Published in final edited form as:
JAMA Cardiol. 2018 October 01; 3(10): 957–966. doi:10.1001/jamacardio.2018.2866.

Genetically-enhanced LPL mediated lipolysis, LDL cholesterol lowering alleles and risk of coronary disease and type 2 diabetes

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Abstract

Importance—Pharmacological enhancers of lipoprotein lipase (LPL) are in preclinical or early clinical development for cardiovascular prevention. Studying whether these agents will reduce cardiovascular events or diabetes risk when added to existing lipid-lowering drugs would require large outcome trials. Human genetics studies can help prioritize or deprioritize these resource-demanding endeavors.

Objective—To investigate the independent and combined associations of genetically-determined differences in LPL-mediated lipolysis and LDL-C metabolism with diabetes and coronary risk.

Design—Population-based cohort and case-cohort.

Setting—This study was conducted in the United Kingdom between 2014 and 2018.

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Declaration of interests

Dr. R. A. S. is an employee and shareholder of GlaxoSmithKline Plc. (GSK). Dr McCarthy received grants from Eli Lilly, Roche, AstraZeneca, Merck, Janssen, Servier, Novo Nordisk, Sanofi-Aventis, Boehringer Ingelheim, Pfizer, and Takeda; and honoraria from Novo Nordisk and Pfzier. Dr O'Rahilly received personal fees from Pfizer, AstraZeneca, iMed, and ERX Pharmaceuticals for serving on advisory boards and scientific panels.

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Participants—Individual-level genetic data from 390,470 people were included.

Exposure—Six conditionally-independent triglyceride-lowering alleles in *LPL*, p.Glu40Lys in *ANGPTL4*, rare loss-of-function variants in *ANGPTL3* and LDL-C lowering polymorphisms at 58 independent genomic regions, including *HMGCR*, *NPC1L1* and *PCSK9*.

Main Outcomes and Measures—Odds ratio for type 2 diabetes and coronary artery disease.

Results—Triglyceride-lowering alleles in *LPL* were associated with protection from coronary disease (~40% lower odds per standard deviation [SD] genetically-lower triglycerides) and type 2 diabetes (~30% lower odds) in people above or below the median of the population distribution of LDL-C lowering alleles at 58 independent genomic regions, *HMGCR*, *NPC1L1* or *PCSK9* ($p<0.001$ in all subgroups). Associations with lower risk were consistent in quintiles of the distribution of LDL-C lowering alleles and 2×2 "factorial" genetic analyses. The 40Lys variant in *ANGPTL4* protected from coronary disease and type 2 diabetes in groups with genetically-higher or lower LDL-C. For a genetic difference of 0.23 SD in LDL-C, *ANGPTL3* loss-of-function variants, which also have beneficial effects on LPL-lipolysis, were associated with greater protection against coronary disease than other LDL-C lowering genetic mechanisms (odds ratio from a meta-analysis of published genetic studies, 0.66, 95% CI, 0.52-0.83 for *ANGPTL3* vs odds ratio, 0.90, 95% CI, 0.89-0.91 for 58 LDL-C lowering variants; $p_{heterogeneity}=0.0089$).

Conclusions and Relevance—Triglyceride-lowering alleles in the LPL pathway are associated with protection against coronary disease and type 2 diabetes independently of LDL-C lowering genetic mechanisms. These findings provide human genetics evidence to support the development of agents that enhance LPL-mediated lipolysis for further clinical benefit in addition to LDL-C-lowering therapy.

Introduction

Lipoprotein lipase (LPL) is an endothelium-bound enzyme that catalyzes the rate-limiting step in the clearance of atherogenic triglyceride-rich particles.1 There is genetic evidence of a causal link between impaired LPL-mediated lipolysis and coronary artery disease. Gain-of-function genetic variants in *LPL*,2,3 or loss-of-function variants in its intravascular inhibitors *ANGPTL3*,4–6 *ANGPTL4*2,7 or *APOC3*8,9 are associated with lower triglyceride levels and lower coronary disease risk, while loss-of-function variants in *LPL*2,3,10 or its natural activator *APOA5*11 are associated with higher triglycerides and higher coronary risk. Impaired LPL-mediated lipolysis has also been linked to insulin resistance12 and a higher type 2 diabetes risk,12–15 but the relationships of this pathway with glucose metabolism are incompletely understood.

There is growing interest around LPL-mediated lipolysis as a target for pharmacological intervention. Several new medicines that enhance LPL-mediated clearance of triglyceride-rich lipoprotein particles by directly activating LPL16,17 or by inhibiting its intravascular inhibitors6,7,18–20 are in pre-clinical7,16,17 or early clinical6,18–21 development for cardiovascular prevention. However, it is not known whether these agents will provide further benefits in addition to low-density lipoprotein cholesterol (LDL-C) lowering therapy, which is the mainstay of lipid-lowering therapy in cardiovascular prevention. Drugs that accelerate LPL-mediated clearance of triglyceride-rich lipoprotein particles are being

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developed for use in addition to statins and, possibly, other LDL-C lowering agents. However, statins,22 ezetimibe23 and PCSK9 inhibitors24–27 also reduce triglyceride-rich particles and this could limit the clinical benefits and utility of LPL-enhancing agents when used in combination with these drugs.

