Common variants associated with plasma triglycerides and risk for coronary artery disease

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

Abstract

Triglycerides are transported in plasma by specific triglyceride-rich lipoproteins; in epidemiologic studies, increased triglyceride levels correlate with higher risk for coronary artery disease (CAD). However, it is unclear whether this association reflects causal processes. We used 185 common variants recently mapped for plasma lipids ($P<5 \times 10^{-8}$ for each) to examine the role of triglycerides on risk for CAD. First, we highlight loci associated with both low-density lipoprotein cholesterol (LDL-C) and triglycerides, and show that the direction and magnitude of both are factors in determining CAD risk. Second, we consider loci with only a strong magnitude of association with triglycerides and show that these loci are also associated with CAD. Finally, in a model accounting for effects on LDL-C and/or high-density lipoprotein cholesterol, a polymorphism’s strength of effect on triglycerides is correlated with the magnitude of its effect on CAD risk. These results suggest that triglyceride-rich lipoproteins causally influence risk for CAD.
LDL-C or HDL-C\textsuperscript{16-18}, violating the “no pleiotropy” assumption of instrumental variable analysis\textsuperscript{8,19}.

Here, we utilize common variants and develop a statistical framework to dissect causal influences among a set of correlated biomarkers. As this approach requires a large set of SNPs where precise measurements of effect on triglycerides, LDL-C, HDL-C, and CAD risk are simultaneously available, we leveraged: 1) 185 common SNPs all representing independent loci that are associated with at least one lipid trait at genome-wide levels of significance; 2) estimates of effect of each SNP on plasma triglycerides, LDL-C, and HDL-C in a sample exceeding 180,000 individuals; and 3) estimates of effect of each SNP on CAD in a sample exceeding 86,000 individuals (22,233 cases and 64,762 controls).

We studied 185 SNPs at 157 one megabase pair intervals with association $P<5\times10^{-8}$ for triglycerides, LDL-C, or HDL-C in a meta-analysis involving 188,578 genotyped individuals (see companion manuscript\textsuperscript{20}). For each SNP, we obtained effect estimates for triglycerides ($\beta_{\text{TRIGLYCERIDES}}$), LDL-C ($\beta_{\text{LDL-C}}$), and HDL-C ($\beta_{\text{HDL-C}}$) (in standard deviation units and estimated using inverse normal transformed residuals of lipid levels after adjusting for covariates; see Supplementary Figure 1 for study design). We also estimated the effect of each SNP on CAD ($\beta_{\text{CAD}}$) from a recently published genome-wide association study (GWAS) involving 86,995 individuals (the CARDIoGRAM study)\textsuperscript{21}. For the 185 SNPs, effect sizes ($\beta$) and $P$-values for triglycerides, LDL-C, HDL-C, and CAD are shown in Supplementary Table 1.

We considered several analytic approaches to investigate whether plasma triglycerides reflect processes causal for CAD. First, we evaluated the direction and magnitude of $\beta_{\text{LDL-C}}$ and $\beta_{\text{TRIGLYCERIDES}}$ in combination, and then compared these to $\beta_{\text{CAD}}$ (Figure 1 and Supplementary Figure 2). Second, to isolate the effect of triglycerides, from the 185 SNPs, we restricted analysis to loci that have moderate to strong effect on triglycerides (large $\beta_{\text{TRIGLYCERIDES}}$) but minimal effect on LDL-C (small $\beta_{\text{LDL-C}}$). Finally, across the 185 SNPs, we formally developed and applied a statistical framework to test if the effect size of a SNP on triglycerides is linearly related to its effect size on CAD, before and after accounting for the same SNP’s potential effect on plasma LDL-C and/or HDL-C.

For each of the 185 independent lipid SNPs, we evaluated joint patterns of associations for triglycerides and LDL-C by examining SNPs that have strong association to both triglycerides and LDL-C ($P<5\times10^{-8}$for each). Among these, we examined SNPs with the same direction and a similar magnitude of association for both lipid traits (within a factor of 5). We observed 11 loci with this pattern of association. Five loci confer risk for CAD ($P<0.05$) and ten of the eleven loci show a direction of effect consistent between the lipid traits and CAD (Table 1). For example, the A allele at rs2954022 in the TRIB1 gene was associated strongly with lower triglycerides ($\beta_{\text{TRIGLYCERIDES}}=-0.078$, $P=2\times10^{-124}$) and lower LDL-C ($\beta_{\text{LDL-C}}=-0.055$, $P=4\times10^{-51}$) and showed the expected association with lower CAD risk ($\beta_{\text{CAD}}=-0.056$, $P=6\times10^{-5}$).

Next, we identified SNPs that had strong association with both triglycerides and LDL-C ($P<5\times10^{-8}$ for each) but had opposite directions for $\beta_{\text{TRIGLYCERIDES}}$ and $\beta_{\text{LDL-C}}$ (within a factor of 5, Table 2). Four SNPs displayed this pattern and none showed significant association with CAD (all $P>0.05$). For example, the A allele at rs2255141 in the GPAM gene was associated with lower triglycerides ($\beta_{\text{TRIGLYCERIDES}}=-0.078$, $P=2\times10^{-124}$) and higher LDL-C ($\beta_{\text{LDL-C}}=0.030$, $P=7\times10^{-14}$) but had no discernible effect on CAD risk ($\beta_{\text{CAD}}=-0.0076$, $P=0.63$).
Secondly, we considered a subset of the 185 SNPs that have moderate to strong effects on triglycerides but minimal effect on LDL-C \( n=44 \) SNPs, all SNPs have large \( \beta_{\text{TRIGLYCERIDES}} > 0.01 \) or \( < -0.01 \) but small \( \beta_{\text{LDL-C}} \) (between \( -0.01 \) and \( 0.01 \)). In regression analysis, we confirmed that \( \beta_{\text{LDL-C}} \) was not associated with \( \beta_{\text{CAD}} \) for this set of SNPs (\( P=0.68 \); see Supplementary Table 2). However, we observed a significant association of \( \beta_{\text{TRIGLYCERIDES}} \) and \( \beta_{\text{CAD}} \) (\( P=3 \times 10^{-5} \); see Supplementary Table 3). These observations suggest that the direction and magnitude of effect of a SNP on both triglycerides and LDL-C impact risk for CAD.

