Journal of the American Heart Association

ORIGINAL RESEARCH

Insights Into Causal Effects of Genetically Proxied Lipids and Lipid-Modifying Drug Targets on Cardiometabolic Diseases

Liwan Fu, PhD; Qin Liu, MM; Hong Cheng, MB; Xiaoyuan Zhao, MB; Jingfan Xiong, MB; Jie Mi, PhD

BACKGROUND: The differential impact of serum lipids and their targets for lipid modification on cardiometabolic disease risk is debated. This study used Mendelian randomization to investigate the causal relationships and underlying mechanisms.

METHODS: Genetic variants related to lipid profiles and targets for lipid modification were sourced from the Global Lipids Genetics Consortium. Summary data for 10 cardiometabolic diseases were compiled from both discovery and replication data sets. Expression quantitative trait loci data from relevant tissues were employed to evaluate significant lipid-modifying drug targets. Comprehensive analyses including colocalization, mediation, and bioinformatics were conducted to validate the results and investigate potential mediators and mechanisms.

RESULTS: Significant causal associations were identified between lipids, lipid-modifying drug targets, and various cardiometabolic diseases. Notably, genetic enhancement of LPL (lipoprotein lipase) was linked to reduced risks of myocardial infarction (odds ratio [OR]$_1$, 0.65 [95% CI, 0.57–0.75], P_1=2.60×10^{-9}; OR$_2$, 0.59 [95% CI, 0.49–0.72], P_2=1.52×10^{-7}), ischemic heart disease (OR$_1$, 0.968 [95% CI, 0.962–0.975], P_1=5.50×10^{-23}; OR$_2$, 0.64 [95% CI, 0.55–0.73], P_2=1.72×10^{-10}), and coronary heart disease (OR$_1$, 0.980 [95% CI, 0.975–0.985], P_1=3.63×10^{-14}; OR$_2$, 0.64 [95% CI, 0.54–0.75], P_2=6.62×10^{-8}) across 2 data sets. Moreover, significant Mendelian randomization and strong colocalization associations for the expression of LPL in blood and subcutaneous adipose tissue were linked with myocardial infarction (OR, 0.918 [95% CI, 0.872–0.967], P=1.24×10^{-3}; PP.H4, 0.99) and coronary heart disease (OR, 0.991 [95% CI, 0.983–0.999], P=0.041; PP.H4=0.92). Glucose levels and blood pressure were identified as mediators in the total effect of LPL on cardiometabolic outcomes.

CONCLUSIONS: The study substantiates the causal role of lipids in specific cardiometabolic diseases, highlighting LPL as a potent drug target. The effects of LPL are suggested to be influenced by changes in glucose and blood pressure, providing insights into its mechanism of action.

Key Words: cardiometabolic diseases ■ colocalization ■ drug targets ■ eQTL ■ lipids ■ Mendelian randomization

Cardiometabolic diseases continue to be the primary reason for illness and death globally, resulting in roughly 18.6 million fatalities each year, constituting nearly a quarter of all deaths worldwide.[1,2] Predominantly, ischemic heart diseases, strokes, and hypertensive heart diseases are the leading causes of these deaths.[3] Despite the potential for lifestyle modifications to mitigate the risk of these conditions,[4,5] pharmacotherapeutic interventions are essential for maintaining cardiometabolic health in

Correspondence to: Jingfan Xiong, Child and Adolescent Chronic Disease Prevention and Control Department, Shenzhen Center for Chronic Disease Control, No.2021, Buxin Road, Luohu District, Shenzhen 518020, China. Email: xiongjingfan@126.com and Jie Mi, Center for Non-Communicable Disease Management, Beijing Children's Hospital, Capital Medical University, 56 Nanlishi Road, Xicheng District, Beijing 100045, China. Email: jiemi12@vip.sina.com

This article was sent to Jacquelyn Y. Taylor, PhD, PNP-BC, RN, FAHA, FAAN, Associate Editor, for review by expert referees, editorial decision, and final disposition.

Supplemental Material is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.124.038857

For Sources of Funding and Disclosures, see page 16.

JAHA is available at: www.ahajournals.org/journal/jaha

RESEARCH PERSPECTIVE

What Is New?

- Different serum lipids and lipid-modifying targets affect the risk of distinct cardiometabolic diseases differently, and a total of 11 cardiometabolic diseases, 5 life course adiposity phenotypes, 3 body compositions, 4 glycemic traits, and 2 blood pressure traits were included to comprehensively explore the relationship of lipids and lipid-modifying drug targets with cardiometabolic diseases and the potential mechanisms.
- Significant effects of lipids and lipid-modifying drug targets on several cardiometabolic diseases were found through the analysis of gene expression, glucose, and blood pressure.
- Lipoprotein lipase is a promising candidate drug target in preventing the risk cardiometabolic diseases. The effects of lipoprotein lipase are suggested to be influenced by changes in glucose and blood pressure. We should take care of glucose and blood pressure when some drug targets are used.

What Question Should Be Addressed Next?

- What are the specific mechanisms through which different serum lipids and lipid-modifying drug targets influence the pathophysiology of distinct cardiometabolic diseases, particularly in relation to glucose metabolism and blood pressure regulation?
- How can personalized treatment approaches, considering individual variations in lipid profiles, glucose, and blood pressure, optimize the use of lipoprotein lipase and other lipid-modifying drug targets to prevent cardiometabolic diseases across different populations and life-course adiposity phenotypes?

Nonstandard Abbreviations and Acronyms

ABCG5	ATP binding cassette subfamily G member 5
ABCG8	ATP binding cassette subfamily G member 8
ANGPTL3	angiopoietin-related protein 3
eQTL	expression quantitative trait locus
HOMA-IR	homeostasis model assessment-insulin resistance
LD	linkage disequilibrium
LPL	lipoprotein lipase
MR	Mendelian randomization
MR-PRESSO	Mendelian randomization pleiotropy residual sum and outlier

high-risk individuals.[6] Moreover, lipid levels are pivotal in the development of cardiometabolic diseases,[7,8] although findings from observational studies may be influenced by reverse causation or confounding variables. Recent advancements in pharmacotherapy targeting blood lipid metabolism have shown promise in the prevention and treatment of cardiometabolic diseases.[9] Notably, therapies that modulate low-density lipoprotein (LDL) cholesterol, such as mipomersen targeting apoB (apolipoprotein B-100), and those affecting triglyceride levels, such as LPL (lipoprotein lipase) and ANGPTL3 (angiopoietin-related protein 3), operate through distinct lipid metabolic pathways,[10] suggesting varied impacts on cardiometabolic outcomes.

