, Sandhu MS

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Genetic variants influencing circulating lipid levels and risk of coronary artery disease

A full list of authors and affiliations appears at the end of the article.

Abstract

Objectives—Genetic studies might provide new insights into the biological mechanisms underlying lipid metabolism and risk of CAD. We therefore conducted a genome-wide association study to identify novel genetic determinants of LDL-c, HDL-c and triglycerides.

Methods and results—We combined genome-wide association data from eight studies, comprising up to 17,723 participants with information on circulating lipid concentrations. We did independent replication studies in up to 37,774 participants from eight populations and also in a population of Indian Asian descent. We also assessed the association between SNPs at lipid loci and risk of CAD in up to 9,633 cases and 38,684 controls.

We identified four novel genetic loci that showed reproducible associations with lipids (P values 1.6×10^{-8} to 3.1×10^{-10}). These include a potentially functional SNP in the *SLC39A8* gene for HDL-c, a SNP near the *MYLIP/GMPR* and *PPP1R3B* genes for LDL-c and at the *AFF1* gene for triglycerides. SNPs showing strong statistical association with one or more lipid traits at the *CELSR2*, *APOB*, *APOE-C1-C4-C2* cluster, *LPL*, *ZNF259-APOA5-A4-C3-A1* cluster and *TRIB1* loci were also associated with CAD risk (P values 1.1×10^{-3} to 1.2×10^{-9}).

Conclusions—We have identified four novel loci associated with circulating lipids. We also show that in addition to those that are largely associated with LDL-c, genetic loci mainly associated with circulating triglycerides and HDL-c are also associated with risk of CAD. These findings potentially provide new insights into the biological mechanisms underlying lipid metabolism and CAD risk.

Keywords

lipids; lipoproteins; genetics; epidemiology

Introduction

Circulating levels of blood lipids have been consistently associated with risk of coronary artery disease (CAD).[1] However, whereas low-density lipoprotein cholesterol (LDL-c) is known to cause atherosclerosis and CAD, the role of circulating high-density lipoprotein

*Correspondence to: Dr Manjinder S Sandhu, Department of Public Health and Primary Care, Strangeways Research Laboratory, University of Cambridge, Cambridge CB1 8RN, UK manj.sandhu@srl.cam.ac.uk.
37Contributed equally
†Members listed at end of paper

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cholesterol (HDL-c) and triglycerides (TG) in the development of atherosclerosis and CAD remains uncertain.[2, 3] In this context, the integration of population genetics and epidemiological approaches could help assess the aetiological role of HDL-c and TG levels in atherosclerosis and CAD.[4] By identifying novel genetic determinants of blood lipids, these integrated approaches can also help provide new insights into the biological mechanisms regulating lipid metabolism and identify potentially novel therapeutic targets for CAD.[5–7]

Recent genome-wide association (GWA) studies have identified several new loci that influence circulating levels of blood lipids, with around 30 genetic loci showing reproducible statistical associations with circulating HDL-c, LDL-c and TG.[5–12] However, given that a substantial proportion of the genetic variance for these traits remains unexplained, these loci are likely to only represent a small proportion of all genetic determinants involved in lipid metabolism. We therefore conducted an extended GWA study of LDL-c, HDL-c and TG levels to identify novel genetic determinants of these traits and validated our associations in independent populations, including a population of Indian Asian descent. We also examined the association between genetic variants showing reproducible statistical association with lipid levels with risk of CAD.

Methods

Study Populations

Genome-wide association meta-analysis of circulating lipid traits—We used data from eight study populations comprising up to 17,723 participants of white European descent. These are the EPIC-Norfolk subcohort (up to 2,269 participants), EPIC-Norfolk obese set (up to 1,009 participants), British 1958 birth cohort (WTCCC controls—up to 1,458 participants), CoLaus study (up to 5,226 participants), Genetic Epidemiology of Metabolic Syndrome (GEMS) study (up to 1,665 participants), a sample from the London Life Sciences Population (LOLIPOP) study (up to 813 participants), controls from the FUSION type 2 diabetes study (up to 1,099 participants), and the SardiNIA Study of Aging (up to 4,184 participants). Individual studies have been described in detail in recent reports and are summarised briefly in Supplementary Table I.[6, 9, 12] Selected descriptive characteristics of all study populations are also provided in Supplementary Table I. We only utilised data from the GEMS study[13, 14] for our GWA analysis of LDL-c as the study comprises cases and controls of dyslipidaemia, defined by high and low percentiles of HDL-c and TG, respectively. (Supplementary Table I).

Lipids replication analyses—Our replication set encompassed individuals of white European descent from eight studies comprising up to 37,774 participants. Individual studies are summarised in Supplementary Table II. Briefly, the replication set comprised the EPIC-Norfolk cohort[15] (up to 19,793 individuals who had DNA and lipid measurements available and did not overlap with the EPIC-Norfolk subcohort or obese set), controls from the Ottawa Heart Study[16] (OHS—up to 1,445 participants), Fenland study[17] (up to 1,402 participants), an additional subset of the LOLIPOP study[9] (up to 710 participants), British 1958 Birth cohort T1DGC controls[18] (2,527 participants—there is no overlap with the control set from the original WTCCC sub-study), Northern Finland Birth Cohort 1966[10] (up to 5,138 participants), National FINRISK Study[7] (up to 910 participants), and Rotterdam study[7] (up to 5,849 participants).

Case-control studies for CAD—For our CAD meta-analysis we combined data from nine studies comprising up to 9,633 cases and 38,684 controls. These studies included two non-overlapping case-control studies of CAD derived from the EPIC-Norfolk cohort[15],

WTCCC CAD study[19, 20], OHS[16], MEDSTAR and PENN CATH studies[21], and nested CAD case-control studies derived from the CoLaus study[6], GEMS study[13, 14] and Rotterdam study[7]. Details of these studies are provided in Supplementary Table III.

Studies of Indian Asian ethnicity—To examine the consistency of our novel association signals for lipids in an ethnically distinct population, we used four non-overlapping subsets of Indian Asian participants from the LOLIPOP study[9]; collectively comprising up to 9,665 participants (see Supplementary Table II for details).

Local ethics committees approved all studies and all participants gave written informed consent.

Genotyping

Genome-wide association genotyping—The eight studies utilised in the GWA meta-analysis of lipid traits have been genotyped with different genome-wide SNP chips (for details see Supplementary Table I). To enable us to combine data from all studies for our GWA meta-analysis we used information on SNP genotypes in our samples and HapMap II data to statistically predict (impute) all SNP genotypes for all individuals. These genome-wide imputation analyses were conducted in each study independently using either IMPUTE[22] or MACH [12] (Supplementary Table I).

Replication genotyping for lipid SNPs—SNPs taken forward for replication for the three lipid traits were genotyped on the EPIC-Norfolk cohort using either the iPLEX Sequenom MassARRAY platform or allelic discrimination on an ABI 7900 instrument (Taqman, Applied Biosystems, Warrington, UK). Criteria for genotyping quality are outlined in Supplementary Table II. For the remaining seven replication studies (Supplementary Table II), genotypes were available *in-silico* using data from genome-wide SNP chips or imputation analyses (Supplementary Table II).

Genotyping of case-control studies for CAD—Genotypes were available for *in-silico* testing of lipid SNPs for association with CAD risk for the nine case-control studies described above (see Supplementary Table III for details).

Studies of Indian Asian ethnicity—Genotypes were available for *in-silico* testing of SNPs with circulating lipid levels for the four non-overlapping subsets of the LOLIPOP study (Supplementary Table II).