To formally investigate whether the strength of a SNP’s association with triglycerides predicts CAD risk, we devised a statistical framework that controls for pleiotropic effects on secondary lipid traits. This approach is particularly important because SNP association signals with triglycerides, LDL-C, and/or HDL-C \( (\beta_{\text{TRIGLYCERIDES}}, \beta_{\text{LDL-C}}, \text{ and } \beta_{\text{HDL-C}}) \) are correlated (Supplementary Figure 3 and Supplementary Table 4).

We tested the role of triglycerides on CAD by first calculating residuals of \( \beta_{\text{CAD}} \) after including as covariates \( \beta_{\text{LDL-C}} \) and \( \beta_{\text{HDL-C}} \) in our regression model (Supplementary Figure 1). We then tested the association of \( \beta_{\text{TRIGLYCERIDES}} \) with \( \beta_{\text{CAD}} \) residuals. Similar models were created to assess the independent roles of LDL-C and HDL-C.

We observed that across the 185 SNPs, \( \beta_{\text{LDL-C}} \) was strongly associated with \( \beta_{\text{CAD}} \), after adjusting for either \( \beta_{\text{TRIGLYCERIDES}} \) individually, \( \beta_{\text{HDL-C}} \) individually, or both \( \beta_{\text{TRIGLYCERIDES}} \) and \( \beta_{\text{HDL-C}} \) (all \( P < 1 \times 10^{-18} \), Table 3). The pattern for \( \beta_{\text{HDL-C}} \) was different. Across the 185 SNPs, \( \beta_{\text{HDL-C}} \) was associated with \( \beta_{\text{CAD}} \), after adjusting for \( \beta_{\text{LDL-C}} \) (\( P=0.005 \)); however, this association was greatly attenuated after adjusting for \( \beta_{\text{TRIGLYCERIDES}} \) individually (\( P=0.057 \)) and rendered non-significant after accounting for both \( \beta_{\text{TRIGLYCERIDES}} \) and \( \beta_{\text{LDL-C}} \) (\( P=0.35 \), Table 3).

The results for triglycerides were similar to those observed for LDL-C. Across the 185 SNPs, \( \beta_{\text{TRIGLYCERIDES}} \) was strongly associated with \( \beta_{\text{CAD}} \), after adjusting for both \( \beta_{\text{LDL-C}} \) and \( \beta_{\text{HDL-C}} \) (\( P=1 \times 10^{-9} \), Table 3).

As an alternative to this approach using residuals, we also tested a single model with the outcome variable of \( \beta_{\text{CAD}} \) and predictor variables of \( \beta_{\text{TRIGLYCERIDES}}, \beta_{\text{LDL-C}}, \text{ and } \beta_{\text{HDL-C}} \) considered jointly (Supplementary Table 5). Results were similar with \( \beta_{\text{TRIGLYCERIDES}} \) and \( \beta_{\text{LDL-C}} \) showing association with \( \beta_{\text{CAD}} \) (\( P=2 \times 10^{-10} \) and \( P=1 \times 10^{-22} \), respectively) but \( \beta_{\text{HDL-C}} \) failing to show association (\( P=0.32 \)).

In summary, we have demonstrated that: 1) SNPs with the same direction and a similar magnitude of association for both triglycerides and LDL-C tend to associate with CAD risk; 2) loci that have an exclusive effect on triglycerides are also associated with CAD; and 3) the strength of a SNP’s effect on triglycerides is correlated with the magnitude of its effect on CAD risk, even after accounting for the same SNP’s effect on LDL-C and/or HDL-C.

Using an analytical approach that accounts for the potential pleiotropic effects of a SNP on triglycerides, LDL-C, and/or HDL-C, we provide evidence that plasma triglycerides likely reflects processes causal for CAD. This finding based on 185 common SNPs is in line with recent reports of specific genes predominantly related to triglycerides also affecting risk for CAD. A promoter SNP in the \textit{APOA5} gene\(^{22}\), a common SNP upstream of the \textit{TRIB1} gene\(^{23}\), and a nonsense polymorphism at the \textit{APOC3} gene\(^{24}\) all predominantly associate with plasma triglycerides and each SNP has been convincingly related to clinical CAD\(^{11,25}\) or subclinical atherosclerosis\(^{24}\).
Our results raise several questions. First, if plasma triglycerides reflect causal processes, what are the specific mechanistic direct links to atherosclerosis? Triglycerides are carried in plasma mostly in VLDL, chylomicrons and remnants of their metabolism and as such, triglycerides capture several physiologic processes that may promote atherosclerosis. One potential link is post-prandial cholesterol metabolism. Plasma triglycerides are highly correlated with the amount of cholesterol in remnant lipoproteins (i.e., VLDL and chylomicron particles after interaction with lipoprotein lipase) and a variety of evidence ranging from the human Mendelian disorder of Type III hyperlipoproteinemia to experimental evidence in cell culture and animal models suggests that cholesterol-rich remnant particles have pro-atherogenic properties similar to LDL (reviewed in 26). Another process reflected by plasma triglycerides is the activity of lipoprotein lipase, a key enzyme that hydrolyzes triglycerides within triglyceride-rich lipoproteins. Higher enzymatic activity of lipoprotein lipase in the circulation leads to lower plasma triglycerides; a gain-of-function nonsense polymorphism in the LPL gene has been shown to not only reduce plasma triglyceride levels but also lower risk for CAD 27.

Second, why are plasma triglycerides not significantly associated with CAD in observational epidemiologic studies when multiple risk factors are considered jointly to predict risk for future CAD2? Multivariable models have known limitations for assessing the etiological relevance for a given exposure. For example, an exposure may be rendered non-significant after multivariable adjustment because of less precise measurement or greater biologic variability when compared with other factors. Plasma triglyceride measurements are more variable than other plasma lipids such as HDL-C 26. Alternatively, downstream effects of an exposure may more completely capture the risk conferred. For example, body mass index does not predict CAD risk in the Framingham model after accounting for blood pressure and type 2 diabetes despite the accepted causal influence of weight on blood pressure and type 2 diabetes 28. Our approach using SNPs as proxies overcomes these limitations of observational epidemiology.