The growing repository of genome-wide association studies (GWAS) data has bolstered the utility of Mendelian randomization (MR) in uncovering the impacts of adverse drug events and aiding drug repurposing.[11] MR mirrors the structure of randomized controlled trials, assigning subjects based on genetic variants, thereby establishing a natural randomization akin to clinical trials.[12] It can investigate the causal association of certain risk factors (referred yo as biomarker MR) or therapeutic drug targets (referred to as drug target MR) with the outcome.[13] In drug target MR, genetic variants that encode protein targets via their respective genes can influence the expression of target genes, similar to the ways in which drugs exert their effects. These insights can anticipate the outcomes of randomized controlled trials.[14] For example, genetic mutations within the *PCSK9* gene that lead to decreased LDL levels have been correlated with a decreased occurrence of coronary heart disease (CHD).[15] MR has become a pivotal tool in investigating the influence of drug targets on both neurological and cardiovascular diseases.[16,17]

The previous studies[18–22] have some shortcomings: (1) most of the mentioned studies are focused on ischemic heart diseases, CHD, and type 2 diabetes, and it is not known what impacts lipids and lipid-targeting drugs have on other cardiometabolic conditions; (2) lipid-drug targets influence the lipid levels through the gene expression, and if gene expression of lipid-drug targets is not included in the study, it is likely to lead to false associations.; and(3) administering lipid-lowering medications might elevate the likelihood of type 2 diabetes[18] and is associated with elevated body mass index.[23] These factors collectively amplify the risk of cardiometabolic diseases.[24] Considerations of adiposity and glucose metabolism can significantly influence the selection of lipid-lowering therapies.[23] Few studies have investigated the potential mediating effect of adiposity and glycemic traits between lipid-drug targets and cardiometabolic diseases.

In the present study, we propose that lipid-drug targets initially influence the expression of their corresponding genes, leading to subsequent alterations in blood lipid levels. These changes are hypothesized to contribute to the development of various cardiometabolic diseases. Additionally, adiposity, blood glucose, and blood pressure are posited to act as mediators in this relationship. Using newly available GWAS information alongside the drug-target MR method, we investigated how lipids and targets for lipid-lowering drugs might affect various cardiometabolic conditions. These conditions encompass myocardial infarction, atrial fibrillation, heart failure, stroke, type 2 diabetes, hypertension, varicose veins, deep vein thrombosis (DVT), pulmonary embolism (PE), ischemic heart diseases, and CHD. This investigation aimed to elucidate unique cardiometabolic relationships with various lipid-lowering drug targets. Complementary analyses employing genetic variants to assess tissue-derived gene expression levels, alongside colocalization and bioinformatics analyses, provided multifaceted evidence to enhance our understanding of these relationships.[25] Additionally, we assessed the genetic proxy impacts of targets for lipid-lowering drugs on life course adiposity, glycemic traits, and blood pressure, which are crucial in understanding the pathways linking therapeutic targets to cardiometabolic risk.

METHODS

Data Sources and Approval

In this study, we employed summary-level GWAS data as depicted in Figure 1 and followed the Strengthening the Reporting of Observational Studies in Epidemiology-Mendelian Randomization guidelines (detailed in Table S1).[26] All data sources, as specified in Table S2, have obtained ethical approvals from their

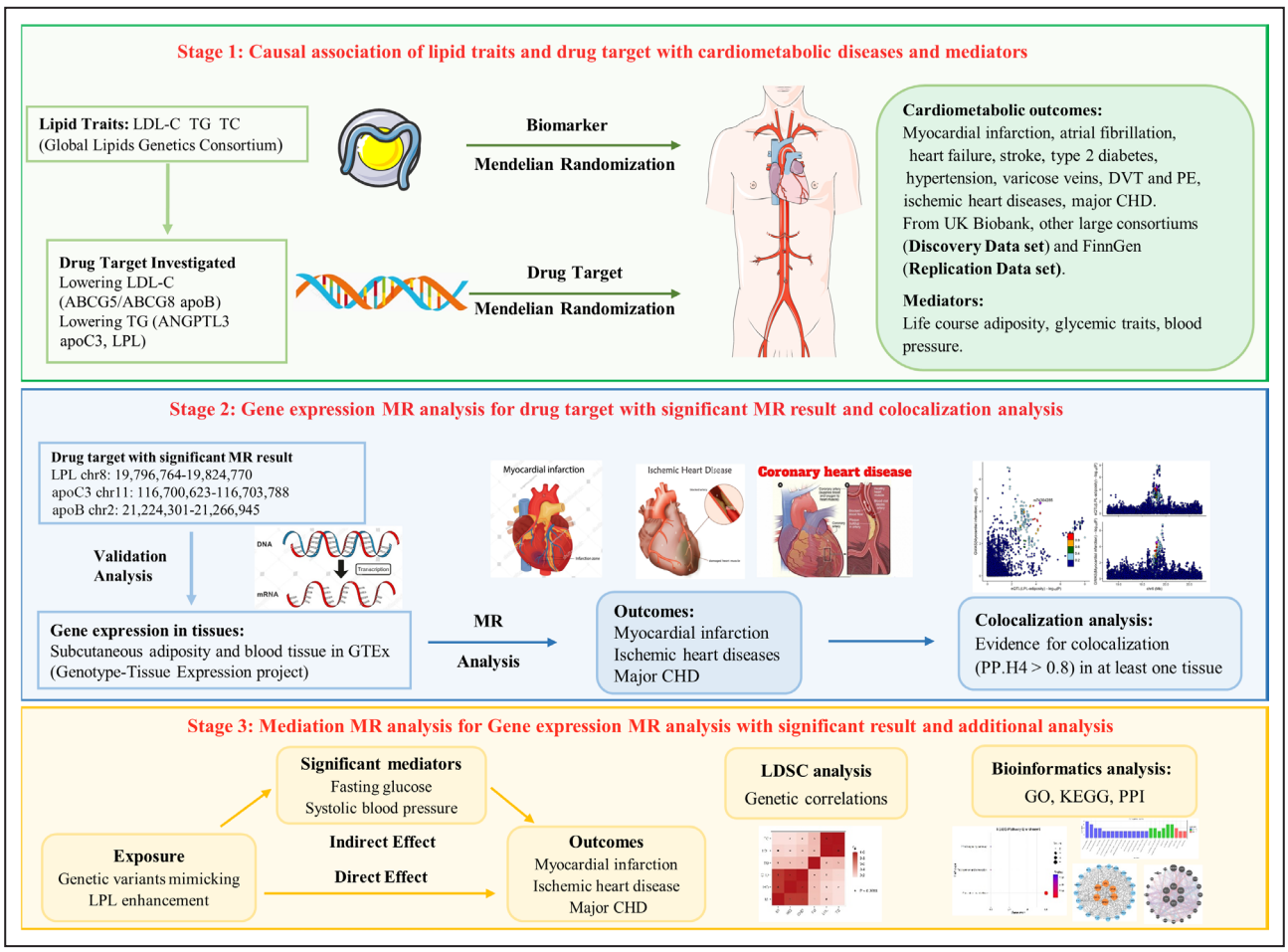


Figure 1. Flow chart for the study design.
ABCG5 indicates ATP binding cassette subfamily G member 5; ABCG8, ATP binding cassette subfamily G member 8; ANGPTL3, angiopoietin-like 3; apoB, apolipoprotein B-100; apoC3, apolipoprotein C-III; CHD, coronary heart disease; DVT, deep vein thrombosis; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; LDL-C, low-density lipoprotein cholesterol; LDSC, linkage disequilibrium score regression; LPL, lipoprotein lipase; MR, Mendelian randomization; PE, pulmonary embolism; PPI, protein–protein interaction networks.; TC, total cholesterol; and TG, triglyceride.

respective institutional review boards and have been made publicly available. Consent was acquired from all participants engaged in these GWAS studies. Due to the nature of the summary-level data used in this MR analysis, no further ethical approval was necessary.