Statistical Analyses

Genome-wide association meta-analysis of circulating lipid traits—Sample and SNP quality control criteria and statistical analysis for each lipid trait was done within each study independently (Supplementary Table I). For the initial GWA screen, analyses were done within study using a uniform analytical strategy. All lipid traits were natural log transformed before GWA analysis across studies. The choice of natural log-transformation was guided by the shape of the phenotype distributions across studies, to minimise skew whilst also retaining a link to the original data—particularly for studies comprising selected populations. This transformation also provided an interpretable regression coefficient. Analyses were conducted using an additive model adjusted for age, sex, and geographical/population covariables where appropriate. Association analysis for both imputed and genotyped SNPs was done using SNPTEST[22] (with the full posterior probability genotype distribution) or MERLIN[12]. Only SNPs with a minor allele frequency of 1% or more and with a posterior-probability score more than 0.90 were considered for these imputed

association analyses. Criteria for imputation quality and genomic control parameters are outlined in Supplementary Table I.

We conducted a GWA meta-analysis by combining summary data from each of the eight studies using a fixed effects model and inverse-variance weighted averages of β coefficients with Stata version 8.2. This provided us with a combined estimate of the overall β coefficient and its standard error. Between-study heterogeneity was assessed with the χ^2 test. To optimise data quality, we only analysed SNPs that passed sample and SNP quality control criteria in each of the eight studies and that had a measure of association (β coefficient and standard error) in all eight studies (see above for details). Data for 2,155,369 autosomal SNPs were available for analysis of circulating HDL-c levels, 2,154,923 for LDL-c and 2,155,784 SNPs for TG. We also calculated an inflation factor (λ) for each study, which was estimated from the mean of the χ^2 tests generated on all SNPs that were tested (Supplementary Table I). The overall genomic control parameter[23] was 1.08, 1.07 and 1.06 in our meta-analysis for HDL-c, LDL-c and TG, respectively. These results suggest that unmodelled relatedness or population stratification are unlikely to materially influence our results.

For the three lipid traits (HDL-c, LDL-c and TG), we only examined SNPs at known, previously reported and novel loci that had a combined $P < 1 \times 10^{-5}$ (an arbitrary statistical threshold) in the meta-analysis and that did not show any heterogeneity among studies ($P < 0.1$).

Replication analyses for lipid SNPs—For each novel locus, the SNP showing the strongest statistical association was taken forward for replication in Stage 2. These comprised 40 SNPs in total: 11 for HDL-c, 13 for LDL-c, 15 for TG and one for both HDL-c and TG. We conducted replication analyses in the EPIC-Norfolk cohort using linear regression using natural log transformed lipid levels and an additive model with adjustment for age and sex. We combined these data with *in-silico* replication sets from the other seven studies using meta-analysis, as above, to obtain an overall estimate of association in the combined datasets. These analyses comprised adjustment for age, sex and population variables, as relevant (Supplementary Table II).

Association analyses for CAD risk—Association analyses were done using either SNPTEST[22], PLINK[24] or ProbABEL[25] for eight studies with genome-wide SNP data available. Analyses for these studies comprised adjustment for at least age, sex and population variables (where relevant—see Supplementary Table III). For the EPIC-Norfolk-2 case-control study, we used logistic regression and a log additive model adjusted for age and sex to test for association of novel lipid SNPs with CAD. We combined summary estimates (log odds ratios and standard errors) for each of the nine studies using meta-analysis, as above, to obtain a combined estimate of the association between SNPs and risk of CAD for a log additive model.

Studies of Indian Asian ethnicity—For association analyses of potentially novel lipid loci in Indian Asian individuals, we combined data from the four LOLIPOP subsets (Supplementary Table II) using the meta-analytical strategy outlined above. We then conducted a formal assessment of the heterogeneity (Q statistic) between the two ethnic groups for our potentially novel lipid loci by comparing overall summary estimates from our white European and Indian Asian studies using meta-analysis, as above.

Results

Known genes influencing lipids

For LDL-c, HDL-c and TG levels, the strongest statistical association signals in our GWA meta-analysis were at loci previously implicated in lipid metabolism or those recently identified as potential lipid genes.[5] Table 1 lists the 28 SNPs with strongest statistical associations for the three lipid traits ($P < 1 \times 10^{-5}$) with no detectable heterogeneity among studies ($P > 0.1$) at these genetic loci. Eighteen SNPs at these known loci reached genome-wide statistical association ($P < 5 \times 10^{-8}$) in our data. As expected, genes showing strong statistical associations with LDL-c included *APOB*, the *APOE-C1-C4-C2* cluster, *CELSR2*, *HMGCR*, *LDLR*, *PCSK9* and *CILP2*, whereas *CETP*, *LIPC*, *LIPG*, *LPL*, *ABCA1*, *LCAT*, *GALNT2* and *MMAB/MVK* showed clear statistical associations with HDL-c. Likewise, the *ZNF259-APOA5-A4-C3-A1* cluster, *LPL*, *ANGPTL3*, *GCKR*, *TRIB1* and *MLXIPL* genetic regions were strongly associated with triglyceride levels. Several genetic loci were associated with more than one lipid trait, including a SNP at the *APOE-C1-C4-C2* cluster, which showed a strong association with all three traits (Table 1).

Recently identified and novel lipid genes

We also found statistical evidence for potentially novel loci that may influence circulating levels of blood lipids. Supplementary Table IV lists SNPs showing the strongest statistical association at 40 potentially novel loci with $P < 1 \times 10^{-5}$ and no detectable heterogeneity among studies ($P > 0.1$). None of these SNPs reached genome-wide statistical association in our meta-analysis. Therefore, to help validate statistical associations found at these potentially novel loci, we examined whether these SNPs showed statistical associations in additional population-based cohorts as part of a replication study (complete results for all 40 SNPs at this stage 2 replication validation step are given in Supplementary Table V).

From our stage 2 analysis, we identified SNPs at eight loci that showed evidence for independent replication ($P < 0.05$) with one or more lipid traits and that showed directional consistency with the discovery studies and no material heterogeneity among studies ($P > 0.1$). Table 2 summarises the results for these SNPs. In a combined analysis of all studies (including the discovery GWA studies), six of these loci reached genome-wide statistical association ($P < 5 \times 10^{-8}$). These were SNPs at the *MYLIP/GMPR* and *PPP1R3B* loci for LDL-c, SNPs at the *SLC39A8*, *TTC39B* and *FADS1* locus for HDL-c, and at *FADS1* for TG. We note that recently published reports have also found SNPs at the *TTC39B* and *FADS1-FAD2-FADS3* loci to be associated with circulating HDL-c and HDL-c/triglyceride levels, respectively.[5, 7] As they were included in our replication strategy, we have retained these SNPs in Table 2 in order to present the relevant data and confirm statistical associations at the genome-wide level in our combined analysis.

MYLIP–GMPR—SNP rs2142672 showed strong statistical association with LDL-c levels. The C allele (frequency 74%) is associated with relatively higher levels of circulating LDL-c. The SNP lies in a distinct block of high LD between two genes— myosin regulatory light chain interacting protein (*MYLIP*) and guanosine monophosphate reductase (*GMPR*) on chromosome 6p23 (Supplementary Figure If). The illustration suggests that this SNP is correlated with other SNPs that also show similar patterns of statistical association and that cluster around the *MYLIP* gene (Supplementary Figure If). Our data confirm results from a recent study that also identifies this locus as one that influences LDL-c.[26] A recent report has also implicated MYLIP (IDOL) in the regulation of circulating LDL-c levels, by its induction of LDL receptor (LDLR) degradation via ubiquitination[27].