Finally, what are the implications of these data for the development of drugs aimed at lowering plasma triglycerides with the hope of reducing CAD risk? Several recent randomized controlled trials have tested whether the lowering of plasma triglycerides with fish oils 29 or with fibrates 30-32 will decrease risk for CAD and in many cases, treatment did not reduce risk 29,31,32. Possible explanations for failed trials are wrong study population, wrong mechanism of lowering triglycerides, insufficient degree of triglyceride-lowering, and limited statistical power.

Our study has several limitations. SNPs associated with triglycerides also relate to other lipid traits and thus, are not ideal instruments for Mendelian randomization analysis. Given that the plasma triglycerides measured in the blood is the end product of several metabolic processes, it is not surprising that triglyceride-related SNPs affect at least one other lipid trait. We have attempted to address this complexity through our statistical approach.

We are unable to distinguish if only specific mechanisms of altering triglycerides affect risk for CAD. Of note, there is strong evidence that at least three mechanisms that robustly influence triglycerides – loss of APOA5 function, loss of TRIB1 function, and gain of APOC3 function – increase risk for CAD.

In summary, we utilize common polymorphisms and employ a statistical framework to dissect causal influences among a set of correlated biomarkers. By applying this framework to a correlated set of plasma lipid measures and CAD risk, we suggest a causal role of triglyceride-rich lipoproteins in the development of CAD.
Online Methods

For the association of a given SNP with a plasma lipid trait, we obtained estimates of the effect size ($\beta_{\text{TRIGLYCERIDES}}$, $\beta_{\text{LDL-C}}$ and $\beta_{\text{HDL-C}}$) and strength of association ($P$-value) from a meta-analysis of association results from genome-wide and custom-array genotyping – the Global Lipids Genetics Consortium (GLGC) Metabochip study (described in companion manuscript, Willer et al.20). All effect sizes are in standard deviation units from inverse normal transformed residuals of lipids after adjusting for covariates. This analysis included up to 188,578 individuals from 60 studies. For the association of a given SNP with coronary artery disease (CAD), we obtained estimates of the effect size ($\beta_{\text{CAD}}$) and strength of association ($P$-value) from a published GWAS study for CAD, the CARDIoGRAM study21. This study included 22,233 cases and 63,762 controls.

We selected independent SNPs associated with plasma lipids using the following criteria. First, we restricted to SNPs with association with at least one of the three lipid traits (triglycerides, LDL-C or HDL-C) at a genome-wide significance level of $P<5\times10^{-8}$. For each lipid locus – defined as a region of the genome that has a cluster of associated SNPs within one megabase from each other – we selected the strongest associated SNP (‘lead’ SNP). For loci with multiple associated SNPs, we calculated pairwise linkage disequilibrium (LD) estimates ($r^2$) of these SNPs using whole genome sequencing data from 85 Utah residents with ancestry from northern and western Europe (CEU) samples from the 1000 Genomes project33, and selected a second SNP if there was very low LD ($r^2<0.05$) with the lead SNP. In total, we selected 185 SNPs that met these criteria. These criteria yield a conservative estimate of the number of independent lipid SNPs. A list of effect sizes and $P$-values for triglycerides, LDL-C, HDL-C and CAD for the 185 selected SNPs is shown in Supplementary Table 1.

To formally investigate whether the strength of a SNP’s association with triglycerides predicts CAD risk, we performed linear regression on the effect sizes of each SNP for triglycerides ($\beta_{\text{TRIGLYCERIDES}}$), LDL-C ($\beta_{\text{LDL-C}}$), HDL-C ($\beta_{\text{HDL-C}}$) as predictor variables, and the effect sizes of CAD ($\beta_{\text{CAD}}$) as the outcome variable. To control for pleiotropic effects, we first calculated the residuals of $\beta_{\text{CAD}}$ after adjusting for covariates of $\beta_{\text{TRIGLYCERIDES}}$, $\beta_{\text{LDL-C}}$ and/or $\beta_{\text{HDL-C}}$. We then performed linear regression analysis in a second model on the effect size of the primary lipid trait ($\beta_{\text{TRIGLYCERIDES}}$, $\beta_{\text{LDL-C}}$ or $\beta_{\text{HDL-C}}$) with the residuals of $\beta_{\text{CAD}}$. For example, to test for the role of LDL-C on CAD, we first calculated residuals of $\beta_{\text{CAD}}$ after including as covariates $\beta_{\text{TRIGLYCERIDES}}$ and $\beta_{\text{HDL-C}}$ in our regression model. In a second regression model, we then performed association of residual $\beta_{\text{CAD}}$ with $\beta_{\text{LDL-C}}$. All possible combinations of linear regression analysis was performed between $\beta_{\text{TRIGLYCERIDES}}$, $\beta_{\text{LDL-C}}$ or $\beta_{\text{HDL-C}}$ on $\beta_{\text{CAD}}$ (see Table 3).