Code Available

The study codes can be accessed at https://github.com/Liwan-Fu/lipids-drugs.

Study Design

As depicted in Figure 1, our study used a 2-sample MR methodology using summary statistics derived from comprehensive GWAS analyses of lipid levels,[27] and cardiometabolic diseases,[28–34] as well as variables like life course adiposity, body composition, glycemic traits, and blood pressure.[33,35–40] Sources included the Global Lipids Genetics Consortium consortium, the UK Biobank study, the FinnGen study, the Genetic Investigation of Anthropometric Traits consortium, and the Meta-Analyses of Glucose and Insulin-Related Traits Consortium consortium, detailed in Table S2. Initially, we calculated genetic correlations among lipid traits, targets for lipid-lowering drugs, and outcomes related to cardiometabolic outcomes including mediators such as adiposity, glycemic traits, and blood pressure. Subsequent MR analysis assessed the effects of genetically inferred lipids and genetically inferred targets for lipid-lowering drugs on these outcomes. For genes associated with these pharmacologic targets that causally affect outcomes, we used data on expression quantitative trait loci (eQTL) from the GTEx-V8 (Genotype-Tissue Expression project version 8)[41] to analyze the causal effects of gene expression on cardiometabolic outcomes within the MR framework. Additionally, colocalization analysis was conducted to assess whether genetic signals for a cardiometabolic trait and an eQTL could be driven by the same causal variant, thereby eliminating misleading signals due to complex linkage disequilibrium (LD) patterns.[42] A mediation MR analysis further examined whether the established causal relationships were direct. Supplementary LD score regression and bioinformatics analyses were performed to provide insights into significant findings.

Data Sources of Lipids and Drug Targets

For the exposure data on serum lipids, we referenced the Global Lipids Genetics Consortium,[27] using single-nucleotide variants (SNVs) associated with triglycerides (triglycerides), total cholesterol (TC), and LDL cholesterol at genome-wide significance levels (*P* value <5E-08). These genetic instruments were selected based on a stringent linkage disequilibrium clumping threshold ($r^2 < 0.001$ and a maximum physical distance of 10 000 kb). To mitigate potential sample overlap biases, we used summary statistics excluding data from UK Biobank participants.

According to current dyslipidemia management recommendations, we chose prevalent lipid-reducing goals and promising therapeutic options like bile acid sequestrants, mipomersen, and antisense oligonucleotides aimed at apoC3 (apolipoprotein C-III) mRNA, and angiopoietin-like 3 (ANGPTL3) inhibitors, as detailed in Table S3.[43,44] We used the DrugBank database (https://go.drugbank.com/) along with multiple reputable reviews[45–47] to identify genes associated with these pharmacologic targets, categorizing them as LDL-C target genes (ie, *APOB*, *ABCG5*, *ABCG8*, *PCSK9*, *NPC1L1*, and *HMGCR*) and triglyceride target genes (ie, *ANGPTL3*, *APOC*,*3* and *LPL*).

Following methodologies established in prior studies,[48,49] we chose SNVs situated within a range of ±100 kb surrounding the target gene locations that were strongly correlated with triglycerides and LDL-C levels (*P* value <5E-08) based on findings from the Global Lipids Genetics Consortium.[27] We assessed LD for these SNVs using the PLINK clumping approach. This analysis used the 1000 Genomes European reference panel, employing an (r^2) threshold <0.20 and a physical distance limit of 250 kb. Due to the proximity of the genes responsible for ABCG8 (ATP binding cassette subfamily G member 8) and ABCG5 (ATP binding cassette subfamily G member 5), variants in these regions were analyzed jointly. Ultimately, this study encompassed 5 pivotal drug targets, specifically:apoB, the combined ABCG5 and ABCG8 complex, LPL, ANGPTL3, and apoC3, as listed in Table S3. A positive control analysis confirmed the correlation of these targets with coronary artery disease, using genetic data obtained from the consortium known as Coronary Artery Disease Genome Wide Replication and Meta-Analysis plusC4D.[34] Relevant eQTL data, indicating genetic variations linked to the levels of gene expression in tissues where target genes are highly expressed, were selected from GTEx-V8[41] using a false discovery rate approach (*P* value <0.05), and additional LD clumping techniques ($r^2 < 0.20$).

Data Sources of Cardiometabolic Diseases

This study incorporated 10 cardiometabolic end points including myocardial infarction, heart failure, atrial fibrillation, stroke, type 2 diabetes, varicose veins, hypertension, DVT and PE, ischemic heart diseases, and CHD. We used discovery and replication data sets to provide robust evidence. Summary-level data from major genetic consortia[29–32] and the UK Biobank study[33] were acquired for the discovery data set concerning these end points with case numbers ranging from 4319 for

DVT and PE to 119 731 for hypertension. The GWAS data for the replication data set were obtained from the FinnGen consortium[28] with case numbers ranging from 7988 for DVT and PE to 55 917 for hypertension. Detailed information regarding the data origins can be found in Table S2. The UK Biobank and other GWAS data sets adjusted for birth year, genetic relatedness, sex, and the initial 4 genetic principal components. The analyses were performed using the R package "SAIGE," accessible at https://github.com/weizhouUMICH/SAIGE. The comprehensive GWAS pipeline used by the UK Biobank is accessible at https://data.bris.ac.uk/data/dataset/pnoat8cxo0u52p6ynfaekeigi. The FinnGen study, a collaborative initiative blending digital health records from Finnish health registries with genotype data from Finnish biobanks, details its methods at https://www.finngen.fi/en. Statistical analyses used mixed-effects logistic regression, incorporating age, sex, 10 principal components, and genotyping batch as covariates for adjustment.

Data Sources of Life Course Adiposity, Glycemic Traits, and Blood Pressure

Life course adiposity data encompassed several metrics: childhood body mass index (BMI; n=39 620), birth weight (n=133 903), waist circumference adjusted for BMI (n=231 353), waist-to-hip ratio adjusted for BMI (n=210 082), and adult BMI (n=454 884). Additional body composition traits included body fat-free mass (n=454 850), body fat mass (n=454 137), and body fat percentage (n=454 633).[33,36–38] The glycemic measures included fasting glucose levels, 2-hour glucose levels, fasting insulin levels, and homeostasis model assessment of insulin resistance (HOMA-IR). Blood pressure data, including systolic and diastolic measurements, covered 757 601 individuals. Information about the data origins for these traits can be found in Table S2.