PPP1R3B—SNP rs2126259 lies upstream of the protein phosphatase 1, regulatory (inhibitor) subunit 3B gene (*PPP1R3B*) on chromosome 8p23 and is statistically associated with circulating LDL-c levels. The A allele (10% frequency) is associated with relatively lower levels of circulating LDL-c. The LD structure of this region is modest with surrounding SNPs also showing statistical association (Supplementary Figure Ig). The PPP1R3B protein is involved in the regulation of glycogen metabolism in both muscle and liver.[28] It is possible that its association with circulating LDL-c levels is a reflection of downstream effects on the bioavailability of TG. In addition, in support of our findings for LDL-c, this locus has also been shown to be associated with VLDL-c levels.[26]

TTC39B—The C allele (14% frequency) of rs643531 at the tetratricopeptide repeat domain 39B (*TTC39B*) locus on chromosome 9p22 is associated with lower HDL-c levels (Table 2). SNP rs643531 lies within intron one of the *TTC39B* gene in a modest LD block that does not contain any other known or putative genes (Supplementary Figure Ic). Again, several highly correlated SNPs in this region show statistical associations with HDL-c levels (Supplementary Figure Ic) in our genome-wide scan. Our data support results from a recent report showing statistical association between a SNP— rs471364— at this locus and HDL-c levels.[5] The two SNPs (rs471364 and rs643531) are correlated at an r^2 of 0.74 and show directional consistent associations. The function of the *TTC39B* gene in humans is presently unknown.

SLC39A8—SNP rs13107325 at the solute carrier family 39 (zinc transporter) member 8 (*SLC39A8*) locus on chromosome 4q22 shows strong statistical association with circulating levels of HDL-c (Table 2). It is a nonsynonymous SNP located in exon 8 of the *SLC39A8* gene, which produces a change in amino acid from alanine to threonine. The T allele (frequency 8%) is associated with relatively lower levels of circulating HDL-c and is not materially correlated with any other SNP across 100 kb of genomic sequence spanning the *SLC39A8* gene in HapMap (Supplementary Figure Id). This gene encodes a zinc transporter that has been shown to function in the cellular importation of zinc at the onset of inflammation, and its expression is induced by TNF-α.[29] It is possible that the SLC39A8 molecule might be associated with HDL-c in an inflammatory context.

FADS1—SNP rs174548 at the fatty acid desaturase 1 (*FADS1*) locus on chromosome 11q12 shows strong statistical association with both HDL-c and triglyceride levels. The G allele (30% frequency) is associated with relatively higher triglyceride and lower HDL-c levels (Table 2). The genomic context of this locus is illustrated in Supplementary Figures Ia and Ib. SNP rs174548 lies in a block of clear linkage disequilibrium (LD) that also contains the *C11orf9/10*, *FEN1* and *FADS2/3* genes. Several highly correlated SNPs within this LD block show statistical association with these traits in our genome-wide scan (Supplementary Figures Ia and Ib), including two SNPs in the 3′ UTR of the *FADS1* gene. Our study supports findings from a recent report showing that a SNP—rs174547— at this locus is also associated with both HDL-c and triglyceride levels.[5] SNP rs174548 in our study is highly correlated (r^2 0.8) to SNP rs174547 and shows directionally consistent associations. Fatty acid desaturases are involved in the metabolism of polyunsaturated fatty acids in humans and SNPs at the *FADS1/2* gene cluster have been linked to changes in the fatty acid composition of serum phospholipids in humans[30].

Examination of lipid associations in an Indian Asian population

In an exploratory analysis, and to provide a wider context to our studies, we examined whether our replicated loci (from Table 2) were also associated with the relevant lipid traits in a population of Indian Asian descent—Stage 3. Table 3 shows the results of these analyses. Only six of the eight SNPs were available for analysis in this population (SNPs at

two loci—*SLC39A8* and *IGF2R/SLC22A1*—were not present or poorly imputed—Supplementary Information). As expected, given the low statistical resolution for this study, we only found evidence for independent replication ($P < 0.05$) at three loci in Indian Asian individuals— *PPP1R3B* for circulating LDL-c levels, *FADS1* for circulating HDL-c and triglyceride levels, , and *AFF1* for circulating triglyceride levels. However, association signals for the other three loci (*TTC39B*, *C5orf35* and *MYLIP/GMPR*) were directionally consistent between ethnic groups (Table 3).

In a combined analysis of all studies (Stages 1, 2 and 3), we identified an additional locus that reached genome-wide statistical association—*AFF1*—a novel locus for circulating TG ($P = 3.1 \times 10^{-10}$ (Table 3)). SNP rs442177 lies in intron 10 of the *AFF1* gene on chromosome 4q21 in a modest LD block with correlated SNPs showing similar levels of statistical association (Supplementary Figure Ie). The A allele (60% frequency in white European populations) is associated with relatively higher levels of circulating TG. The *AFF1* gene encodes a protein involved in the regulation of cyclin-dependent kinase inhibitor *CDKN1B* and may therefore be involved in cell cycle regulation.[31] Its function with respect to triglyceride metabolism is unknown. For three of our eight novel lipid loci we did not observe a statistical association with lipids in the Indian Asian population, and for two SNPs data were not available (Table 3). These observations could denote limited statistical resolution, differences in linkage disequilibrium patterns in Indian Asians compared to Europeans, or that there are no association signals at these loci in Indian Asian populations. However, in further exploratory analyses, examining association signals across a 10 kb region spanning these five SNPs in our studies, we found evidence indicating that additional association signals may be present at some of these loci (see Supplementary Information).

Association with risk of coronary artery disease

Given the causal link between circulating LDL-c levels and risk of CAD, and the consistent associations between circulating levels of TG and HDL-c with subsequent risk of CAD, we assessed the association between these known, recently identified and potentially novel genetic lipid loci and risk of CAD. Table 4 shows the association between these 36 SNPs linked to lipid metabolism in our data and risk of CAD in up to 9,633 cases and 38,684 controls.

As expected, and given the prior associations between these loci and blood lipids, a much greater proportion of these SNPs showed statistical associations with CAD risk at $P < 0.05$ than expected by chance alone, taking into account any correlated SNPs (Table 4). We identified six genetic loci that showed both genome-wide statistical association with blood lipids and statistical association with CAD after adjustment for multiple testing ($P < 0.0013$ after testing 36 SNPs) (Table 4). Specifically, we confirm the association between variation at the *CELSR2*[32] and *APOB* genes and at the *APOE-C1-C4-C2* cluster, which influence mainly LDL-c levels, and risk of CAD (Figure 1). None of the genetic variants largely or specifically associated with HDL-c showed statistical association with CAD risk after correction for multiple testing. Notably, SNPs at the *ZNF259-APOA5-A4-C3-A1* cluster—which reached genome-wide statistical association—and at the *TRIB1* and *LPL* loci, which show strongest association with triglyceride levels (Figure 2), were also statistically associated with risk of CAD after adjustment for multiple testing (Table 4 and Figure 1). The direction of association with CAD risk for all of these SNPs was consistent with their association with lipid levels (Table 1). However, several of the SNPs at these loci were associated with more than one lipid trait (Figure 2 and Table 1). Of note, only SNPs at *CELSR2* and *APOB* showed specific associations with LDL-c. By contrast, only SNPs at the *LPL* locus showed clear associations with HDL-c and TG, but were not associated with LDL-c in our studies (Figure 2 and Table 1).

Discussion

Our studies have identified three novel loci (*PPP1R3B* for LDL-c, *SLC39A8* for HDL-c, and AFF1 for TG) associated with variation in circulating LDL-c, HDL-c and TG. We also provide strong statistical evidence for six loci that influence levels of blood lipids and risk of CAD. In addition to those that are largely associated with LDL-c concentrations, we show that genetic loci mainly associated with circulating TG are also associated with risk of CAD. Collectively, these studies potentially provide new insights into biological regulation of lipid metabolism and the aetiology of CAD.

We provide robust statistical evidence for the association of three novel genetic loci with circulating LDL-c, HDL-c and TG levels, in addition to confirming the recently reported novel associations for circulating LDL-c with SNPs at *MYLIP/GMPR*, HDL-c levels with SNPs at the *TTC39B* locus, and for both circulating HDL-c and triglyceride levels at the *FADS1* locus[5, 26]. For the three novel loci, *PPP1R3B*, *SLC39A8*, and *AFF1*, their function in lipid metabolism is not known. However, consistent with our results, a recent study has shown that *PPP1R3B* is also associated with VLDL-c levels[26]. *F*ine-mapping and functional studies, including large-scale resequencing to help identify common and rare functional variants[33], might help clarify the role of proteins encoded by these genes in lipid metabolism and relevant disorders.