As an alternative to this residuals approach, we also tested a single model where the outcome variable of $\beta_{\text{CAD}}$ was tested with the predictor variables of $\beta_{\text{TRIGLYCERIDES}}$, $\beta_{\text{LDL-C}}$ and $\beta_{\text{HDL-C}}$ jointly considered (Supplementary Table 5). We also performed several sensitivity analyses to test for the effect of using different thresholds on $\beta_{\text{TRIGLYCERIDES}}$ and $\beta_{\text{LDL-C}}$ when highlighting loci with associations for both triglycerides and LDL-C (Supplementary Table 6, 7 and 8). We used thresholds that yielded the highest number of SNPs for each statistical analysis (factor threshold of 5 in Table 1 and Table 2, and $\beta$ cutoff value of 0.01 in Supplementary Table 2 and 3). Furthermore, we assessed the effect of extreme influential outliers using Cook’s D statistic34 (Supplementary Figure 4 and Supplementary Table 9) on our conditional regression models (Table 3). A list of the number of SNPs included in each of the different analyses are shown in Supplementary Table 10.
Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Authors

Affiliations

1Cardiovascular Research Center, Massachusetts General Hospital, Boston, Massachusetts 02114, USA 2Center for Human Genetic Research, Massachusetts General Hospital, Boston, Massachusetts 02114, USA 3Department of Medicine, Harvard Medical School, Boston, Massachusetts 02115, USA 4Program in Medical and Population Genetics, Broad Institute, 7 Cambridge Center, Cambridge, MA 02142, USA 5Department of Internal Medicine, Division of Cardiovascular Medicine, University of Michigan, Ann Arbor, Michigan 48109, USA 6Department of Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, Michigan 48109, USA 7Department of Human Genetics, University of Michigan, Ann Arbor, Michigan 48109, USA 8Center for Statistical Genetics, Department of Biostatistics, Boston University School of Public Health, Boston, Massachusetts 02118, USA 9Department of Medical Sciences, Molecular Epidemiology, Uppsala University, Uppsala, Sweden 10Science for Life Laboratory, Uppsala University, Uppsala, Sweden 11Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, CB10 1SA, Hinxton, United Kingdom 12Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden 13Department of Genetics, University of North Carolina, Chapel Hill, NC 27599 USA 14Division of Preventive Medicine, Brigham and Women’s Hospital, 900 Commonwealth Ave., Boston MA 02215, USA 15Harvard Medical School, Boston MA 02115, USA 16Service of Medical Genetics, Lausanne University Hospital, Lausanne, Switzerland 17Department of Medical Genetics, University of Lausanne, Lausanne, Switzerland 18Division of Preventive Medicine and Health Services Research, Institute of Population Health Sciences, National Health Research Institutes, Zhunan, Taiwan 19Genetic Epidemiology Unit, Department of Epidemiology, Erasmus University Medical Center, Rotterdam, The Netherlands
21Department of Neurology, Erasmus Medical Center, Rotterdam, The Netherlands
22Medical Research Institute, University of Dundee, Ninewells Hospital and Medical
School, Dundee, DD1 9SY, United Kingdom 23Cardiology, Department of
Specialities of Medicine, Geneva University Hospital, Rue Gabrielle-Perret-Gentil 4,
1211 Geneva 14, Switzerland 24Center for Complex Disease Genomics, McKusick-
Nathans Institute of Genetic Medicine, Johns Hopkins University School of
Medicine, Baltimore, MD 21205, USA 25Estonian Genome Center of the University
of Tartu, Tartu, Estonia 26Institute of Molecular and Cell Biology, University of Tartu,
Tartu, Estonia 27Department of Genetics, Washington University School of
Medicine, USA 28Wellcome Trust Centre for Human Genetics, University of Oxford,
Oxford, OX3 7BN, United Kingdom 29Centre for Population Health Sciences,
University of Edinburgh, Teviot Place, Edinburgh, EH8 9AG, Scotland, United
Kingdom 30Department of Public Health and Primary Care, University of Cambridge,
Cambridge, United Kingdom 31Hjelt Institute, Department of Public Health,
University of Helsinki, Finland 32Centre For Paediatric Epidemiology and
Biostatistics/MRC Centre of Epidemiology for Child Health, University College of
London Institute of Child Health, London, United Kingdom 33Centre for Medical
Systems Biology, Leiden, the Netherlands 34Department of Immunology, Genetics
and Pathology, Uppsala University, Uppsala, Sweden 35Uppsala Clinical Research
Center, Uppsala University, Uppsala, Sweden 36Genome Centre, Barts and The
London School of Medicine and Dentistry, Queen Mary University of London,
London, UK 37Clinical Pharmacology, NIHR Cardiovascular Biomedical Research
Unit, William Harvey Research Institute, Barts and The London School of Medicine
and Dentistry Queen Mary University of London, London, UK 38Biocenter Oulu,
University of Oulu, Oulu, Finland 39Institute of Health Sciences, University of Oulu,
Finland 40Institute for Molecular Medicine Finland FIMM, University of Helsinki,
Finland 41Public Health Genomics Unit, National Institute for Health and Welfare,
Helsinki, Finland 42Department of Internal Medicine II – Cardiology, University of
UlM Medical Centre, Ulm, Germany 43Mannheim Institute of Public Health, Social
and Preventive Medicine, Medical Faculty of Mannheim, University of Heidelberg,
Ludolf-Krehl-Strasse 7-11, 68167 Mannheim, Germany 44Medical Genetics Institute,
Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA 45MRC Epidemiology
Unit, Institute of Metabolic Science, Box 285, Addenbrooke’s Hospital, Hills Road,
Cambridge, CB2 OQQ, United Kingdom 46Department of Clinical Chemistry, Fimlab
Laboratories, Tampere 33520, Finland 47Department of Clinical Chemistry,
University of Tampere School of Medicine, Tampere 33014, Finland 48Department
of Twin Research and Genetic Epidemiology, King’s College London, London,
United Kingdom 49Division of Endocrinology, Diabetes, and Nutrition, Department of
Medicine, University of Maryland, School of Medicine, Baltimore, Maryland
50Institute of Genetic Epidemiology, Helmholtz Zentrum München, Neuherberg
85764, Germany 51Department of Medicine I, University Hospital Grosshadern,
Ludwig-Maximilians University, Munich, Germany 52Institute of Medical Informatics,
Biometry and Epidemiology, Ludwig-Maximilians-University of Munich, Munich,
Germany 53Department of Epidemiology, University of Groningen, University
Medical Center Groningen, The Netherlands 54Division of Endocrinology, Children’s
Hospital Boston, Boston, Massachusetts 02115, USA 55Division of Genetics,
Program in Genomics, Children’s Hospital Boston, Boston, Massachusetts 02115,
USA 56Istituto di Ricerca Genetica e Biomedica, Consiglio Nazionale delle Ricerche,
Monserrato, 09042, Italy 57Center for Neurobehavioral Genetics, The Semel
Institute for Neuroscience and Human Behavior, University of California, Los
Angeles, USA 58Genetic Epidemiology Group, Department of Epidemiology and