Statistical Analysis

Mendelian Randomization Methods

This study followed the Strengthening the Reporting of Observational Studies in Epidemiology-Mendelian Randomization guidelines,[26] employing traditional MR methods on the basis of 3 core assumptions for causality[50]: the relevance of genetic variants to the exposure, independence from confounders, and an exclusive pathway through the exposure to affect the outcome. Additionally, we consider essential criteria specific to drug-target MR, such as the localization of genetic variants near drug-regulating genes, the pathological relevance of the exposure as a disease marker, and robust associations with disease biomarkers. These conditions are crucial for interpreting the effects of genetic predispositions on clinical outcomes. Besides the fundamental principles of MR, certain assumptions, such as linearity between exposure and outcomes and absence of interaction between exposure and mediators must also be satisfied.[51] Of particular importance in drug-target MR are these considerations: (1) genetic variants need to be situated within or adjacent to genes that regulate drug targets; (2) the exposure ought to act as an indicator of the disease and faithfully mirror the effects of interventions, such as LDL cholesterol and triglycerides; and (3) genetic variations should demonstrate a robust association with the disease biomarker, although this requirement is not essential for traditional MR analyses.[11]

One fundamental principle of MR is that genetic instruments affect the outcome exclusively via the exposure, thereby mitigating concerns of horizontal pleiotropy.[52] Horizontal pleiotropy occurs when genetic variants influence both the exposure and outcome through separate mechanisms (pleiotropy without correlation) or through a common genetic pathway (correlated pleiotropy).[53] This study adopted several complementary methods to account for horizontal pleiotropy assumptions. Primarily, the inverse variance-weighted method is employed for analysis.[54] Additionally, the weighted median approach[55] addresses potential horizontal pleiotropy; MR-Egger[56] and MR pleiotropy residual sum and outlier (MR-PRESSO)[57] methods assess pleiotropy by detecting horizontal pleiotropic effects that are uncorrelated with the genetic instruments' associations with the outcome; the mode-based estimate[58] provides well-calibrated and not particularly biased results even if most instruments are deemed invalid.

In this study, methods including inverse variance-weighted, mode-based estimate, weighted median, and MR-Egger were executed using the "MendelianRandomization" version 0.5.1[59] by default parameters. The MR-PRESSO analysis was conducted using version 1.0 of its dedicated R package, accessible for download from https://github.com/rondolab/MR-PRESSO. The analysis involved 10 000 simulations, providing a corrected estimate post outlier removal if the global test *P* value from MR-PRESSO indicated statistical significance below the threshold of 0.05, alongside confirmation by a distortion test.

Colocalization Analysis

Colocalization analysis evaluates whether 2 traits share a common genetic variant. In Bayesian colocalization analyses, priors are essential for guiding the inference process by incorporating prior knowledge or assumptions about the data. The primary priors typically used are:

1. Effect size priors: For each trait or phenotype, the effect size of genetic variants (eg, SNVs) is modeled using normal distributions, often centered at 0 with a large variance. This allows for a wide range of possible effect sizes. For example:

$$\beta \sim N\left(0, \tau^2\right)$$

where β is the effect size and τ^2 is the variance.

2. Correlation priors: To assess whether 2 traits share the same causal variant, a prior is placed on the correlation between the effect sizes of SNVs for the 2 traits. This is commonly modeled using a Beta distribution:

$$\gamma \sim \text{Beta}(\alpha, \beta)$$

where α and β determine the distribution's shape, reflecting beliefs about the correlation between traits.

3. Colocalization probability prior: A Beta distribution is often used to model the probability that 2 traits share a causal variant:

$$P(\text{colocalization}) \sim \text{Beta}(1, 1)$$

This reflects the belief that colocalization is equally likely or unlikely, unless prior evidence suggests otherwise. These priors help inform the Bayesian model, guiding the posterior inference about shared genetic signals between traits.

Five hypotheses are tested: (1) H0: the variant shows no association with either phenotype; (2) H1: the variant exhibits association solely with the first phenotype; (3) H2: the variant shows association exclusively with the second phenotype; (4) H3: the variant is associated with both phenotypes; and (5) H4: the variant is strongly associated with both phenotypes.[60] This analysis, conducted by the "coloc" package (version 5.2.2, available at https://github.com/chr1swallace/coloc),[60,61] considers a posterior probability >0.8 for H4 as evidence supporting colocalization. Genetic associations of gene expression for lipid-lowering drug targets with cardiometabolic outcomes were the inputs. Drug targets demonstrating robust colocalization (PP.H4 > 0.8) with cardiometabolic diseases are considered potential target genes.

Mediation Analysis

To ascertain whether the direct association between drug targets and cardiometabolic diseases exists, we investigated the connections involving genetically proxied targets of lipid-lowering drugs and potential mediators—namely, life course adiposity, glycemic traits, and blood pressure—using MR analyses. By exploring the exposure-mediator-outcome pathway, we identified possible mediation effects for significant mediators. For an accurate assessment of the direct impact of genetic lipid drug targets on cardiometabolic disease risks, we applied the Two-Step Cis-MR method, ensuring adjustment for intermediary variables.[62] This approach mitigates the bias introduced by strong LD correlations among genetic variants, a common occurrence in cis-MR analyses.[62] We evaluated the indirect impacts of genetic proxy lipid drug targets on cardiometabolic diseases and the mediated proportions using the Product of Coefficients approach, with indirect effect SEs calculated using the Delta method.[51]

Statistical Test and Criterion Used in This Study

The main analysis employed the inverse variance-weighted method, supplemented by sensitivity assessments via the weighted median, mode-based estimate, MR-Egger, and MR-PRESSO methods. Associations were considered statistically significant if they showed nominal significance ($P \leq 0.05$) and were consistent across sensitivity analyses. Results were considered robust if they remained statistically significant after Bonferroni correction for multiple comparisons (0.05/10=0.005 for cardiometabolic diseases; 0.05/14=0.00357 for mediators) and if there was no evidence of significant pleiotropy. Pleiotropy was assessed using the MR-Egger test, where results were considered free of significant pleiotropic effects if the Egger intercept term had a P value ≥0.05, or if the MR-Egger regression P value was <0.05. However, the MR-Egger method was excluded from drug-target MR analyses to avoid potential violations of the Instrument Strength Independent of Direct Effect assumption by instruments from the same gene region.[63] Genetic proxies for drug targets exhibited low linkage disequilibrium, with r^2 values <0.2. For significant MR findings related to the drug target, we applied stricter LD thresholds ($r^2 < 0.01$ and $r^2 < 0.001$) to ensure the robustness of our findings. The LD relationship between genetic variants was computed via the LDmatrix Tool, and adjustments for LD structure were incorporated in both the inverse variance-weighted method and sensitivity analyses. F-statistics were computed to evaluate the potential for bias from instruments with weak explanatory power, and statistical power was evaluated using the "mRnd" tool, a method designed to assess the power of MR studies. The analysis was conducted by simulating different sample sizes and effect sizes to determine the likelihood of detecting a true association given the study's design and available data. The power calculations accounted for factors such as the number of genetic instruments used, the strength of these instruments, and the expected causal effect sizes. Based on these simulations, the results indicated that the study had sufficient power to detect associations with an effect size greater than a certain value, with a power of

calculated value (%) at the significance level of 0.05. For example, when the outcome is a binary variable, the sample size is 140 000, the type I error rate is 0.05, the proportion of cases is 0.2, and the odds ratio (OR) value is 0.82 or 1.18, the power value can reach 0.8. Similarly, when the outcome is a continuous variable, the sample size is 140 000, the type I error rate is 0.05, the regression coefficient between the exposure and outcome variables is 0.07, and the proportion of variance in the exposure variable explained by SNVs is 0.01, the power value can reach 0.75. We employed both discovery data set and replication data set to ensure that the analysis is adequately powered to detect meaningful genetic associations in the main context of the study. Effect estimates, including ORs for cardiometabolic diseases and regression coefficients for mediators, were standardized to represent the impact of a 1-mmol/L change in lipid levels, corresponding to specific mg/dL values for LDL, triglycerides, and TC. The eQTL data were normalized based on the SD of gene expression per each additional effect allele.