Recent reports have identified several potentially novel loci for circulating lipids.[5, 7] One of these reports presents an updated meta-analysis and has used the same threshold for Stage 2 SNP selection as our study ($P < 1 \times 10^{-5}$).[5] By using this arbitrary threshold for selection, we will undoubtedly have missed some additional novel loci. For example, one report identified a SNP—rs1501908—that lies between the *TIMD4* and *HAVCR1* loci and is reproducibly associated with circulating levels of LDL-c. We selected this SNP for Stage 2 replication testing but it did not reach statistical association in our Stage 2 samples ($P = 0.1$) (Supplementary Tables IV and V). However, the association signal in our data is directionally consistent with that found in the original report. These findings suggest that further novel loci involved in the regulation of blood lipids exist—providing opportunities for additional insights into lipid biology and potential therapeutic targets—and therefore highlight the need for a more comprehensive analysis of all available studies to gain appropriate statistical resolution to identify these loci.

We identified six lipid genes that show strong statistical association with CAD risk. Associations at these loci were directionally consistent with their associations with blood lipids. Three of these are predominantly associated with circulating LDL-c levels—*APOB*, *APOE* cluster and *CELSR2*—reiterating the causal link between LDL-c and CAD,[34] and as previously reported[12, 32, 35]. Interestingly, our variant at the APOE locus, rs4420638, was correlated (data not shown) with one of the canonical APOE variants ($r2 = 0.71$ with E4), but showed no correlation with E2 ($r2 = 0.018$), suggesting that other independent variants may contribute to the variation in LDL-c and risk of CAD.

Whereas previous studies or reviews have provided only suggestive or inconsistent evidence for the *APOB* locus and CAD risk[12, 35], we confirm that common variation at the *APOB* locus is associated with risk of CAD—in line with the effect of rare, highly deleterious mutations at this gene[36]. However, some genes known to be implicated in Mendelian forms of hypercholesterolaemia and more recently myocardial infarction, including *LDLR* and *PCSK9*[21, 37, 38], showed only suggestive evidence for association with CAD risk in our data. Because of limited statistical power to detect associations, larger scale studies of these and other genetic variants that influence LDL-c levels may help reliably determine their association with CAD risk.

We also provide compelling statistical evidence that genetic variants at loci predominantly associated with both circulating blood TG and HDL-c are also associated with risk of CAD—specifically at the *ZNF259-APOA5-A4-C3-A1* cluster, *TRIB1* and *LPL* loci. The *TRIB1* locus is a recently identified lipid gene, which predominantly influences TG, but is also associated with LDL-c and HDL-c. One previous report has shown suggestive evidence for an association between a SNP at this locus and CAD risk[12]. We provide convincing evidence for association with CAD risk in our studies. The *LPL* variant in our study (rs325) is in perfect linkage disequilibrium with the known S447X variant—a gain-of-function mutation, which causes a two amino acid truncation in the enzyme and increases its activity[39]. Our data are consistent with these observations and suggest that LPL activity may be causally linked to CAD risk. By contrast, the CAD-risk variant we identified in the *ZNF259-APOA5-A4-C3-A1* cluster was largely uncorrelated with variants at the *APOA5* and related genes that have been previously linked to triglyceride levels and CAD risk[40]. A recent systematic review of known genetic variants at the *LPL* locus and CAD risk provided only suggestive evidence for association with CAD risk[41]. Similarly, previous reports have provided only weak and inconsistent evidence to suggest that variation at the *APOA5* cluster is implicated in CAD risk[40, 42]. Collectively, our data, based on an unbiased analytical framework, confirm that the *LPL*, *TRIB1* and *ZNF259-APOA5-A4-C3-A1* cluster are CAD susceptibility loci.

Importantly, consistent with its biological role,[43] SNPs at the *LPL* locus were not associated with LDL-c levels in our large scale analysis, suggesting that the association with CAD risk is independent of LDL-c. However, other loci that were associated with CAD risk showed robust associations with potentially multiple lipid traits (including LDL-c). By contrast, some loci showed comparable magnitudes of association with one or more lipid traits, but showed inconsistent magnitudes of association with CAD risk. These differences might be due to limited statistical power or the differential impact of comparable differences in these lipids on risk of CAD. Statistical analyses adjusting for these intermediate phenotypes (lipid levels) when examining SNP–CAD risk associations may help disentangle the impact of these genetic variants on lipid levels and CAD risk. However, these analyses would require large-scale prospective studies with information on genetic variants, biomarkers and subsequent disease risk, which are not available across most of the studies used in the current analysis.

These lipid and CAD risk loci may also have pleiotropic actions.[5] As a result, interpretation of interpretation of these findings is complex. From a qualitative perspective, these findings may suggest that some, but not all, biological mechanisms involved in TG and HDL-c regulation and metabolism or their correlates (including atherogenic VLDL remnant lipoproteins[44]) may be implicated in the aetiology of CAD[44–46]. In this context, these data suggest that therapeutic approaches that target specific lipid pathways might have a potentially greater impact on reducing risk of CAD—particularly in the context of our findings for the *LPL* locus.

None of the genetic loci showing reproducible and specific association with HDL-c levels (including *CETP*), showed strong evidence for association with CAD risk. Because of limited statistical power, in terms of the expected magnitudes of the associations among HDL-c levels, HDL-c SNPs and CAD risk,[47] we may have missed HDL-c genetic loci that also show association with CAD risk. Furthermore, the functional relationship of HDL-c to CAD risk is inherently complex and plasma concentrations of HDL-c are not always a reliable marker of reverse cholesterol transport or other biological functions of HDL, including anti-inflammatory effects.[48, 49]

We assessed the generalisability of our novel SNP-lipid associations in a population of Indian Asian ethnicity and found that there was directional consistency between the two populations for statistically associated SNPs, with no strong heterogeneity between the two ethnic groups. However, we only had limited statistical resolution to detect any differences in the magnitudes of these associations between ethnic groups. It will be important to fully characterise the associations among all known genetic regulators of blood lipids and their link to CAD risk in this and other ethnically distinct populations. Importantly, genetic epidemiological approaches may help determine whether the marked differences in the prevalence of some metabolic diseases among populations have a genetic basis.[50]

In conclusion, our studies have identified four novel loci associated with variation in circulating lipid concentrations, and indicate that, with the caveats outlined above, genetic variants that influence lipid concentrations, primarily those that are associated with circulating LDL-c, or specific metabolic and regulatory pathways for both TG and HDL-c, are also associated with risk of CAD. These findings potentially provide new insights into the biological mechanisms underlying lipid metabolism and CAD risk.