Nat Genet. Author manuscript; available in PMC 2014 May 01.
Public Health, UCL, London WC1E 6BT, United Kingdom 59 Department of Clinical Sciences, Genetic & Molecular Epidemiology Unit, Lund University Diabetes Center, Scania University Hospital, Malmö, Sweden 60 Department of Odontology, Umeå University, Umeå, Sweden 61 Department of Public Health and Primary Care, Unit of Medicine, Umeå University, Umeå, Sweden 62 Dipartimento di Scienze Biomediche, Universita di Sassari, 07100 SS, Italy 63 Atherosclerosis Research Unit, Department of Medicine Solna, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden 64 Center for Molecular Medicine, Karolinska University Hospital, Stockholm, Sweden 65 Clinical Research Branch, National Institute Health, Baltimore, MD, USA 66 deCODE Genetics/Amgen, 101 Reykjavik, Iceland 67 Department of Genetics, University of Pennsylvania - School of Medicine, Philadelphia PA, 19104, USA 68 Department of Systems Pharmacology and Translational Therapeutics, University of Pennsylvania - School of Medicine, Philadelphia PA, 19104, USA 69 Human Genetics Center, University of Texas Health Science Center - School of Public Health, Houston, TX 77030, USA 70 HudsonAlpha Institute for Biotechnology, Huntsville, AL, USA 71 MRC Unit for Lifelong Health and Ageing, 33 Bedford Place, London, WC1B 5JU, United Kingdom 72 Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, United Kingdom 73 Ealing Hospital, Southall, Middlesex UB1 3HW, United Kingdom 74 MRC/UVRI Uganda Research Unit on AIDS, Entebbe, Uganda 75 University of Cambridge Metabolic Research Laboratories and NIHR Cambridge Biomedical Research Centre, Level 4, Institute of Metabolic Science Box 289 Addenbrooke's Hospital Cambridge CB2 0QQ, UK 76 Department of Pediatrics, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA 77 Genome Technology Branch, National Human Genome Research Institute, NIH, Bethesda, MD 20892, USA 78 Department of Experimental Medicine, University of Milano Bicocca, Italy 79 MedStar Health Research Institute, 6525 Belcrest Road, Suite 700, Hyattsville, MD 20782, USA 80 Research Centre on Public Health, University of Milano Bicocca, Italy 81 Department of Dietetics-Nutrition, Harokopio University, 70 El. Venizelou Str, Athens, Greece 82 Institute of Epidemiology I, Helmholtz Zentrum München, Neuherberg 85764, Germany 83 Institute of Epidemiology II, Helmholtz Zentrum München, Neuherberg 85764, Germany 84 MRC Health Protection Agency (HPA) Centre for Environment and Health, School of Public Health, Imperial College London, UK 85 The Laboratory in Mjodd, 108 Reykjavik, Iceland 86 Division of Cardiovascular Epidemiology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden 87 Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA 88 Research Unit of Molecular Epidemiology, Helmholtz Zentrum München, Neuherberg 85764, Germany 89 Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, OX3 7LJ, United Kingdom 90 Department of Public Health and Clinical Medicine, Nutritional research, Umeå University, Umeå, Sweden 91 Department of Clinical Sciences/Obstetrics and Gynecology, Oulu University Hospital, Oulu, Finland 92 MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, Western General Hospital, Edinburgh, Scotland, United Kingdom 93 Laboratory of Neurogenetics, National Institute on Aging, Bethesda, MD 20892, USA 94 Center for Biomedicine, European Academy Bozen/Bolzano (EURAC), Bolzano, Italy - Affiliated Institute of the University of Lübeck, Lübeck, Germany 95 Division of Endocrinology & Metabolism, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan 96 Hannover Unified Biobank, Hannover Medical School, Hannover 30625, Germany 97 Department of Vascular Medicine, Academic Medical Center,
Amsterdam, The Netherlands 98Clinical Gerontology Unit, University of Cambridge, Cambridge, United Kingdom 99Kuopio Research Institute of Exercise Medicine, Kuopio, Finland 100Division of Endocrine and Metabolism, Department of Internal Medicine, Taichung Veterans General Hospital, School of Medicine, National Yang-Ming University, Taipei, Taiwan 101Diabetes Prevention Unit, National Institute for Health and Welfare, 00271 Helsinki, Finland 102The Genetics of Obesity and Related Metabolic Traits Program, The Icahn School of Medicine at Mount Sinai, New York, USA 103The Charles Bronfman Institute for Personalized Medicine, The Icahn School of Medicine at Mount Sinai, New York, USA 104The Mindich Child Health and Development Institute, The Icahn School of Medicine at Mount Sinai, New York 105School of Social and Community Medicine, University of Bristol, Oakfield House, Oakfield Grove, Bristol BS8 2BN, United Kingdom 106Institute for Medical Informatics and Biometrics, University of Dresden, Medical Faculty Carl Gustav Carus, Fetscherstrasse 74, 01307 Dresden, Germany 107Laboratory of Genetics, National Institute on Aging, Baltimore, MD21224, USA 108Department of Clinical Pharmacology, University of Tampere School of Medicine, Tampere 33014, Finland 109Department of Internal Medicine, Päijät-Häme Central Hospital, Lahti, Finland 110Division of Cardiology, Helsinki University Central Hospital, Helsinki, Finland 111Department of Clinical Biochemistry, Landspitali University Hospital, 101 Reykjavik, Iceland 112Department of Medical Genetics, Haartman Institute, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland 113Genetic Epidemiology Group, Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom 114Department of Statistical Sciences, University College of London, London, United Kingdom 115National Institute for Health and Welfare, Oulu, Finland 116Cardiovascular Institute, Perelman School of Medicine at the University of Pennsylvania, 3400 