Large-scale clinical trials and the investment of massive resources would be required to study the impact on cardiovascular outcomes of each of these LPL-enhancing agents in the context of LDL-C lowering therapy. In advance of outcome trials, human genetic approaches can provide evidence of whether or not genetically-determined differences in LPL-mediated lipolysis and LDL-C metabolism have independent contributions to cardio-metabolic disease risk, which can help prioritize or deprioritize these resource-intensive efforts.28,29

Methods

Study design

The aims of this study were: (1) to investigate associations of genetically-enhanced LPL-mediated lipolysis with cardio-metabolic risk factors, coronary artery disease and type 2 diabetes (eFigure 1A); and (2) to estimate the independent and combined cardiovascular and metabolic associations of genetically-enhanced LPL-mediated lipolysis and of LDL-C lowering genetic variants (eFigure 1B-C). For the first aim, we estimated associations from summary-level genetic data including up to 672,505 individuals in non-stratified analyses (eFigure 1A). For the second aim, we used individual-level genetic data from up to 390,470 individuals to perform 2×2 “factorial” (eFigure 1B) or stratified genetic analyses (eFigure 1C). We also investigated the associations of naturally-occurring variation in the genes encoding LPL-inhibitors.

Participants and studies

In non-stratified analyses (eFigure 1A), we used genetic association data on up to 672,505 people from EPIC-InterAct,30 EPIC-Norfolk,31 UK Biobank32 and large-scale genetic consortia, including the CARDIoGRAMplusC4D,33 DIAGRAM,34 GIANT,35,36 MAGIC37,38 and GLGC consortia.39

In factorial and stratified analyses (eFigure 1B-C), we used individual-level data from up to 390,470 individuals of EPIC-InterAct, EPIC-Norfolk, and UK Biobank (Table 1). EPIC-InterAct30 is a case-cohort study of type 2 diabetes nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) study.40 EPIC-Norfolk is a prospective cohort study of over 20,000 individuals aged 40-79 and living in the Norfolk county in the United Kingdom at recruitment.31 UK Biobank is a population-based cohort of 500,000 people aged between 40-69 years who were recruited in 2006-2010 from several centers across the United Kingdom.32 Detailed characteristics of the participants with individual level genotype data included in this study are presented in Table 1, and details about the cohorts participating in each analysis, phenotype definitions and data sources are in eNote 1 and eTable 1.

Factorial and stratified genetic analyses

The similarities between the random allocation of genetic variants at conception and that of participants in a randomized trial41 have been used as rationale to study associations of alleles in different genes to gain insights into the likely consequences of the pharmacological modulation of the gene products in a way that simulates a factorial randomized controlled trial.42,43 In this study, for each participant, we calculated a weighted *LPL* genetic score and a weighted LDL-C genetic score by adding the number of triglyceride-lowering *LPL*-alleles or LDL-C–lowering alleles at 58 LDL-C-associated genetic loci, weighted by their effect on the corresponding lipid levels. These genetic scores were dichotomized at the median value to "naturally randomize" participants into four groups: (1) reference, (2) genetically-lower triglycerides via *LPL*-alleles, (3) genetically-lower LDL-C via alleles at 58 independent genetic loci, or (4) *both* genetically-lower triglycerides via *LPL*-alleles *and* genetically-lower LDL-C via the 58 genetic loci. We studied associations with lipid traits and cardio-metabolic outcomes between groups using a 2×2 "factorial" design (eFigure 1B). Further details about this approach are in the eNote 2.

In stratified analyses (eFigure 1C), we studied the associations of *LPL*-alleles in quantiles of the population distribution of 58 LDL-C lowering alleles or alleles at three genes encoding the targets of current lipid-lowering therapy, including *HMGCR* (encoding the target of statins), *NPC1L1* (ezetimibe) and *PCSK9* (PCSK9 inhibitors). We considered groups above or below the median of overall and gene-specific LDL-C lowering genetic scores, as well as quintiles of the general LDL-C lowering genetic score.

Selection of genetic variants

As a proxy for genetically-enhanced LPL-lipolysis, we used six genetic variants in the *LPL* gene previously reported to be strongly and independently associated with triglyceride levels ($p<5.0\times10^{-08}$ for each variant in conditional analyses from the Global Lipids Genetics Consortium [GLGC]10; eTable 2).