Supplementary Material

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Authors

Dawn M Waterworth, PhD[1,37], Sally L Ricketts, PhD[2,37], Kijoung Song, PhD[1], Li Chen, MSc[3], Jing Hua Zhao, PhD[4], Samuli Ripatti, PhD[5], Yurii S Aulchenko, PhD[6], Weihua Zhang, PhD[7], Xin Yuan, PhD[1], Noha Lim, PhD[1], Jian'an Luan, PhD[4], Sofie Ashford, MSc[2], Eleanor Wheeler, PhD[8], Elizabeth H Young, PhD[2,8], David Hadley, MSc[9], John R Thompson, PhD[10], Peter S Braund, MSc[10], Toby Johnson, PhD[11], Maksim Struchalin, MSc[12], Ida Surakka, MSc[5,13], Robert Luben, BSc[2], Kay-Tee Khaw, FRCP[2], Sheila A Rodwell, PhD[14], Ruth J F Loos, PhD[4], S. Matthijs Boekholdt, MD, PhD[15], Michael Inouye, MSc[8], Panagiotis Deloukas, PhD[8], Paul Elliott, FRCP[16], David Schlessinger, PhD[17], Serena Sanna, PhD[18], Angelo Scuteri, MD, PhD[18,19], Anne Jackson, PhD[20], Karen L Mohlke, PhD[21], Jaako Tuomilehto, MD, MPolSc, PhD[22], Robert Roberts, MD, FRCPC[3], Alexandre Stewart, PhD[3], Y Antero Kesäniemi, PhD[23], Robert W Mahley, PhD[24], Scott M Grundy, PhD[25], Albert Hofman, MD, PhD[6], Wellcome Trust Case Control Consortium[†], Wendy McArdle, PhD[26], Lon Cardon, PhD[1], Gérard Waeber, MD[27], Peter Vollenweider, MD[27], John C Chambers, PhD[7], Michael Boehnke, PhD[20], Gonçalo R Abecasis, PhD[28], Veikko Salomaa, MD[29], Marjo-Riitta Järvelin, MD[7], Inês Barroso, PhD[8], Stephen E Epstein, MD[30], Hakon H Hakonarson, MD, PhD[31], Daniel J Rader, MD[32,33], Muredach P Reilly, MB[32,33], Jacqueline C M Witteman, PhD[6], Alistair S Hall, FRCP[34], Nilesh J Samani, FRCP[10], David P Strachan, MD[9], Philip Barter, PhD[35], Cornelia M van Duijn, PhD[6], Jaspal S Kooner, FMedSci[16], Leena Peltonen, MD, PhD[5,8,36], Nicholas J Wareham, FRCP[4], Ruth McPherson, MD, PhD, FRCPC[3], Vincent Mooser, MD[1], and Manjinder S Sandhu, PhD[2,4,8,*]

Affiliations

[1]Genetics Division, GlaxoSmithKline R&D, King of Prussia, PA, USA [2]Department of Public Health and Primary Care, Strangeways Research Laboratory, University of Cambridge, Cambridge, UK [3]Division of Cardiology, University of Ottawa Heart Institute, Ottawa, ON, Canada [4]MRC Epidemiology Unit, Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, UK [5]Institute for Molecular Medicine (FIMM), University of Helsinki, Finland [6]Department of Epidemiology, Erasmus

Medical Center, Rotterdam, The Netherlands [7]Division of Epidemiology, Public Health and Primary Care, Imperial College London, London, UK [8]Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK [9]Division of Community Health Sciences, St George's, University of London, London, UK [10]Departments of Health Sciences and Genetics, University of Leicester, Leicester, UK [11]William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University Of London, London, UK [12]Department of Forensic Molecular Biology, Erasmus University Medical Center, Rotterdam, The Netherlands [13]National Public Health Institute, Biomedicum, Helsinki, Finland [14]MRC Centre for Nutritional Epidemiology in Cancer Prevention and Survival, Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK [15]Departments of Vascular Medicine & Cardiology, Academic Medical Center, Amsterdam, The Netherlands [16]National Heart and Lung Institute, Imperial College London, London, UK [17]Laboratory of Genetics, NIA, NIH, Baltimore, MD, USA [18]Istituto di Neurogenetica e Neurofarmacologia, CNR, Monserrato, Cagliari, Italy [19]Gerontology Research Center, National Institute on Aging, Baltimore, MD, USA [20]Department of Biostatistics, School of Public Health, University of Michigan, Ann Arbor, MI, USA [21]Department of Genetics, University of North Carolina, Chapel Hill, NC, USA [22]National Public Health Institute, Department of Epidemiology and Health Promotion, Helsinki, Finland [23]Department of Internal Medicine and Biocenter Oulu, University of Oulu, Oulu, Finland [24]Gladstone Institute of Neurological Disease and Gladstone Institute of Cardiovascular Disease, San Francisco, CA, USA [25]Center for Human Nutrition, Department of Clinical Nutrition, University of Texas Southwestern Medical Center, Dallas, TX, USA [26]Avon Longitudinal Study of Parents and Children, University of Bristol, Bristol, UK [27]Department of Internal Medicine, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland [28]Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, MI, USA [29]National Institute for Health and Welfare, Helsinki, Finland [30]Cardiovascular Research Institute, MedStar Research Institute, Washington Hospital Center, Washington, DC, USA [31]The Center for Applied Genomics, Children's Hospital of Philadelphia, PA, USA [32]The Institute for Translational Medicine and Therapeutics, School of Medicine, University of Pennsylvania, Philadelphia, PA, USA [33]The Cardiovascular Institute, University of Pennsylvania, Philadelphia, PA, USA [34]LIGHT, University of Leeds, Leeds, UK [35]Heart Research Institute, Camperdown, Sydney, NSW, Australia [36]Broad Institute of Harvard and MIT, Cambridge, MA, USA

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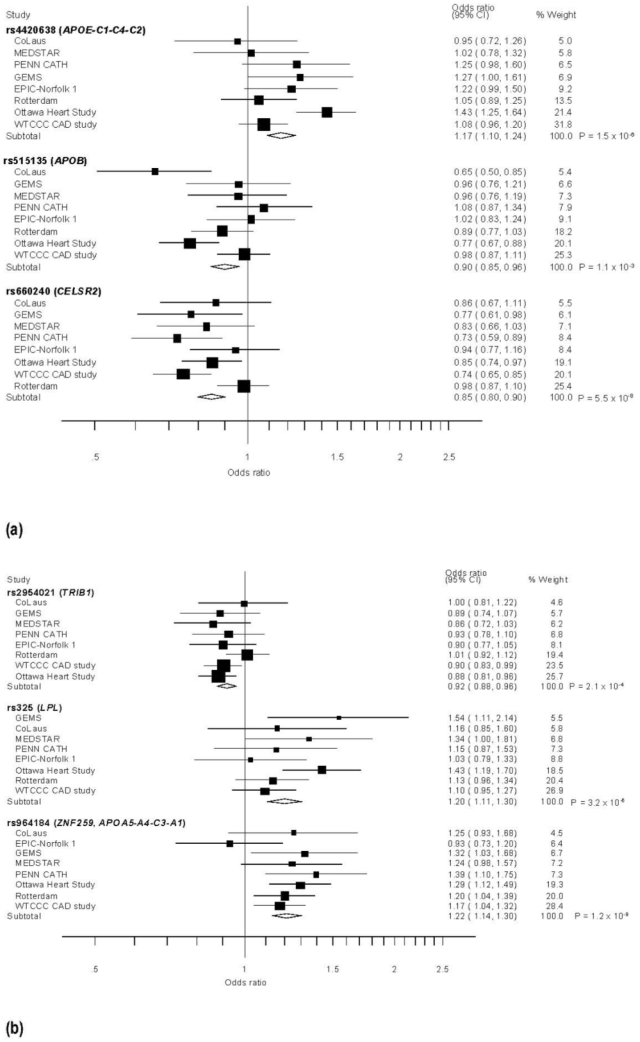

(a)

(b)

Figure 1.

Associations between SNPs at loci predominantly associated with circulating levels of (a) LDL-c and (b) TG/HDL-c with risk of CAD in eight studies comprising up to 7,018 cases and 20,765 controls (see **Methods** for details). There was no material heterogeneity among studies for these associations (Table 4).