Civic Center Blvd, Building 421, Translational Research Center, Philadelphia, PA 19104-5158, USA 117Division of Translational Medicine and Human Genetics, Perelman School of Medicine at the University of Pennsylvania, 3400 Civic Center Blvd, Building 421, Translational Research Center, Philadelphia, PA 19104-5158, USA 118Department of Epidemiology, Erasmus University Medical Center, Rotterdam, the Netherlands 119Department of Internal Medicine, Erasmus University Medical Center, Rotterdam, the Netherlands 120Netherlands Genomics Initiative (NGI)-sponsored Netherlands Consortium for Healthy Aging (NCHA), Leiden, The Netherlands 121Department of Clinical Sciences/Clinical Chemistry, University of Oulu, Oulu, Finland 122National Institute for Health Research Leicester Cardiovascular Biomedical Research Unit, Glenfield Hospital, Leicester LE3 9QP, UK 123Department of Cardiovascular Sciences, University of Leicester, Glenfield Hospital, Leicester, LE3 9QP, UK 124Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Austria 125School of International Development, University of East Anglia, Norwich NR4 7TJ, United Kingdom 126University of Eastern Finland and Kuopio University Hospital, 70210 Kuopio, Finland 127INSERM UMRS 937, Pierre and Marie Curie University, Paris, France 128Department of Laboratory Medicine, University of Groningen, University Medical Center Groningen, The Netherlands 129LifeLines Cohort Study, University of Groningen, University Medical Center Groningen, The Netherlands 130Department of Genetics, University of Groningen, University Medical Center Groningen, The Netherlands 131Department of Biological Psychology, VU Univ, Amsterdam, The Netherlands 132Department of Community Medicine, Faculty of Health Sciences, University of Tromsø, Tromsø, Norway 133Department of Nutrition, University of North Carolina, Chapel Hill, NC, USA 134Department of Epidemiology and Public Health, EA 3430, University of
Strasbourg, Faculty of Medicine, Strasbourg, France
Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA
Geriatric Unit, Azienda Sanitaria Firenze (ASF), Florence, Italy
Chemical Pathology, Department of Pathology, University of the West Indies, Mona, Kingston 7, Jamaica
Institute of Social and Preventive Medicine (IUMSP), Lausanne University Hospital, Route de la Corniche 10, 1010 Lausanne, Switzerland
Division of Endocrinology and Diabetes, Department of Internal Medicine, Ulm University Medical Centre, Ulm, Germany
Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore
Department of Medicine III, University of Dresden, Medical Faculty Carl Gustav Carus, Fetscherstrasse 74, 01307 Dresden, Germany
Ministry of Health, Victoria, Republic of Seychelles
Service of Nephrology, Lausanne University Hospital, Lausanne, Switzerland
Imperial College Healthcare NHS Trust, London, United Kingdom
Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, Cedars-Sinai Medical Center, Los Angeles, California, USA
Department of Medicine, University of California Los Angeles, Los Angeles, California, USA
Department of Preventive Medicine and Epidemiology, Loyola University Medical School, Maywood, Illinois 60153, USA
Office of Population Studies Foundation, University of San Carlos, Talamban, Cebu City, Philippines
Department of Cardiology, Toulouse University School of Medicine, Rangueil Hospital, Toulouse, France
Department of Psychiatry, University of California, Los Angeles, USA
Department of Clinical Sciences, Lund University, SE-20502, Malmö, Sweden
Department of Medicine, Helsinki University Hospital, FI-00029 Helsinki, Finland
ICelandic Heart Association, Kopavogur, Iceland
Department of Cardiology, Karolinska University Hospital, Stockholm, Sweden
Laboratory of Epidemiology, Demography, and Biometry, National Institute on Ageing, Bethesda, MD, USA
Institute of Population Health Sciences, National Health Research Institutes, Zhunan, Taiwan
Cardiovascular Genetics, BHF Laboratories, Institute Cardiovascular Science, University College London, London, United Kingdom
Cardiovascular Genetics, University of Utah School of Medicine, Salt Lake City, UT, USA
HUNT Research Centre, Department of Public Health and General Practice, Norwegian University of Science and Technology, Levanger, Norway
Kaiser Permanente, Division of Research, Oakland, CA, USA
Unit of Primary Care, Oulu University Hospital, Oulu, Finland
Department of Chronic Disease Prevention, National Institute for Health and Welfare, Turku, Finland
Department of Clinical Physiology, University of Tampere School of Medicine, Tampere 33014, Finland
Department of Mental Health and Substance Abuse Services, National Institute for Health and Welfare, Helsinki, Finland
Institute of Clinical Medicine, Department of Medicine, University of Oulu and Clinical Research Center, Oulu University Hospital, Oulu, Finland
National Heart & Lung Institute, Imperial College London, Hammersmith Hospital, London, United Kingdom
Children’s Hospital Oakland Research Institute, 5700 Martin Luther King Junior Way, Oakland, CA 94609, USA
Department of Medicine, University of Eastern Finland and Kuopio University Hospital, 70210 Kuopio, Finland
Institute of Regional Health Services Research, University of Southern Denmark, Odense, Denmark
Odense Patient data Explorative Network (OPEN), Odense University Hospital, Odense, Denmark
Institute of Biomedicine/Physiology, University of Eastern Finland, Kuopio Campus, Finland
Department of Medical Sciences, Uppsala University, Uppsala, Sweden
Queensland Institute of Medical Research, Locked Bag 2000, Royal Brisbane Hospital, Queensland 4029, Australia
Synlab Academy, Synlab Services GmbH, Gottlieb-Daimler-Straße 25, 68165 Mannheim, Germany
Acknowledgments