In factorial or stratified analyses, as instruments for genetically-lower LDL-C we used 58 genetic variants from independent genomic regions associated with LDL-C levels in up to 188,577 participants of GLGC39 ($p<5.0\times10^{-08}$ for LDL-C in each region; all variants >500 kb away from each other and low linkage disequilibrium with pairwise $R^2<0.01$; eTable 2). In sensitivity analyses, we used a subset of 22 of the 58 variants that had no association with triglycerides in GLGC39 ($p>0.05$). We also considered six *HMGCR*,43 five *NPC1L1*42 or seven *PCSK9*43 genetic variants previously used by Ference et al. as genetic proxies for statin, ezetimibe or PCSK9 inhibitor therapy42,43 (eTable 2). Quality checks of genetic data and of analyses presented in this manuscript are described in eNote 3.

Loss-of-function variants in the inhibitors of lipoprotein lipase

We estimated associations with cardio-metabolic outcomes of a low-frequency variant in *ANGPTL4* (p.Glu40Lys, 40Lys allele frequency, 1.9%). The 40Lys allele disrupts the inhibitory effect of ANGPTL4 on LPL *in vitro*44 and is strongly associated with lower triglyceride levels (~0.27 standard deviations [SD] lower triglycerides per 40Lys allele;

p=4.2×10^{-175}) but not with LDL-C (p=0.70) in GLGC.14 The variant is also associated with protection from cardio-metabolic disease.2,7,14,45

Rare loss-of-function alleles in the LPL-inhibitor *ANGPTL3* are associated with lower LDL-C and triglyceride levels,4–6 offering a unique genetic model for the combined reduction of LDL-C levels and enhancement of LPL-mediated lipolysis. Genetic studies and clinical trials show that different LDL-C-lowering mechanisms protect against coronary disease with a log-linear relationship that is observed independently of the mechanism by which this reduction is attained.42,46,47 If the association with lower risk of *ANGPTL3* variants is only via lower LDL-C, one would expect their association to be the same as that of LDL-C lowering variants in other genes, for a given genetic difference in LDL-C levels. We investigated this hypothesis by meta-analyzing and modelling data from previously published genetic studies about the association of rare loss-of-function variants of *ANGPTL3* with LDL-C and coronary disease risk (eNote 4).5,6

We also attempted to estimate the associations with cardio-metabolic outcomes of a rare loss-of-function variant in the *APOC3* gene captured by direct genotyping in UK Biobank, but the analysis was uninformative likely due to low statistical power (eNote 5).

Statistical analysis

In non-stratified and stratified genetic analyses, associations of the six triglyceride-lowering genetic variants in *LPL* and outcomes were estimated using weighted generalized linear regression models that accounts for correlation between genetic variants.48 Estimates of (a) *LPL*-alleles to triglyceride levels associations and of (b) *LPL*-alleles to outcome associations were used to calculate estimates of (c) genetically-lower triglyceride levels via *LPL*-alleles to outcome associations. Correlation values were obtained from the LDlink software (eTable 3).49 Results were scaled to represent the beta coefficient or the odds ratio (OR) per SD in genetically-predicted triglyceride levels via *LPL*-alleles. Triglyceride associations are expressed in ln-transformed and standardized units.

In factorial genetic analyses (eFigure 1B), the associations of each group relative to the reference group were estimated using linear regression for plasma LDL-C and triglyceride levels, and either logistic or Prentice-weighted Cox regression (as appropriate for the study design) for coronary artery disease and type 2 diabetes.

All analyses were adjusted for age, sex and genetic principal components. Analyses were conducted within each study and pooled using fixed-effect inverse variance weighted meta-analysis. Statistical analyses were performed using STATA v14.2 (StataCorp, College Station, Texas 77845 USA) and R v3.2.2 (The R Foundation for Statistical Computing). A two-tailed p<0.05 was considered statistically significant.

Results

Associations of LPL-alleles with cardio-metabolic risk factors and outcomes

Triglyceride-lowering alleles in *LPL* were associated with lower risk of type 2 diabetes both in combined (OR per SD genetically-lower triglycerides, 0.69; 95% confidence interval [CI],

0.62-0.76; $p=2.6\times10^{-13}$; eFigure 2 and eTable 4) and individual-variant analyses (eFigure 3 and eTable 5). Comparisons with estimates from multiple triglyceride-lowering genetic mechanisms50 showed that this association is specific to *LPL* and does not reflect a general association in a protective direction of lower triglyceride levels (eNote 6 and eTable 6). Associations with lower coronary risk (OR per SD genetically-lower triglycerides, 0.59; 95% CI, 0.53-0.66; $p=1.3\times10^{-22}$; eFigures 2-3 and eTables 4-5) were consistent with previous studies.10

Triglyceride-lowering *LPL*-alleles were associated with lower fasting insulin, fasting plasma glucose and a lower BMI-adjusted WHR (i.e. a more favorable fat distribution; $p=7.9\times10^{-05}$; eFigure 2), a novel association consistent with evidence of the preferential LPL-mediated lipid distribution to peripheral, rather than central adipocytes.51