We conducted all statistical analyses using 2-sided tests within R (version 4.1.0), specifically using the following packages: LdlinkR,[64] coloc,[65] locuscomparer,[42] MendelianRandomization,[59] and MR-PRESSO.[57] The code for this study is publicly available at Liwan-Fu/lipids-drugs on GitHub.

Bioinformatics Analysis

Bioinformatics analyses were conducted to elucidate the biological mechanisms underlying lipid and lipid-drug target interactions. Genome-wide genetic correlations between LDL-C, TC, triglycerides, and cardiometabolic diseases were estimated using LD score regression.[66] Analyses for pathway enrichment, encompassing both Gene Ontology and Kyoto Encyclopedia of Genes and Genomes,[67,68] were conducted to identify biological processes potentially associated with drug-target genes. The network of protein–protein interactions for lipid-drug targets was built using the STRING database, available at https://string-db.org/, with a minimum confidence score of 0.4, a maximum of 20 interactors, and default settings for other parameters.[69] Results from the protein–protein interactions network were visualized using Cytoscape (V3.10.1)[70] and supplemented by analyses conducted on GeneMANIA (https://genemania.org/)[71] with detailed data set information available elsewhere.[71] For bioinformatics analyses, we deemed a significant adjusted *P* value threshold of <0.05 after applying multiple testing corrections using the false discovery rate method.

RESULTS

We tested for the effects on these outcomes (myocardial infarction, atrial fibrillation, heart failure, stroke, type 2 diabetes, hypertension, varicose veins, DVT, PE, ischemic heart diseases, and CHD) and we report the ones that were significant in the results discussion.

Lipid Traits and Cardiometabolic Diseases

In this research, we identified instrumental variables for lipid traits: 80 SNVs for LDL, 87 SNVs for TC, and 55 SNVs for triglycerides, as detailed in Table S4. The discovery data set revealed significant associations:

LDL levels correlated with increased risks of myocardial infarction (OR, 1.51 [95% CI, 1.35–1.67], $P=4.38\times10^{-14}$), heart failure (OR, 1.15 [95% CI, 1.08–1.22], $P=5.91\times10^{-6}$), ischemic heart diseases (OR, 1.024 [95% CI, 1.018–1.03], $P=2.34\times10^{-16}$), and CHD (OR, 1.011 [95% CI, 1.008–1.015], $P=8.50\times10^{-13}$; Figure 2A and Table S5). These findings were corroborated in a replication data set (Figure 2B and Table S6). Genetic predisposition to triglycerides was linked with heightened risks of myocardial infarction (OR, 1.30 [95% CI, 1.17–1.44], $P=4.31\times10^{-7}$), heart failure (OR, 1.15 [95% CI, 1.08–1.23], $P=2.57\times10^{-5}$), hypertension (OR, 1.03 [95% CI, 1.01–1.05], $P=6.24\times10^{-4}$), ischemic heart diseases (OR, 1.02 [95% CI, 1.01–1.03], $P=1.41\times10^{-9}$), and CHD (OR, 1.01 [95% CI, 1.005–1.013], $P=3.86\times10^{-6}$; Figure 2A and Table S5). Again, replication data set results supported these associations (Figure 2B and Table S6).

TC enhancements were significantly correlated with increased risks of myocardial infarction (OR, 1.47 [95% CI, 1.32–1.64], $P=5.45\times10^{-12}$), heart failure (OR, 1.15 [95% CI, 1.08–1.22], $P=8.12\times10^{-6}$), ischemic heart diseases (OR, 1.024 [95% CI, 1.018–1.03], $P=9.09\times10^{-17}$), CHD (OR, 1.012 [95% CI, 1.009–1.015], $P=4.26\times10^{-13}$), and a decreased risk of type 2 diabetes (OR 0.87 [95% CI, 0.79–0.96], $P=4.64\times10^{-3}$; Figure 2A and Table S5). Replication data set findings confirmed these relationships (Figure 2B and Table S6). Additional investigation into the causal impact of genetic predisposition to lipids on adiposity throughout life, glycemic traits, and blood pressure is illustrated in Figure 3A and detailed in Table S7.

Lipid-Lowering Drug Targets and Cardiometabolic Diseases

Genetic instrumental analysis identified several SNVs linked to lipid-lowering drug targets, including 15 within the *APOB* gene, 12 within *APOC3*, 3 within *ANGPTL3*, 7 within ABCG5/ABCG8, and 15 within the *LPL* gene, as detailed in Table S8. Notably, we observed correlations indicating increased risk of coronary artery disease for all targets except ANGPTL3, validating the genetic instruments used (Table S9).[10,72] These instruments demonstrated robustness with F statistics ranging from 34.2 to 916.1 ($F>10$), confirming their reliability for further analysis (Table S8).