We thank the Global Lipids Genetics Consortium for early access to the association results of the Metabochip study. S.Kathiresan is supported by a Research Scholar award from the Massachusetts General Hospital (MGH), the Howard Goodman Fellowship from MGH, the Donovan Family Foundation, R01HL107816, and a grant from Fondation Leducq. R.D. is supported by a Banting Fellowship from the Canadian Institutes of Health Research. G.P. is supported by Award Number T32HL007208 from the National Heart, Lung, and Blood Institute. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Heart, Lung, And Blood Institute or the National Institutes of Health.

References


**Sequence accession numbers**


**Disclosures**

**CHS**

Bruce Psaty serves on the DSBM of a clinical trial funded by the manufacturer (Zoll), and he serves on the Steering Committee of the Yale Open-Data Project funded by the Medtronic.

**CoLaus**

Peter Vollenweider received an unrestricted grant from GSK to build the CoLaus study

**deCODE**

Authors affiliated with deCODE Genetics/Amgen, a biotechnology company, are employees of deCODE Genetics/Amgen

**GLACIER**

Inês Barroso and spouse own stock in GlaxoSmithKline and Incyte Ltd.
S. Kathiresan serves on scientific advisory boards for Merck, Celera, American Genomics and Catabasis. He has received unrestricted research grants from Merck and Pfizer.

Author Contributions

R.D. carried out primary data analyses and prepared the supplementary information. R.D. and C.G. prepared figures and tables. C.W., E.M.S., S.Sebanti, G.R.A. contributed meta-analysis results. R.D., M.J.D, B.M.N., S.Kathiresan contributed to the design and conduct of the study. R.D., M.J.D, B.M.N., S.Kathiresan wrote the manuscript.

All authors contributed to the research and reviewed the manuscript.

Design, management and coordination of contributing cohorts


Genotyping of contributing cohorts

Phenotype definition of contributing cohorts


Primary analysis from contributing cohorts

C. Song, E.I.; (PROMIS) J.D., D.F.F., K. Stirrups; (Rotterdam Study) A.I.; (SardiNIA)
C. Sidore, J.L. Bragg-Gresham, S. Sanna; (SCARFSHEEP) R.J.S.; (SEYCHELLES) G.B.E.,
M. Bochud; (SUvimax) T.J.; (Swedish Twin Reg.) C. Song, E.I.; (TAICHI) D. Absher,
(TWINGENE) A.G., E.I.; (ULSAM) C. Song, E.I., S.G.; (WGHS) D.I.C.; (Whitehall II)
S. Shah
Figure 1. Effect of a single nucleotide polymorphism on triglycerides, low-density lipoprotein cholesterol, and risk for coronary artery disease. Black dots represent SNPs with CAD $P < 0.001$; B. Red dots represent SNPs with $0.01 < CAD P < 0.001$; C. Grey dots represent CAD $P > 0.10$). Loci strongly associated with CAD tend to have consistent directions for both triglycerides and LDL-C (bottom left and top right quadrants). In contrast to the grey points, the black and red points are concentrated in the bottom left and top right quadrants. Betas are in standard deviation units. SNPs with $-0.10 < \beta_{\text{LDL-C}} < 0.10$ and $-0.10 < \beta_{\text{TRIGLYCERIDES}} < 0.10$ are shown.
Table 1

SNPs with consistent direction of genetic effects on LDL-C and triglycerides and their subsequent relationship to risk for CAD.

<table>
<thead>
<tr>
<th>Locus</th>
<th>rs ID</th>
<th>A1</th>
<th>( \beta_{\text{LDL-C}} )</th>
<th>( P )</th>
<th>( \beta_{\text{TRIGLYCERIDES}} )</th>
<th>( P )</th>
<th>( \beta_{\text{CAD}} )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANGPTL3</td>
<td>rs4587594</td>
<td>A</td>
<td>-0.049</td>
<td>3\times10^{-57}</td>
<td>-0.069</td>
<td>3\times10^{-57}</td>
<td>0.017</td>
<td>0.26</td>
</tr>
<tr>
<td>APOB</td>
<td>rs1367117</td>
<td>A</td>
<td>0.12</td>
<td>2\times10^{-196}</td>
<td>0.025</td>
<td>3\times10^{-12}</td>
<td>0.035</td>
<td>0.02</td>
</tr>
<tr>
<td>GCKR</td>
<td>rs3817588</td>
<td>T</td>
<td>0.026</td>
<td>3\times10^{-8}</td>
<td>0.067</td>
<td>7\times10^{-58}</td>
<td>0.034</td>
<td>0.08</td>
</tr>
<tr>
<td>TIMD4</td>
<td>rs6882076</td>
<td>T</td>
<td>-0.046</td>
<td>5\times10^{-33}</td>
<td>-0.029</td>
<td>1\times10^{-16}</td>
<td>-0.021</td>
<td>0.15</td>
</tr>
<tr>
<td>HLA-B</td>
<td>rs2247056</td>
<td>T</td>
<td>-0.025</td>
<td>6\times10^{-9}</td>
<td>-0.038</td>
<td>2\times10^{-22}</td>
<td>-0.030</td>
<td>0.06</td>
</tr>
<tr>
<td>TRIB1</td>
<td>rs2980885</td>
<td>A</td>
<td>-0.031</td>
<td>4\times10^{-12}</td>
<td>-0.058</td>
<td>5\times10^{-45}</td>
<td>-0.041</td>
<td>0.02</td>
</tr>
<tr>
<td>TRIB1</td>
<td>rs2954022</td>
<td>A</td>
<td>-0.055</td>
<td>4\times10^{-51}</td>
<td>-0.078</td>
<td>2\times10^{-124}</td>
<td>-0.056</td>
<td>6\times10^{-5}</td>
</tr>
<tr>
<td>ABCA1</td>
<td>rs1883025</td>
<td>T</td>
<td>-0.030</td>
<td>1\times10^{-11}</td>
<td>-0.022</td>
<td>3\times10^{-8}</td>
<td>-0.014</td>
<td>0.41</td>
</tr>
<tr>
<td>APOA1</td>
<td>rs10790162</td>
<td>A</td>
<td>0.076</td>
<td>3\times10^{-26}</td>
<td>0.23</td>
<td>1\times10^{-276}</td>
<td>0.13</td>
<td>2\times10^{-6}</td>
</tr>
<tr>
<td>CETP</td>
<td>rs9989419</td>
<td>A</td>
<td>0.028</td>
<td>8\times10^{-13}</td>
<td>0.024</td>
<td>3\times10^{-12}</td>
<td>0.010</td>
<td>0.61</td>
</tr>
<tr>
<td>CILP2</td>
<td>rs10401969</td>
<td>T</td>
<td>0.12</td>
<td>2\times10^{-60}</td>
<td>0.12</td>
<td>3\times10^{-76}</td>
<td>0.11</td>
<td>2\times10^{-4}</td>
</tr>
</tbody>
</table>

Shown are SNPs that have strong association with both LDL-C and triglycerides (\( P<5\times10^{-8} \) for each), have consistent direction of effect size for LDL-C and triglycerides, and have a ratio of magnitude of effect size of LDL-C to triglycerides within a factor of 5. Five loci confer risk for CAD (\( P<0.05 \)) and ten of the eleven loci show consistent direction of effect size for both lipid traits with the effect size of CAD.