Independent and combined associations of LPL-alleles and LDL-C lowering alleles

In factorial genetic analyses, people naturally-randomized to genetically-lower triglycerides via *LPL* had lower triglycerides but similar LDL-C levels compared to the reference group (eFigure 4). The association with lipid levels was additive to that of LDL-C lowering alleles (eFigure 4), which were also associated with lower triglyceride levels, consistent with the observed reduction in triglyceride-rich particles observed in people taking statins,22 ezetimibe23 or PCSK9 inhibitors.24–27

People naturally randomized to lower LDL-C levels, lower triglycerides via *LPL* or both had a lower risk of coronary artery disease compared to the reference group (Figure 1), with lowest odds in people naturally randomized to both genetic exposures (odds ratio, 0.73; 95% CI, 0.70-0.76; $p=2.8\times10^{-52}$; Figure 1). In this group, the odds ratio for coronary disease compared to the reference group was a further 7% lower than what expected on the basis of the association of the two exposures alone (95% CI, 12%-1% lower odds ratio; $p_{interaction}=0.018$). However, stratified analyses in groups above or below the median or in quintiles of the distribution of LDL-C lowering alleles were not consistent with an interaction (Figures 2A and 3; $p_{interaction}>0.05$).

People naturally-randomized to lower LDL-C had a higher risk of type 2 diabetes compared to the reference group (Figure 1), consistent with previous studies.43,50,52–55 However, people naturally randomized to both genetic exposures had a similar risk of type 2 diabetes compared to the reference group (Figure 1), as the association of *LPL*-alleles with lower risk "cancelled-out" the risk-increasing association of LDL-C lowering alleles. Consistently, triglyceride-lowering *LPL* alleles were strongly associated with lower diabetes risk also in people with genetically-lower LDL-cholesterol (Figure 2A).

In stratified analyses, triglyceride-lowering *LPL*-alleles were strongly and consistently associated with protection from diabetes and coronary disease in subgroups of people above or below the median of the population distribution of the 58 LDL-C lowering alleles (Figure 2A), 22 of the 58 LDL-C lowering alleles that were not associated with triglyceride levels in GLGC (eTable 7), *HMGCR*, *NPC1L1* or *PCSK9* alleles ($p<0.001$ for all comparisons; Figure 2 and eFigure 5). Associations of *LPL*-alleles with lower risk were consistent in

quintiles of the population distribution of the 58 LDL-C lowering alleles (Figure 3 and eFigure 6).

Evidence from ANGPTL4 and ANGPTL3 genetic variants

The *ANGPTL4* p.Glu40Lys variant was associated with protection from coronary disease and diabetes, with effect estimates nearly identical to the ones of triglyceride-lowering alleles in *LPL* for a given genetic difference in triglycerides (Figure 4A, eFigure 2). Associations were consistent in people above or below the median of the 58-variant LDL-C lowering genetic score (Figure 4A). Also, the 40Lys allele was associated with a more favorable fat distribution in UK Biobank (SD of BMI-adjusted waist-to-hip ratio per allele, -0.024; standard error, 0.0086; p=0.0046; N=350,450).

In previous sequencing studies, carrying a rare loss-of-function variant in *ANGPTL3* has been associated with 0.4 mmol/L (36 mg/dL) lower triglycerides and 0.23 SD lower LDL-C (~0.23 mmol/L or 9 mg/dL).6 In this study, for variants at *HMGCR*, *NPC1L1*, *PCSK9* and for the 58-variant LDL-C lowering genetic score, a genetic difference of 0.23 SD in LDL-C was consistently associated with ~10% lower odds of coronary disease (OR, 0.90; 95% CI, 0.89, 0.91; I^2=0%, $p_{heterogeneity}$ in effect estimates=0.86; eFigure 7). In a meta-analysis of published genetic studies5,6 on rare loss-of-function variants in *ANGPTL3* we found an association with ~34% lower odds of coronary disease (OR, 0.66; 95% CI, 0.52-0.83; p=0.00046; I^2=0%, $p_{heterogeneity}$=0.99; eFigure 8). For a given genetic-difference in LDL-C, the association of *ANGPTL3* variants with lower coronary disease risk was stronger than that of the LDL-C lowering genetic score ($p_{heterogeneity}$ in effect estimates=0.0089; Figure 4B, eFigure 7 and eTable 8).

Discussion

By analyzing individual-level genetic data in close to 400,000 people, we provide strong evidence that triglyceride-lowering alleles in the lipoprotein lipase pathway and LDL-C lowering genetic mechanisms independently contribute to a lower risk of coronary artery disease. This is of relevance to the future clinical development and positioning of LPL-enhancing drugs, given that these agents are being developed for use in addition to statins and other existing LDL-C lowering drugs. Because the LDL-C lowering alleles studied here included those at genes encoding the targets of current LDL-C lowering therapy, this study supports the hypothesis that pharmacologically enhancing LPL-mediated lipolysis is likely to provide further cardiovascular benefits in addition to existing LDL-C lowering agents.