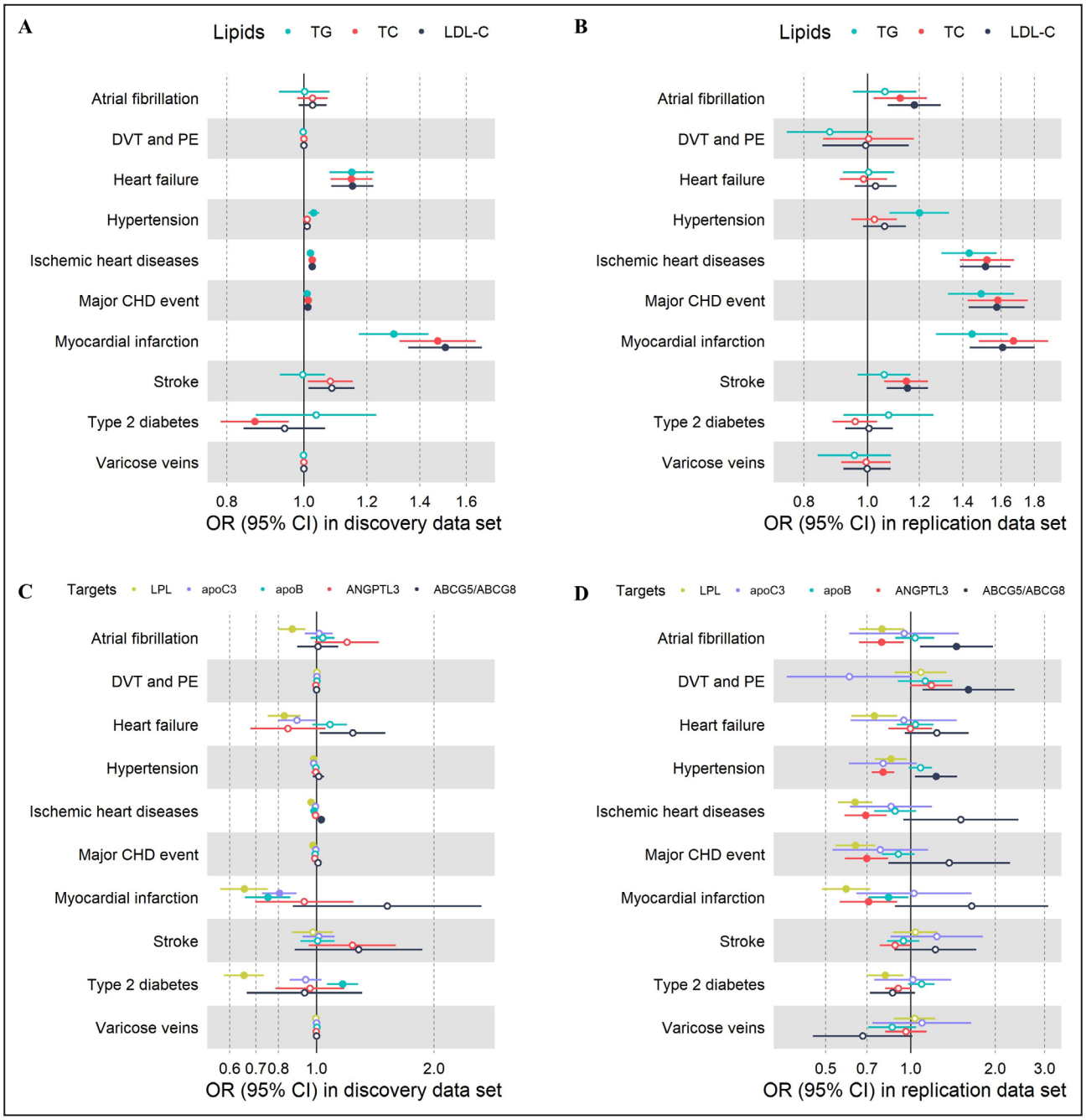


Figure 2. Forest plots illustrating causal effects of genetically proxied lipids and lipid-lowering drug targets on cardiometabolic diseases in the main analysis.

Causal association between a 1-mmol/L (LDL-C, 38.7 mg/dL; triglycerides, 88.9 mg/dL; TC, 41.8 mg/dL; >170 000 subjects) change in the lipid level with cardiometabolic risk (>360 000 subjects) in the discovery data set **(A)**, cardiometabolic risk (>180 000 subjects) in the replication data set **(B)**. Causal association between a 1-mmol/L (LDL-C, 38.7 mg/dL; triglycerides, 88.9 mg/dL; >170 000 subjects) change in the lipid levels of 5 lipid-lowering drug targets with cardiometabolic risk (>360 000 subjects) in the discovery data set **(C)**, cardiometabolic risk (>180 000 subjects) in the replication data set **(D)**. Data are displayed as odds ratio with 95% CI (error bars). Entries with larger than *P* value threshold for statistical significance after Bonferroni correction (0.005 for cardiometabolic diseases in discovery data set and 0.05 for cardiometabolic diseases in replication data set) are drawn with a hollow point. ABCG5 indicates ATP binding cassette subfamily G member 5; ABCG8, ATP binding cassette subfamily G member 8; ANGPTL3, angiopoietin-like 3; apoB, apolipoprotein B-100; apoC3, apolipoprotein C-III; CHD, coronary heart disease; DVT, deep vein thrombosis; LDL-C, low-density lipoprotein cholesterol; LPL, lipoprotein lipase; OR, odds ratio; PE, pulmonary embolism; TC, total cholesterol; and TG, triglyceride.