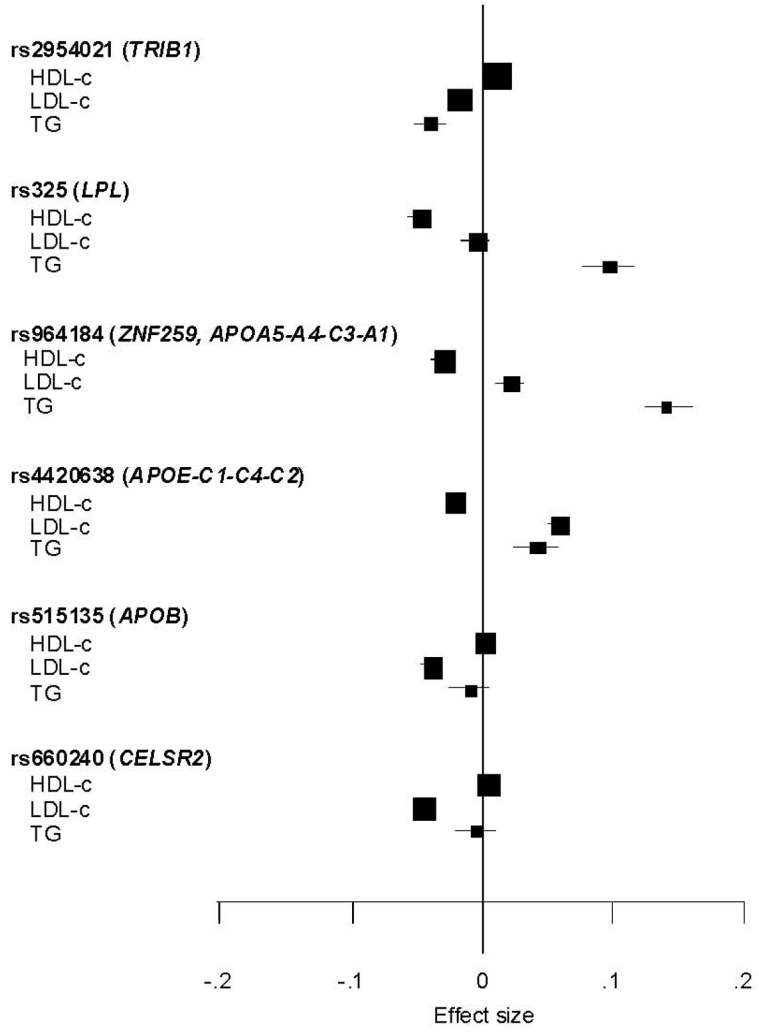

Figure 2.

Associations between SNPs and circulating lipids at known or recently identified loci that show statistical association with risk of CAD in Figure 1 and Table 4. Associations and effect sizes are based on Stage 1 meta-analyses and natural log transformed data (see Table 1 and Supplementary Table 6 for details).

Table 1

Statistical associations between SNPs showing the strongest association signal ($P < 1 \times 10^{-5}$ with no heterogeneity among studies ($P > 0.1$)) in Stage 1 with one or more lipid traits at known lipid loci

						HDL-c			TG			LDL-c		
SNP	**Chr**	**Pos (Mb)***	**Nearest locus (loci)**	**Effect allele†**	**Effect allele freq.†**	**β-coefficient (se)‡**	**P-value**	**P-value for heterogeneity**	**β-coefficient (se)‡**	**P-value**	**P-value for heterogeneity**	**β-coefficient (se)‡**	**P-value**	**P-value for heterogeneity**
LDL-c														
rs11206510	1	55.2	*PCSK9*	T	0.77	0.002 (0.004)	0.52	0.35	0.016 (0.008)	0.04	0.30	**0.026 (0.004)**	**1.2×10^{-10}**	**0.69**
rs660240	1	109.5	*CELSR2*	A	0.21	0.005 (0.004)	0.22	0.31	−0.004 (0.008)	0.56	0.67	**−0.044 (0.004)**	**1.2×10^{-26}**	**0.67**
rs515135	2	21.2	*APOB*	A	0.19	0.003 (0.004)	0.47	0.52	−0.009 (0.008)	0.25	0.67	**−0.038 (0.004)**	**2.4×10^{-20}**	**0.15**
rs12916	5	74.7	*HMGCR*	T	0.62	0.001 (0.003)	0.80	0.57	0.003 (0.006)	0.64	0.79	**−0.023 (0.003)**	**1.4×10^{-11}**	**0.67**
rs2954021	8	126.6	*TRIB1*	G	0.50	0.011 (0.003)	1.3×10^{-4}	0.24	−0.039 (0.006)	6.3×10^{-11}	0.13	**−0.017 (0.003)**	**1.4×10^{-7}**	**0.40**
rs1558861	11	116.1	*BUD13, ZNF259, APOA5-A4-C3-A1*	T	0.94	0.031 (0.006)	1.7×10^{-7}	0.09	−0.142 (0.012)	2.0×10^{-30}	5.4×10^{-3}	**−0.031 (0.006)**	**2.0×10^{-6}**	**0.28**
rs2738459	19	11.1	*LDLR*	C	0.48	−0.003 (0.004)	0.34	0.66	0.007 (0.007)	0.31	0.58	**−0.018 (0.004)**	**6.6×10^{-6}**	**0.40**
rs10401969	19	19.3	*SF4–CILP2*	T	0.91	−0.007 (0.006)	0.26	0.07	0.095 (0.013)	8.4×10^{-14}	0.04	**0.046 (0.007)**	**9.5×10^{-12}**	**0.74**
rs4420638	19	50.1	*APOE-C1-C4-C2*	G	0.18	**−0.021 (0.004)**	**2.0×10^{-7}**	**0.72**	**0.042 (0.008)**	**5.5×10^{-7}**	**0.14**	**0.059 (0.004)**	**1.7×10^{-40}**	**0.16**
HDL-c														
rs10489615	1	226.6	*GALNT2*	G	0.60	**0.018 (0.003)**	**3.8×10^{-9}**	**0.19**	−0.023 (0.006)	2.4×10^{-4}	0.24	0.004 (0.003)	0.25	0.99
rs11902417	2	21.1	*APOB*	G	0.78	**−0.017 (0.003)**	**3.7×10^{-7}**	**0.35**	0.036 (0.007)	2.7×10^{-7}	0.20	0.011 (0.004)	4.0×10^{-3}	0.16
rs325	8	19.9	*LPL*	T	0.89	**−0.047 (0.005)**	**7.8×10^{-25}**	**0.23**	0.097 (0.010)	4.9×10^{-24}	0.08	−0.005 (0.005)	0.34	0.19
rs3890182	9	104.7	*ABCA1*	G	0.88	**0.022 (0.004)**	**4.7×10^{-7}**	**0.74**	0.013 (0.009)	0.16	0.69	0.004 (0.005)	0.43	0.84
rs964184	11	116.2	*ZNF259, APOA5-A4-C3-A1*	G	0.12	**−0.029 (0.004)**	**1.6×10^{-11}**	**0.17**	0.142 (0.009)	9.0×10^{-53}	1.3×10^{-3}	0.022 (0.005)	6.4×10^{-6}	5.6×10^{-3}
rs9943753	12	108.3	*MYO1H, KCTD10, UBE3B, MMAB, MVK*	G	0.63	**0.016 (0.003)**	**3.2×10^{-6}**	**0.94**	−0.005 (0.007)	0.51	0.89	−0.005 (0.004)	0.20	0.92
rs261334	15	56.5	*LIPC*	G	0.20	**0.034 (0.004)**	**4.9×10^{-22}**	**0.72**	0.019 (0.007)	0.01	0.79	−0.002 (0.004)	0.65	0.59
rs9989419	16	55.5	*CETP*	G	0.60	**0.035 (0.003)**	**1.3×10^{-32}**	**0.28**	0.003 (0.006)	0.67	0.38	−0.002 (0.003)	0.58	0.82
rs12449157	16	66.3	*GFOD2–LCAT*	G	0.17	**0.019 (0.004)**	**2.3×10^{-7}**	**0.56**	−0.018 (0.008)	0.02	0.10	0.004 (0.004)	0.31	0.24
rs2156552	18	45.4	*LIPG*	T	0.81	**0.028 (0.004)**	**1.7×10^{-12}**	**0.73**	−0.020 (0.008)	0.02	0.87	0.011 (0.004)	0.01	0.20
TG														
rs1168013	1	62.7	*DOCK7, ANGPTL3*	G	0.65	0.0001 (0.003)	0.97	0.88	**0.035 (0.007)**	**6.4×10^{-8}**	**0.88**	0.009 (0.003)	6.7×10^{-3}	0.78
rs6544366	2	21.1	*APOB*	T	0.22	0.016 (0.003)	5.3×10^{-7}	0.34	**−0.036 (0.007)**	**1.9×10^{-7}**	**0.20**	−0.011 (0.004)	3.8×10^{-3}	0.14
rs1260333	2	27.7	*GCKR*	C	0.55	0.005 (0.003)	0.08	0.87	**−0.054 (0.006)**	**1.7×10^{-19}**	**0.22**	−0.003 (0.003)	0.36	0.99
rs1178979	7	72.3	*BAZ1B, BCL7B, TBL2, MLXIPL*	A	0.80	−0.010 (0.004)	8.0×10^{-3}	0.83	**0.054 (0.008)**	**2.3×10^{-12}**	**0.71**	−0.012 (0.004)	2.5×10^{-3}	0.82
rs10105606	8	19.9	*LPL*	C	0.68	−0.023 (0.003)	1.7×10^{-14}	0.68	**0.067 (0.006)**	**3.6×10^{-25}**	**0.14**	0.0003 (0.003)	0.94	0.37
rs2954029	8	126.6	*TRIB1*	T	0.46	0.012 (0.003)	4.5×10^{-5}	0.33	**−0.040 (0.006)**	**1.8×10^{-11}**	**0.13**	−0.015 (0.003)	9.2×10^{-7}	0.14