By studying the interplay of these pathways with a study design that is directly relevant to the future clinical development of LPL-enhancing agents, this study adds to previous analyses which have investigated the impact on cardio-metabolic disease of *LPL*-pathway alleles2,3,10,12,14 or LDL-C lowering alleles separately.50,53,56–58 The independent associations of genetically-enhanced LPL-mediated lipolysis and of mechanisms that lower LDL-C via *PCSK9*, *NPC1L1* and *HMGCR* provide direct support to the development of direct enhancers of LPL16,17 for use in the context of existing LDL-C-lowering therapy, but also provide general support for other agents that enhance LPL activity via inhibition of its natural inhibitors in this therapeutic context.6,7,18–21

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We also investigated variation at two intravascular inhibitors of LPL, *ANGPTL4* and *ANGPTL3*, making two important observations. First, the level of protection from diabetes and coronary disease associated with *ANGPTL4* p.Glu40Lys is the same as that of *LPL* alleles, for a given genetic-difference in triglycerides, and is consistent across the population distribution of LDL-C lowering alleles. These findings are relevant for drugs that inhibit ANGPTL47 or directly enhance LPL by disrupting the inhibitory activity of ANGPTL4.17 Second, rare loss-of-function variants in *ANGPTL3* are associated with a greater level of protection from coronary disease than other genetic mechanisms, for a given genetic difference in LDL-C. This result suggests that ANGPTL3 inhibition may be an exception to the “LDL paradigm”, the mechanism-independent log-linear relationship between LDL-C lowering and coronary disease protection that has been consistently found in genetic studies and clinical trials.42,46 In phase 1 trials, ANGPTL3 inhibitors reduced LDL-C by amounts similar to or greater than currently approved LDL-C lowering drugs.6,20,21 Our findings suggest that ANGPTL3 inhibitors may be more effective than current agents for a given magnitude of LDL-C reduction.

Triglyceride-lowering *LPL*-alleles were also associated with protection against type 2 diabetes. The strong and consistent association in a protective direction of multiple independent *LPL*-alleles found in our study extends and reinforces previous reports by us and others limited to the rs180117712 and rs32812,14,15 alleles. We also provide evidence consistent with the association with lower incidence of diabetes being specific to the LPL pathway and not being a general association of lower triglycerides. In factorial analyses, this association was in a protective direction with a magnitude equivalent to the association of LDL-C lowering alleles with increased risk of type 2 diabetes. Therefore, our data suggest that enhancing LPL activity may also ameliorate glucose metabolism, while further reducing the risk of cardiovascular disease, also in people taking LDL-C lowering therapy.

Triglyceride-lowering alleles in *LPL* were also associated with greater insulin sensitivity, lower glucose levels, and a more favorable body fat distribution pattern, strengthening the link of this pathway with insulin and glucose metabolism.12, 45 The novel finding from this study of robust associations of triglyceride-lowering *LPL* alleles and *ANGPTL4* p.Glu40Lys with a lower waist-to-hip ratio is consistent with the known role of LPL as a lipid-buffering molecule51 and corroborates the notion that the association of this pathway with insulin sensitivity and lower diabetes risk may be due at least partially to improved capacity to preferentially store excess calories in peripheral adipose compartments.12

A number of assumptions and possible limitations of the genetic approach used in this study are worth considering when interpreting its results. “Mendelian randomization” generally assumes that genetic variants are associated with the endpoint exclusively via the risk factor of interest.41 In this case, the risk factor of interest is genetic differences in LPL-mediated lipolysis of which triglyceride levels are a proxy and therefore the association of LPL-alleles with different metabolic risk factors and diseases does not invalidate the approach. The consequences of modest genetically-determined differences in LPL-mediated lipolysis over several decades, as assessed in this study, may differ from the short-term pharmacological modulation of LPL-mediated lipolysis in randomized controlled trials or clinical practice. While our analyses show a strong association of *LPL*-alleles with diabetes and coronary

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disease, this does not necessarily mean that pharmacologically enhancing lipolysis over a short time will yield clinically-relevant changes in future risk of coronary disease or new-onset diabetes in high-risk adults for whom these agents are being developed. Therefore, the effect estimates from our genetic analysis reflect a life-long exposure to genetic differences in LPL-mediated lipolysis, and should not be interpreted as an exact prediction of the magnitude of the clinical effect for studies of the short-term pharmacological modulation of this pathway.