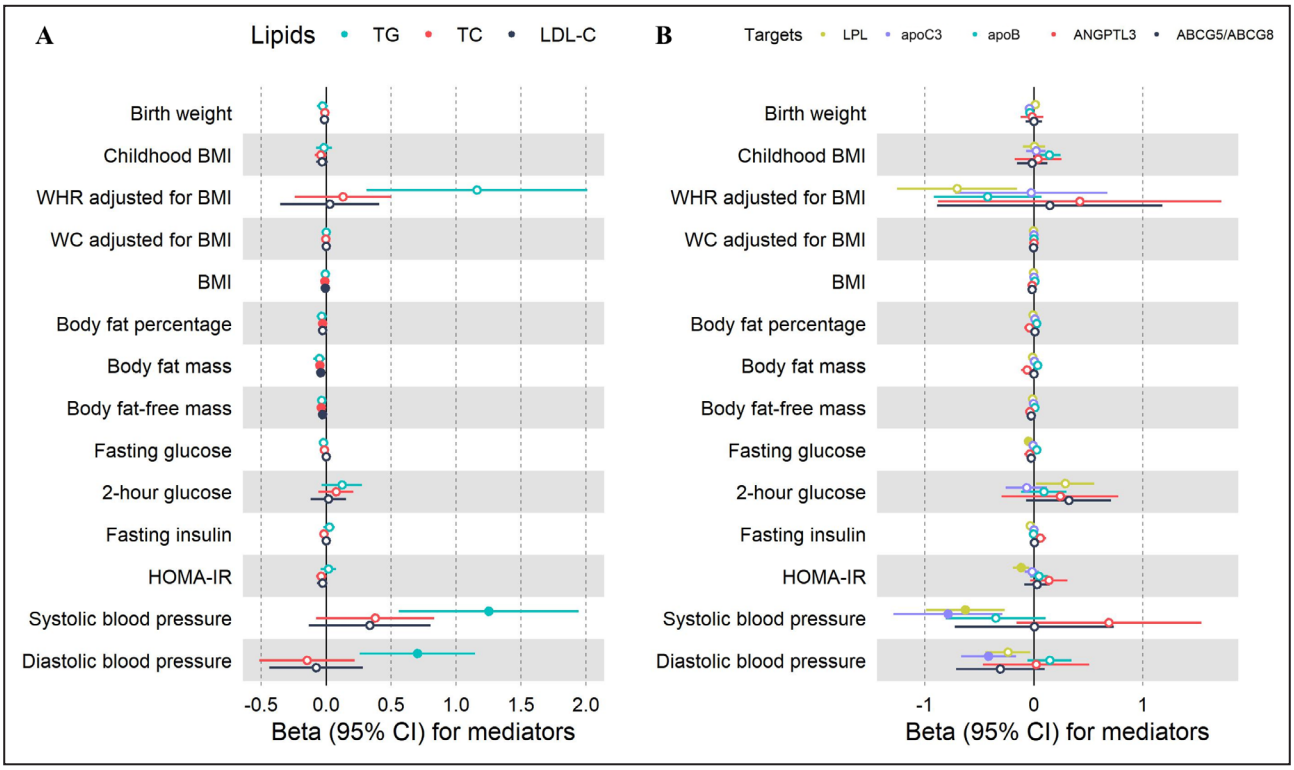


Figure 3. Forest plots illustrating causal effects of genetically proxied lipids and lipid-lowering drug targets on mediators (life course adiposity, glycemic traits, and blood pressure, more than 37 000 subjects) in the main analysis.
Causal association between a 1-mmol/L (LDL-C, 38.7 mg/dL; triglycerides, 88.9 mg/dL; TC, 41.8 mg/dL; >170 000 subjects) change in the lipid level with mediators **(A)**, and causal association between a 1-mmol/L (LDL-C, 38.7 mg/dL; triglycerides, 88.9 mg/dL; >170 000 subjects) change in the lipid levels of 5 lipid-lowering drug targets with mediators **(B)**. Data are displayed as beta with 95% CI (error bars). Entries with larger than *P* value threshold for statistical significance after Bonferroni correction (0.0036 for mediators) are drawn with a hollow point. ABCG5 indicates ATP binding cassette subfamily G member 5; ABCG8, ATP binding cassette subfamily G member 8; ANGPTL3, angiopoietin-like 3; apoB, apolipoprotein B-100; apoC3, apolipoprotein C-III; BMI, body mass index; HOMA-IR, homeostatic model assessment for insulin resistance; LDL-C, low-density lipoprotein cholesterol; LPL, lipoprotein lipase; TC, total cholesterol; TG, triglyceride; WC, waist circumference; and WHR, waist-to-hip ratio.

The study also examined genetic correlations with SNVs of drug targets (Table S10), leading to comprehensive main and sensitivity analyses to assess causal effects on cardiometabolic diseases. Significant findings across both discovery and replication data sets included (Figures 2C, 2D, Tables S11 and S12) are discussed next.

Genetic proxies indicative of an LPL increase, similar to reducing triglycerides by 1 mmol/L (equivalent to 88.9 mg/dL), significantly lowered the risk of myocardial infarction (OR, 0.65 [95% CI, 0.57–0.75]; $P=2.60\times10^{-9}$), atrial fibrillation (OR, 0.87 [95% CI, 0.80–0.94]; $P=4.49\times10^{-4}$), heart failure (OR, 0.83 [95% CI, 0.75–0.91]; $P=1.18\times10^{-4}$), type 2 diabetes (OR, 0.65 [95% CI, 0.58–0.73]; $P=1.11\times10^{-12}$), hypertension (OR, 0.983 [95% CI, 0.973–0.994]; $P=2.51\times10^{-3}$), ischemic heart diseases (OR, 0.968 [95% CI, 0.962–0.975]; $P=5.50\times10^{-23}$) and CHD (OR, 0.980 [95% CI, 0.975–0.985]; $P=3.63\times10^{-14}$).

ApoB inhibition was linked to protective effects against myocardial infarction (OR, 0.75 [95% CI, 0.66–0.86]; $P=2.59\times10^{-5}$) and ischemic heart diseases (OR, 0.985 [95% CI, 0.980–0.991]; $P=6.26\times10^{-7}$), whereas apoC3 inhibition correlated with reduced myocardial infarction risk (OR, 0.80 [95% CI, 0.73–0.89]; $P=2.69\times10^{-5}$).

Replication analyses were similar with discovery analyses and were both 2-sample MR, and the data in replication cohort were independent with the discovery data sets for both exposure and outcomes. The results in discovery data set were mostly corroborated by the replication cohort, with some variations observed in the effects of apoB and apoC3 on myocardial infarction. Moreover, the alternative MR approaches showed consistency in findings, except for the causal associations of LPL with atrial fibrillation and heart failure, where 1 MR method failed to achieve nominal significance (Table S11). In summary, through drug target MR analysis, we observed causal effects of apoB and apoC3 on myocardial infarction and of LPL on myocardial infarction, type 2 diabetes, ischemic heart diseases, hypertension, and CHD, as validated

across discovery and replication data sets as well as sensitivity MR analyses.

Correlation Between Drug Targets for Lowering Lipids and Life Course Adiposity, Glycemic Traits, and Blood Pressure

For continuous outcomes, we used "β" to represent the causal effects of drug targets on outcomes. Figure 3B illustrates the causal effects (β) of 5 genetically determined lipid-lowering drug targets on life course adiposity, glycemic traits, and blood pressure. Specifically, genetic enhancement of LPL was associated with significant protective effects on fasting glucose (β, −0.05 [95% CI, −0.074 to −0.026], P=4.72×10^{-5}), HOMA-IR (β, −0.116 [95% CI, −0.191 to −0.042], P=2.24×10^{-3}), and systolic blood pressure (β, −0.627 [95% CI, −0.986 to −0.267], P=6.38×10^{-4}; Table S13). ApoC3 mimicry also significantly reduced systolic (β, −0.786 [95% CI, −1.285 to −0.287], P=2.01×10^{-3}) and diastolic blood pressure (β, −0.414 [95% CI, −0.667 to −0.162], P=1.31×10^{-3}; Table S13). After Bonferroni correction, additional drug targets like ABCG5/ABCG8, apoB, and ANGPTL3 exhibited no significant impact on these traits.

Gene Expression and Cardiometabolic Diseases

Investigating the relationship between genetic variants that lower triglycerides levels in the *LPL* and *APOC3* genes, alongside genetic variants related to LDL in the *APOB* gene, significant correlations with certain cardiometabolic diseases were observed. These variants, expressed predominantly in whole blood and subcutaneous fat tissues, served as instrumental variables for further analysis. Despite using the GTEx-V8 database,[41] no significant loci related to the *APOB* gene in whole blood tissue were found, and all genetic variants of *APOC3* expression in these tissues did not meet the threshold for statistical significance (P≤0.05 following correction for false discovery rate; Table S14).