						HDL-c		TG			LDL-c		
SNP	**Chr**	**Pos (Mb)***	**Nearest locus (loci)**	**Effect allele†**	**Effect allele freq.†**	**β-coefficient (se)‡**	**P-value**	**β-coefficient (se)‡**	**P-value**	**P-value for heterogeneity**	**β-coefficient (se)‡**	**P-value**	**P-value for heterogeneity**
rs4938303	11	116.1	*BUD13, ZNF259, APOA5-A4-C3-A1*	T	0.75	0.018 (0.003)	9.6×10^{-8}	0.37	**−0.067 (0.007)**	**4.1×10^{-21}**	**0.52**	−0.008 (0.004)	0.02	0.28
rs16965220	16	55.6	*CETP, LOC100130044, NLRC5*	C	0.68	−0.006 (0.003)	0.04	0.07	**0.028 (0.006)**	**9.6×10^{-6}**	**0.16**	0.009 (0.003)	0.01	0.92
rs2304130	19	19.7	*CILP2–ZNF101*	G	0.09	0.004 (0.006)	0.55	0.78	**−0.070 (0.013)**	**3.9×10^{-8}**	**0.36**	−0.036 (0.007)	1.1×10^{-7}	0.51

The genome-wide association meta-analyses (Stage 1) for HDL-c and TG are based on data from seven study populations comprising up to 16,056 and 16,058 participants, respectively. For LDL-c, the meta-analysis is based on data from eight study populations comprising up to 17,543 participants. The strongest SNP association for each lipid trait with no heterogeneity among studies (P > 0.1) is denoted by bold typeface.’

* Based on NCBI Build 35.

† Based on Study 1 (EPIC-Norfolk sub-cohort). Effect allele corresponds to forward strand of NCBI Build 36.3.

‡ Beta-coefficients represent the change in circulating lipid level (natural log) per additional effect allele, adjusted for age and sex.

Table 2

SNPs at novel or recently identified lipid loci that show replication of statistical associations with circulating lipid levels

						Stage 2				Combined			
SNP	**Chr**	**Pos (Mb)***	**Nearest locus**	**Effect allele†**	**Effect allele freq.†**	**No. participants**	**β-coefficient (se) ‡**	**P-value**	**P-value for heterogeneity**	**No. participants**	**β-coefficient (se)‡**	**P-value**	**P-value for heterogeneity**
LDL-c													
rs2142672	6	16.3	*MYLIP, GMPR*	C	0.74	28,112	0.010 (0.003)	2.0×10^{-4}	0.30	45,655	0.013 (0.002)	2.7×10^{-9}	0.30
rs456598	6	160.5	*IGF2R, SLC22A1*	G	0.87	19,882	−0.010 (0.004)	0.01	0.79	37,425	−0.015 (0.003)	8.4×10^{-7}	0.58
rs2126259	8	9.2	*PPP1R3B*	A	0.10	28,145	−0.014 (0.004)	9.5×10^{-5}	0.13	45,688	−0.018 (0.003)	1.4×10^{-9}	0.10
HDL-c													
rs13107325	4	103.5	*SLC39A8*	T	0.08	22,128	−0.017 (0.006)	2.1×10^{-3}	0.24	38,184	−0.023 (0.004)	1.6×10^{-8}	0.37
rs643531	9	15.3	*TTC39B*	C	0.14	34,152	−0.009 (0.003)	2.6×10^{-3}	0.60	50,208	−0.013 (0.002)	4.1×10^{-8}	0.24
rs174548	11	61.3	*FADS1*	G	0.30	33,930	−0.008 (0.002)	7.6×10^{-5}	0.78	49,986	−0.011 (0.002)	9.9×10^{-10}	0.59
TG													
rs442177	4	88.4	*AFF1*	A	0.60	28,676	0.014 (0.004)	1.2×10^{-3}	0.99	44,734	0.019 (0.004)	1.5×10^{-7}	0.71
rs6867983	5	55.9	*C5orf35*	T	0.14	23,957	0.014 (0.007)	0.04	0.15	40,015	0.024 (0.005)	6.1×10^{-6}	0.10
rs174548	11	61.3	*FADS1*	G	0.30	31,066	0.019 (0.004)	2.0×10^{-5}	0.98	47,124	0.024 (0.004)	8.9×10^{-11}	0.78

The replication analysis (Stage 2) is based on data from up to eight study populations comprising up to 37,774 participants.

The combined analysis is based on data from up to 15 studies from Stages 1 and 2 and comprising up to 55,497 participants.

* Based on NCBI Build 35.

† Based on Study 1 (EPIC-Norfolk sub-cohort). Effect allele corresponds to forward strand of NCBI Build 36.3.

‡ Beta-coefficients represent the change in circulating lipid level (natural log) per additional effect allele, adjusted for age and sex.

Table 3

Statistical associations between Stage 1 SNPs at putative novel or recently identified lipid loci with circulating lipid levels in individuals of Indian Asian ethnicity and a combined analysis of all studies

						Stage 3			Combined			
SNP	**Chr**	**Pos (Mb)***	**Nearest locus**	**Effect allele†**	**Effect allele freq.†**	**β-coefficient (se)‡**	**P-value**	**P-value for heterogeneity**	**β-coefficient (se)‡**	**P-value**	**P-value for heterogeneity between ethnic groups**	**P-value for overall heterogeneity between studies**
LDL-c												
rs2142672	6	16.3	*MYLIP, GMPR*	C	0.68	0.002 (0.005)	0.72	0.93	0.011 (0.002)	2.2×10^{-8}	0.04	0.28
rs456598	6	160.5	*IGF2R, SLC22A1*	G	-	-	-	-	-	-	-	-
rs2126259	8	9.2	*PPP1R3B*	A	0.13	−0.023 (0.007)	9.6×10^{-4}	0.02	−0.019 (0.003)	6.5×10^{-12}	0.54	0.03
HDL-c												
rs13107325	4	103.5	*SLC39A8*	T	-	-	-	-	-	-	-	-
rs643531	9	15.3	*TTC39B*	C	0.07	−0.013 (0.007)	0.07	0.77	−0.013 (0.002)	7.3×10^{-9}	0.99	0.39
rs174548	11	61.3	*FADS1*	G	0.17	−0.017 (0.004)	1.1×10^{-4}	0.24	−0.011 (0.002)	1.2×10^{-12}	0.19	0.44
TG												
rs442177	4	88.4	*AFF1*	A	0.50	0.027 (0.007)	2.9×10^{-4}	0.27	0.020 (0.003)	3.1×10^{-10}	0.31	0.62
rs6867983	5	55.9	*C5orf35*	T	0.12	0.016 (0.012)	0.16	0.97	0.023 (0.005)	2.6×10^{-6}	0.56	0.21
rs174548	11	61.3	*FADS1*	G	0.17	0.041 (0.010)	2.6×10^{-5}	0.13	0.026 (0.003)	4.5×10^{-14}	0.09	0.44

The Stage 3 analysis for circulating HDL-c, LDL-c and TG levels is based on data from up to 9,665 participants from four subsets of the LOLIPOP study (see Supplementary Table 2 for details).