Conclusions

Triglyceride-lowering alleles in the LPL pathway are associated with protection against coronary disease and type 2 diabetes independently of LDL-C lowering genetic mechanisms. These findings provide human genetics evidence to support the development of agents that enhance LPL-mediated lipolysis for further clinical benefit in addition to LDL-C-lowering therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

This research has been conducted using the UK Biobank resource. This study has been conducting using data from the EPIC-InterAct and EPIC-Norfolk studies. This study was funded by the United Kingdom's Medical Research Council through grants MC_UU_12015/1, MC_PC_13046, MC_PC_13048 and MR/L00002/1. This work was supported by the MRC Metabolic Diseases Unit (MC_UU_12012/5) and the Cambridge NIHR Biomedical Research Centre and EU/EFPIA Innovative Medicines Initiative Joint Undertaking (EMIF grant: 115372). EPIC-InterAct Study funding: funding for the InterAct project was provided by the EU FP6 programme (grant number LSHM_CT_2006_037197). S. B. is supported by a Sir Henry Dale Fellowship jointly funded by the Wellcome Trust and the Royal Society (Grant Number 204623/Z/16/Z). D. B. S. is supported by the Wellcome Trust grant n. 107064. M. McC. is a Wellcome Trust Senior Investigator and is supported by the following grants from the Wellcome Trust: 090532 and 098381. M. McC. was supported by the National Institute for Health Research (NIHR) Oxford Biomedical Research Centre (BRC) and the views expressed are those of the Author(s) and not necessarily those of the NHS, the NIHR or the Department of Health. S. O. R. acknowleges funding from the Wellcome Trust (Wellcome Trust Senior Investigator Award: 095515/Z/11/Z and Wellcome Trust Strategic Award: 100574/Z/12/Z).

The authors gratefully acknowledge the help of the MRC Epidemiology Unit Support Teams, including Field, Laboratory and Data Management Teams.

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Key Points

- **Question**: Are genetically-determined differences in lipoprotein lipase (LPL) mediated lipolysis and low-density lipoprotein cholesterol (LDL-C) lowering pathways independently associated with risk of coronary disease and diabetes?
- **Findings**: Triglyceride-lowering alleles in *LPL* or its inhibitor *ANGPTL4* were associated with lower risk of coronary artery disease (~40% lower odds per standard deviation genetically-lower triglycerides) and type 2 diabetes (~30% lower odds) in a consistent fashion across quantiles of the population distribution of LDL-C lowering alleles. For a given genetic difference in LDL-C, the association with protection against coronary disease conveyed by rare loss-of-function variants in *ANGPTL3*, which are associated with lower LDL-C and enhanced LPL-lipolysis, was greater than that conveyed by other LDL-C lowering genetic mechanisms.
- **Meaning**: LPL-mediated lipolysis and LDL-C lowering mechanisms independently contribute to the risk of coronary disease and diabetes, which supports the development of LPL-enhancing agents for use in the context of LDL-C lowering therapy.

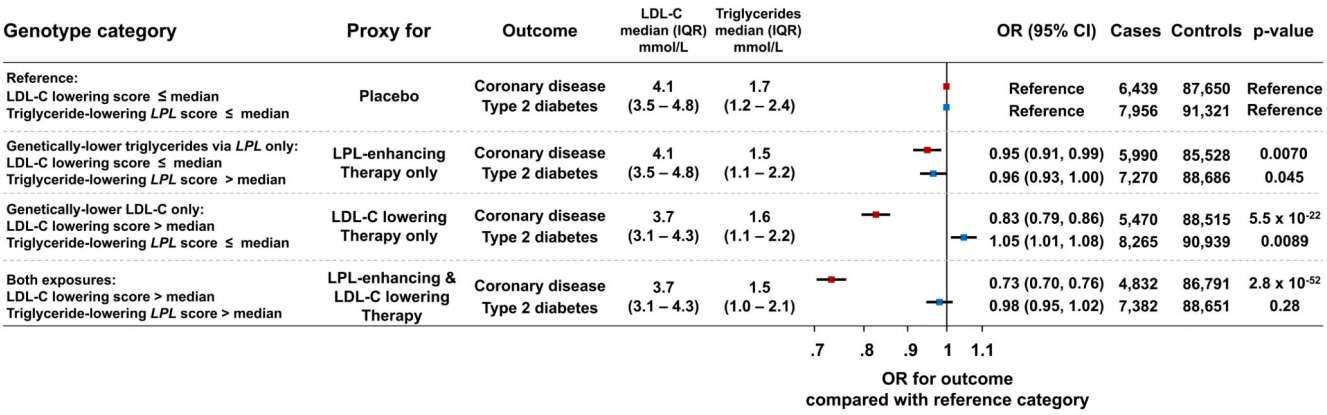

Figure 1. Associations with cardio-metabolic disease outcomes in 2×2 "factorial" genetic analyses.

The figure shows associations with risk of coronary artery disease and type 2 diabetes for each group compared to the reference group. Analyses include individual-level genetic data from 390,470 participants of the UK Biobank, EPIC-Norfolk and EPIC-InterAct studies. Median values and interquartile ranges for lipid levels are from the EPIC-Norfolk study. Abbreviations: IQR, interquartile range; OR, odds ratio; CI, confidence interval; LDL-C, low-density lipoprotein cholesterol.