A1: All beta estimates were calculated with respect to this allele.
SNPs with opposite direction of genetic effects on LDL-C and triglycerides and their subsequent relationship to risk for CAD.

<table>
<thead>
<tr>
<th>Locus</th>
<th>rs ID</th>
<th>A1</th>
<th>(\beta_{\text{LDL-C}})</th>
<th>(P)</th>
<th>(\beta_{\text{TRIGLYCERIDES}})</th>
<th>(P)</th>
<th>(\beta_{\text{CAD}})</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIR148A</td>
<td>rs4722551</td>
<td>T</td>
<td>-0.039</td>
<td>7 \times 10^{-16}</td>
<td>0.027</td>
<td>2 \times 10^{-5}</td>
<td>-0.033</td>
<td>0.23</td>
</tr>
<tr>
<td>GPAM</td>
<td>rs2255141</td>
<td>A</td>
<td>0.080</td>
<td>7 \times 10^{-14}</td>
<td>-0.021</td>
<td>1 \times 10^{-8}</td>
<td>-0.0076</td>
<td>0.63</td>
</tr>
<tr>
<td>FADSI-2-3</td>
<td>rs1535</td>
<td>A</td>
<td>0.053</td>
<td>3 \times 10^{-43}</td>
<td>-0.046</td>
<td>1 \times 10^{-40}</td>
<td>0.0019</td>
<td>0.90</td>
</tr>
<tr>
<td>APOE</td>
<td>rs7254892</td>
<td>A</td>
<td>-0.49</td>
<td>8 \times 10^{-38.5}</td>
<td>0.12</td>
<td>4 \times 10^{-31}</td>
<td>-0.14</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Shown are SNPs that have strong association with both LDL-C and triglycerides (\(P<5 \times 10^{-8}\) for each), but have opposite direction of effect size for LDL-C and triglycerides, and have a ratio of magnitude of effect size of LDL-C to triglycerides within a factor of 5. Four SNPs displayed this pattern and none showed significant association with CAD (all \(P>0.05\)).

A1: All beta estimates were calculated with respect to this allele.
Table 3

Association of the strength of a SNP's effect on plasma lipids with its strength of effect on CAD risk.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Predictor</th>
<th>Covariate</th>
<th>Beta</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>βCAD</td>
<td>βLDL-C</td>
<td>-</td>
<td>0.41</td>
<td>0.039</td>
<td>4×10⁻²⁰</td>
</tr>
<tr>
<td>βCAD</td>
<td>βLDL-C</td>
<td>βHDL-C</td>
<td>0.38</td>
<td>0.039</td>
<td>9×10⁻¹⁹</td>
</tr>
<tr>
<td>βCAD</td>
<td>βLDL-C</td>
<td>βTRIGLYCERIDES</td>
<td>0.40</td>
<td>0.034</td>
<td>1×10⁻²³</td>
</tr>
<tr>
<td>βCAD</td>
<td>βLDL-C</td>
<td>βHDL-C βTRIGLYCERIDES</td>
<td>0.38</td>
<td>0.034</td>
<td>2×10⁻²²</td>
</tr>
<tr>
<td>βCAD</td>
<td>βHDL-C</td>
<td>-</td>
<td>-0.18</td>
<td>0.052</td>
<td>0.0006</td>
</tr>
<tr>
<td>βCAD</td>
<td>βHDL-C</td>
<td>βLDL-C</td>
<td>-0.12</td>
<td>0.041</td>
<td>0.005</td>
</tr>
<tr>
<td>βCAD</td>
<td>βHDL-C</td>
<td>βTRIGLYCERIDES</td>
<td>-0.09</td>
<td>0.048</td>
<td>0.057</td>
</tr>
<tr>
<td>βCAD</td>
<td>βHDL-C</td>
<td>βLDL-C βTRIGLYCERIDES</td>
<td>-0.04</td>
<td>0.037</td>
<td>0.35</td>
</tr>
<tr>
<td>βCAD</td>
<td>βTRIGLYCERIDES</td>
<td>-</td>
<td>0.44</td>
<td>0.074</td>
<td>2×10⁻⁸</td>
</tr>
<tr>
<td>βCAD</td>
<td>βTRIGLYCERIDES</td>
<td>βLDL-C</td>
<td>0.42</td>
<td>0.057</td>
<td>5×10⁻¹²</td>
</tr>
<tr>
<td>βCAD</td>
<td>βTRIGLYCERIDES</td>
<td>βHDL-C</td>
<td>0.36</td>
<td>0.074</td>
<td>3×10⁻⁶</td>
</tr>
<tr>
<td>βCAD</td>
<td>βTRIGLYCERIDES</td>
<td>βLDL-C βHDL-C</td>
<td>0.36</td>
<td>0.057</td>
<td>1×10⁻⁹</td>
</tr>
</tbody>
</table>

Residuals for βCAD were calculated after adjustment of a SNP's effect on the denoted lipid trait. A total of 185 SNPs identified from GWAS for LDL-C, HDL-C and triglycerides were included in regression analysis. βLDL-C, βHDL-C, and βTRIGLYCERIDES represent the effect sizes for a SNP on LDL-C, HDL-C and triglycerides in the GWAS meta-analysis for lipids. Regression was performed with the predictor variable of the effect size on lipid traits (β from predictor column) and the outcome variable of residual CAD effect size after adjusting for covariates. SE: standard error.