* Based on NCBI Build 35.

† Effect allele corresponds to forward strand of NCBI Build 36.3 and effect allele frequency is based on the control subset of LOLIPOP participants genotyped by the Wellcome chip (Supplementary Table 2).

‡ Beta-coefficients represent the change in circulating lipid level (natural log) per additional effect allele, adjusted for age and sex.

Table 4

Statistical associations between Stage 1 SNPs at known/recently identified and putative novel loci with risk of CAD in individuals of white European ethnicity

SNP	Chr	Pos (Mb)*	Nearest locus (loci)	Effect allele†	Effect allele freq.†	No. cases/controls	Odds ratio (95% confidence interval)‡	P-value	P-value for heterogeneity
rs11206510	1	55.2	*PCSK9*	T	0.77	6,988/19,945	1.07 (1.01–1.13)	0.02	0.31
rs1168013	1	62.7	*DOCK7, ANGPTL3*	G	0.65	7,002/20,029	0.96 (0.91–1.00)	0.06	0.07
rs660240	**1**	**109.5**	***CELSR2***	**A**	**0.21**	**6,207/19,638**	**0.85 (0.80–0.90)**	**5.5×10^{-8}**	**0.06**
rs10489615	1	226.6	*GALNT2*	G	0.60	6,330/19,838	0.97 (0.93–1.02)	0.28	0.21
rs11902417	2	21.1	*APOB*	G	0.78	7,002/20,040	1.01 (0.96–1.07)	0.63	0.06
rs6544366	2	21.1	*APOB*	T	0.22	6,976/20,018	0.98 (0.93–1.04)	0.56	0.13
rs515135	**2**	**21.2**	***APOB***	**A**	**0.19**	**6,449/20,031**	**0.90 (0.85–0.96)**	**1.1×10^{-3}**	**0.01**
rs1260333	2	27.7	*GCKR*	C	0.55	6,955/19,981	0.94 (0.90–0.98)	6.9×10^{-3}	0.20
rs442177	4	88.4	*AFF1*	A	0.60	8,187/32,167	1.02 (0.98–1.06)	0.40	0.41
rs13107325	4	103.5	*SLC39A8*	T	0.08	4,328/20,585	0.89 (0.79–0.99)	0.04	0.69
rs6867983	5	55.9	*C5orf35*	T	0.14	8,744/32,520	1.02 (0.97–1.08)	0.40	0.49
rs12916	5	74.7	*HMGCR*	T	0.62	6,928/19,936	0.94 (0.90–0.99)	0.01	0.76
rs2142672	6	16.3	*MYLIP, GMPR*	C	0.74	8,125/32,346	1.03 (0.98–1.07)	0.26	0.90
rs456598	6	160.5	*IGF2R, SLC22A1*	G	0.87	5,593/22,096	0.90 (0.84–0.97)	7.3×10^{-3}	0.39
rs1178979	7	72.3	*BAZ1B, BCL7B, TBL2, MLXIPL*	A	0.80	6,990/20,026	1.03 (0.97–1.09)	0.31	0.36
rs2126259	8	9.2	*PPP1R3B*	A	0.10	8,258/32,517	1.01 (0.95–1.08)	0.73	0.09
rs325	**8**	**19.9**	***LPL***	**T**	**0.89**	**6,881/19,882**	**1.20 (1.11–1.30)**	**3.2×10^{-6}**	**0.20**
rs10105606	8	19.9	*LPL*	C	0.68	6,825/19,797	1.07 (1.02–1.12)	5.6×10^{-3}	0.67
rs2954029	8	126.6	*TRIB1*	T	0.46	6,997/20,734	0.93 (0.89–0.97)	7.4×10^{-4}	0.30
rs2954021	**8**	**126.6**	***TRIB1***	**G**	**0.50**	**7,018/20,765**	**0.92 (0.88–0.96)**	**2.1×10^{-4}**	**0.56**
rs643531	9	15.3	*TTC39B*	C	0.14	9,075/34,589	0.98 (0.93–1.04)	0.50	0.37
rs3890182	9	104.7	*ABCA1*	G	0.88	7,003/20,036	0.96 (0.90–1.03)	0.28	0.23
rs174548	11	61.3	*FADS1*	G	0.30	9,068/34,364	1.01 (0.97–1.06)	0.52	0.45
rs4938303	11	116.1	*BUD13, ZNF259, APOA5-A4-C3-A1*	T	0.75	6,601/19,638	0.93 (0.89–0.98)	9.5×10^{-3}	0.11
rs1558861	11	116.1	*BUD13, ZNF259, APOA5-A4-C3-A1*	T	0.94	4,654/13,359	0.88 (0.77–1.00)	0.04	0.75

SNP	Chr	Pos (Mb)*	Nearest locus (loci)	Effect allele†	Effect allele freq.†	No. cases/controls	Odds ratio (95% confidence interval)‡	P-value	P-value for heterogeneity
rs964184	**11**	**116.2**	***ZNF259, APOA5-A4-C3-A1***	**G**	**0.12**	**6,958/20,001**	**1.22 (1.14–1.30)**	**1.2×10^{-9}**	**0.42**
rs9943753	12	108.3	*MYO1H, KCTD10, UBE3B, MMAB, MVK*	G	0.63	3,540/15,657	1.01 (0.94–1.09)	0.75	0.51
rs261334	15	56.5	*LIPC*	G	0.20	6,414/19,980	1.05 (0.99–1.11)	0.10	0.71
rs9989419	16	55.5	*CETP*	G	0.60	6,991/20,009	1.01 (0.96–1.06)	0.71	0.16
rs16965220	16	55.6	*CETP, LOC100130044, NLRC5*	C	0.68	7,001/20,029	1.00 (0.95–1.05)	0.94	0.49
rs12449157	16	66.3	*GFOD2–LCAT*	G	0.17	6,935/19,961	0.96 (0.90–1.02)	0.16	0.73
rs2156552	18	45.4	*LIPG*	T	0.81	6,991/20,034	0.97 (0.91–1.03)	0.32	0.25
rs2738459	19	11.1	*LDLR*	C	0.48	3,540/15,657	0.96 (0.89–1.03)	0.23	0.05
rs10401969	19	19.3	*SF4–CILP2*	T	0.91	6,723/19,654	1.08 (0.97–1.20)	0.17	0.58
rs2304130	19	19.7	*CILP2–ZNF101*	G	0.09	3,540/15,657	1.02 (0.90–1.16)	0.74	0.22
rs4420638	**19**	**50.1**	***APOE-C1-C4-C2***	**G**	**0.18**	**7,004/20,033**	**1.17 (1.10–1.24)**	**1.5×10^{-6}**	**0.02**

The meta-analysis is based on data from nine studies comprising up to 9,633 cases and 38,684 controls.

SNPs showing both genome-wide statistical association with lipids (Table 1) and statistical association with CAD risk after adjustment for multiple testing ($P < 0.0013$ after testing 36 SNPs) are denoted by bold typeface.

* Based on NCBI Build 35.

† Based on Study 1 (EPIC-Norfolk sub-cohort). Effect allele corresponds to forward strand of NCBI Build 36.3

‡ Odds ratios are based on the additive model