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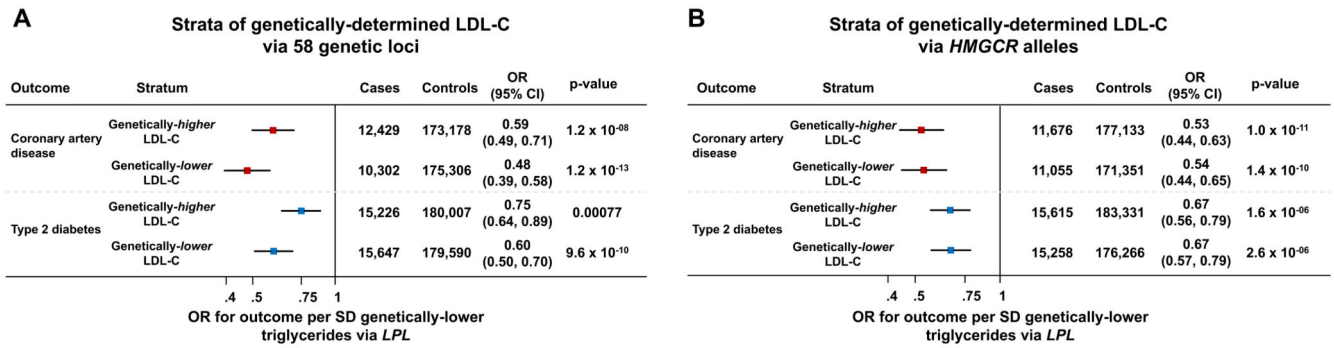

Figure 2. Associations of triglyceride-lowering *LPL* alleles with cardio-metabolic disease outcomes in individuals above or below the median of the population distribution of genetic variants at 58 LDL-C associated genetic loci (Panel A) or *HMGCR* (Panel B).

Analyses include individual-level genetic data from 390,470 participants of the UK Biobank, EPIC-Norfolk and EPIC-InterAct studies. Abbreviations: LDL-C, low-density lipoprotein cholesterol.

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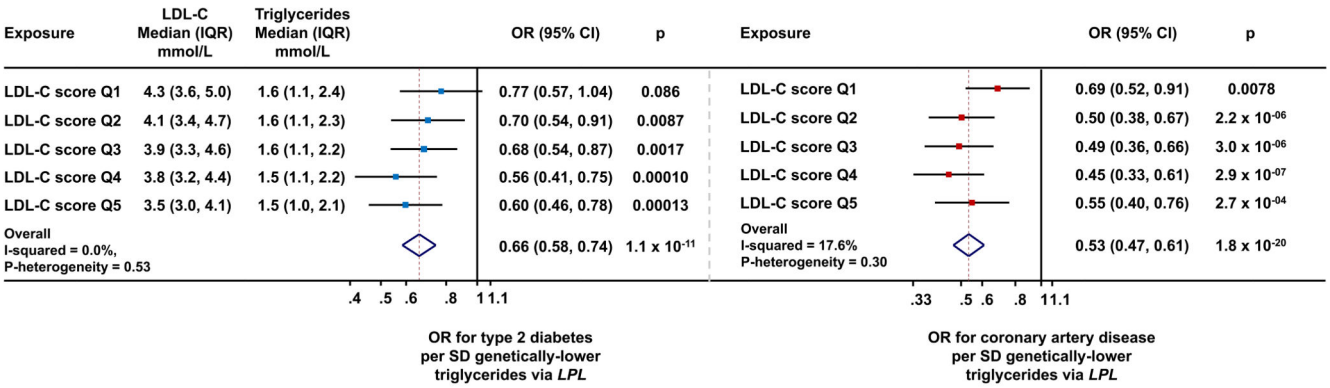

Figure 3. Associations of triglyceride-lowering *LPL* alleles with cardio-metabolic disease outcomes within quintiles of the population distribution of genetic variants at 58 LDL-C associated genetic loci.

Data are from the UK Biobank, EPIC-Norfolk and EPIC-InterAct studies. Median values and interquartile ranges for lipid levels are from the EPIC-Norfolk study. Abbreviations: IQR, interquartile range; OR, odds ratio; CI, confidence interval; LDL-C, low-density lipoprotein cholesterol; Q, quintile.

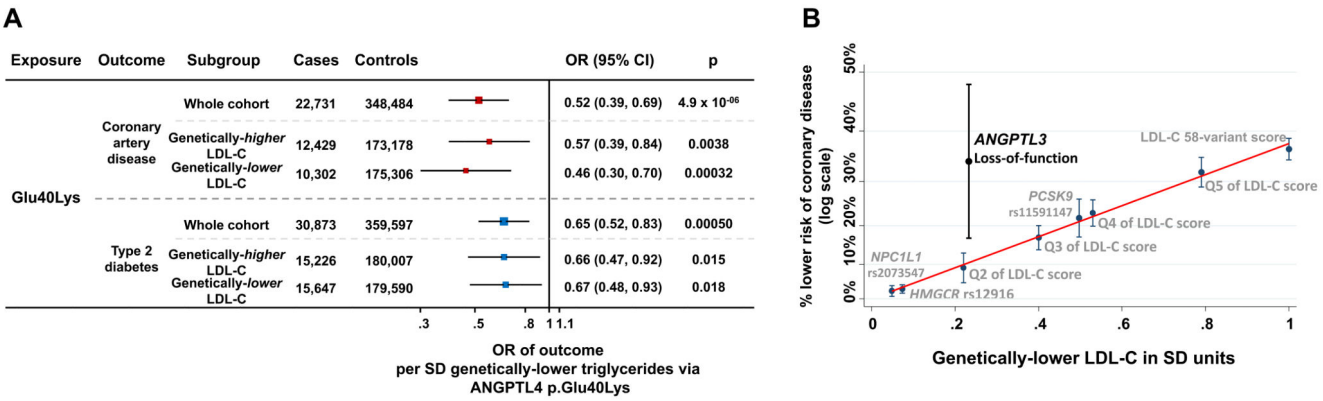

Figure 4. Associations of loss-of-function alleles in *ANGPTL4* and *ANGPTL3*.

Panel A shows associations with cardio-metabolic disease outcomes of the *ANGPTL4* p.Glu40Lys loss-of-function allele in the UK Biobank, EPIC-Norfolk and EPIC-InterAct studies. Associations are scaled to represent the odds ratio per standard deviation genetically-lower triglyceride levels. Data are from the UK Biobank, EPIC-Norfolk and EPIC-InterAct studies. Abbreviations: OR, odds ratio; CI, confidence interval; LDL-C, low-density lipoprotein cholesterol; SD, standard deviation. ***Panel B*** shows associations with protection against coronary disease for different genetic exposures associated with lower LDL-C levels. A clear log-linear relationship between genetic-difference in LDL-C and association with lower risk is observed for several mechanisms, while *ANGPTL3* loss-of-function variants are outliers in this relationship. The graphs display the % lower risk for coronary disease (log scale) and the difference in standard deviations of genetically-lower LDL-C. For individual variants the estimates represent per-allele differences; for quintiles of the LDL-C score the difference compared to the bottom quintile; for the overall genetic score the difference per standard deviation genetically-lower LDL-C; for *ANGPTL3* variants the difference in carriers compared to non-carriers. Abbreviations: Q, quintile; SD, standard deviation; LDL-C, low-density lipoprotein cholesterol.

Table 1

Characteristics of the participants of UK Biobank, EPIC-InterAct, and EPIC-Norfolk included in this study.

Study	UK Biobank	EPIC-InterAct	EPIC-InterAct	EPIC-Norfolk
Study group	Cohort	Incident type 2 diabetes	Non-cases	Cohort
Country	United Kingdom	Multiple European countries	Multiple European countries	United Kingdom
Genotyping chip	Affymetrix UK BiLEVE and UK Biobank Axiom arrays	Illumina 660w quad and Illumina CoreExome chip	Illumina 660w quad and Illumina CoreExome chip	Affymetrix UK Biobank Axiom array
Imputation panel	Haplotype Reference Consortium	Haplotype Reference Consortium	Haplotype Reference Consortium	Haplotype Reference Consortium, UK10K and 1000 Genomes
Participants, N	352,070	9,400	11,593	19,157
Age at baseline, mean years (SD)	57 (8)	55 (7)	52 (9)	59 (9)
Female sex, N (%)	189,755 (54)	4,754 (51)	7,231 (62)	10,175 (53)
Smoking status, current smokers N (%)	36,464 (10)	2,733 (29)	3,115 (27)	2,174 (11)
BMI in kg/m^2, mean (SD)	27.4 (4.8)	29.8 (4.8)	25.8 (4.1)	26.3 (3.8)
Waist-to-hip ratio, mean (SD)	0.87 (0.09)	0.92 (0.09)	0.85 (0.09)	0.86 (0.09)
Systolic blood pressure in mmHg, mean (SD)	138 (19)	144 (20)	132 (19)	135 (18)
Diastolic blood pressure in mmHg, mean (SD)	82 (10)	87 (11)	81 (11)	83 (11)
LDL cholesterol in mmol/L, mean (SD)	NA[a]	4.0 (1)	3.8 (1)	4.0 (1)
HDL cholesterol in mmol/L, mean (SD)	NA[a]	1.2 (0.4)	1.5 (0.4)	1.4 (0.4)
Triglycerides in mmol/L, median (IQR)	NA[a]	1.7 (1.2 – 2.4)	1.1 (0.8 – 1.6)	1.5 (1.1 – 2.2)

Abbreviations: N, number of participants; BMI, body mass index; SD, standard deviation; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; IQR, interquartile range; NA, not available.

[a]Blood lipids concentrations are still being measured in the UK Biobank study and results are not currently available.