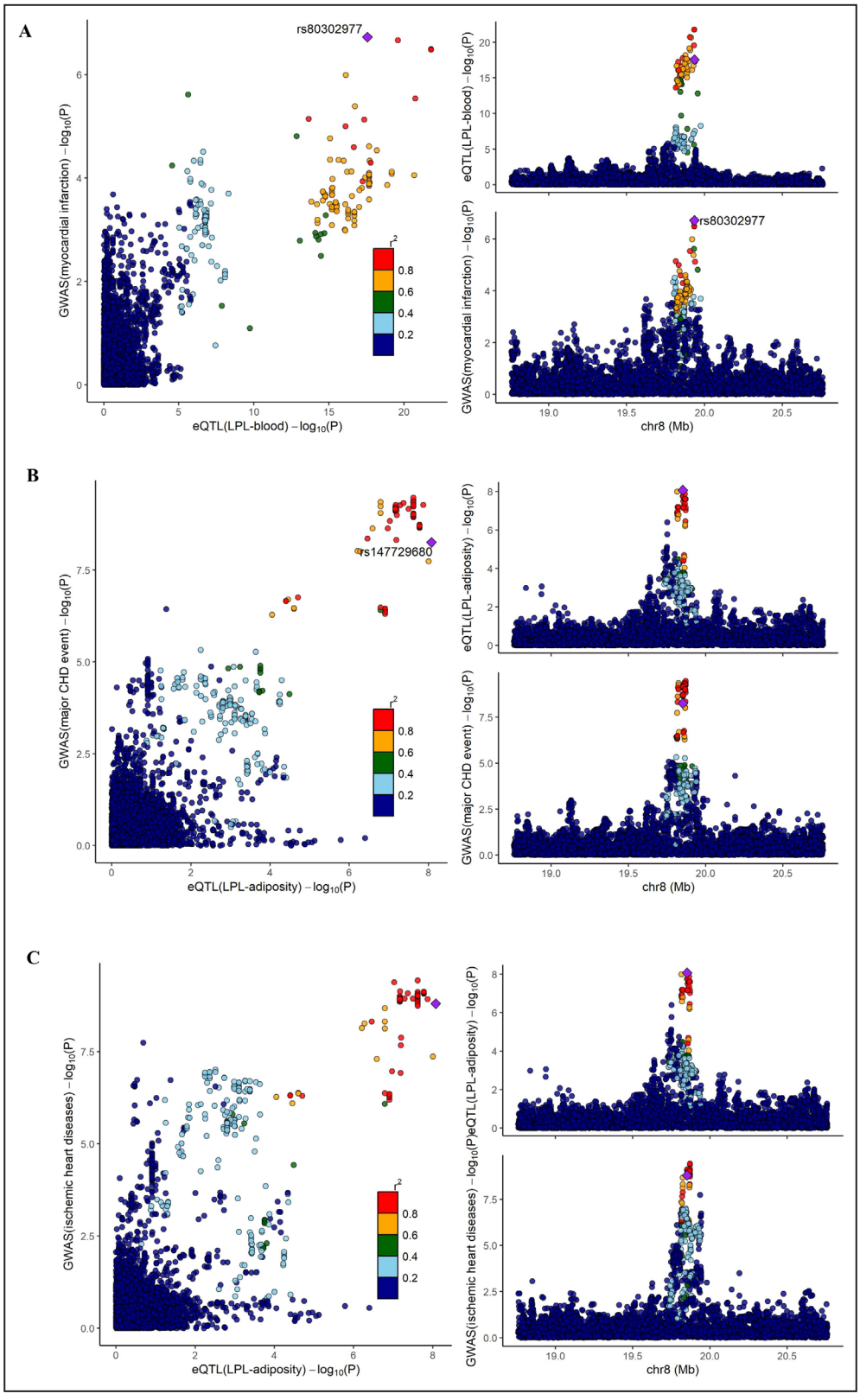
Genetic variations affecting *APOB* expression in subcutaneous adipose tissue and *LPL* levels observed in both whole blood and subcutaneous adipose tissues were used as instruments for further studies. An increase of 1 SD in adipose tissue *APOB* expression was identified to a modest rise in the likelihood of ischemic heart disease (OR, 1.002 [95% CI, 1.001–1.004]; P=4.70×10^{-3}; Table S15). Conversely, an elevation of 1 SD in LPL expression within adipose tissue correlated with reduced chances of myocardial infarction (OR, 0.788 [95% CI, 0.679–0.915]; P=1.70×10^{-3}) and CHD (OR, 0.991 [95% CI, 0.983–0.999]; P=0.041), and similar protective effects were found for *LPL* expression in blood tissue against myocardial infarction (OR, 0.918 [95% CI, 0.872–0.967]; P=1.24×10^{-3}), ischemic heart disease (OR, 0.995 [95% CI, 0.993–0.997]; P=5.02×10^{-5}), and CHD (OR, 0.997 [95% CI, 0.995–0.998]; P=1.95×10^{-5}; Table S15). The findings remained stable under more stringent linkage disequilibrium thresholds (r^2<0.01 and r^2<0.001; Table S16).

Colocalization analyses were conducted to determine whether genetic variations affecting the expression of *APOB* and *LPL* in pertinent tissues and cardiometabolic diseases share causal SNVs. A common causal variant linking LPL expression in blood tissue with myocardial infarction was highly probable (PP.H4=0.99; Figure 4A and Table S17). Furthermore, the presence of *LPL* expression in subcutaneous fat tissue showed a shared causal variant with ischemic heart disease and CHD, with posterior probabilities indicating strong colocalization (ischemic heart disease: PP.H4=0.93; CHD: PP.H4=0.92) as shown in Figures 4B, 4C, and Table S17. However, the colocalization for *APOB* expression related to myocardial infarction, ischemic heart disease, and CHD was less significant (myocardial infarction: PP.H4=0.09; ischemic heart disease: PP.H4=0.08; CHD: PP.H4=0.02; Table S17). Causal variants (rs80302977 and rs147729680) were linked with LPL expression in relevant tissues and cardiometabolic diseases, suggesting these associations are not affected by different SNVs that are in LD.

Mediation Analysis

The established role of life course adiposity, glycemic traits, and blood pressure as risk factors for cardiometabolic diseases suggested these factors likely mediate the effects of LPL on cardiometabolic risks including myocardial infarction, ischemic heart disease, and CHD. A 2-stage MR analysis delineated the pathways connecting *LPL* expression to these diseases. Notably, causal relationships were identified between LPL and fasting glucose, HOMA-IR, and systolic blood pressure (Figure 3B and Table S13). The indirect impact of LPL on myocardial infarction via systolic blood pressure was

Figure 4. Illustration of the colocalization results.
Genetic colocalization between eQTL of LPL in blood tissue (n=670) and GWAS of myocardial infarction (n=171 875) **(A)**. Genetic colocalization between eQTL of LPL in subcutaneous adipose tissue (n=581) and GWAS of major CHD event (n=361 194) **(B)**. Genetic colocalization between eQTL of LPL in subcutaneous adipose tissue (n=581) and GWAS of ischemic heart diseases (n=361 194) **(C)**. The labeled SNV is the lead SNV, and other SNVs are colored according to their LD with the lead SNP. CHD indicates coronary heart disease; eQTL, expression quantitative trait loci; GWAS, genome-wide association study; LD, linkage disequilibrium; LPL, lipoprotein lipase; and SNV, single nucleotide variant.



quantified at 0.974 (95% CI, 0.961–0.988; P=1.96×10^{-4}; Figure 5A). Adjusting for systolic blood pressure, the direct impact of LPL on myocardial infarction decreased, suggesting partial mediation by systolic blood pressure reduction (Table S18). Reducing levels of fasting glucose modestly mediated the effects of LPL on CHD (mediation proportion, 2.76% [95% CI, 0.127%–6.04%]; Figure 5B) and ischemic heart disease (mediation

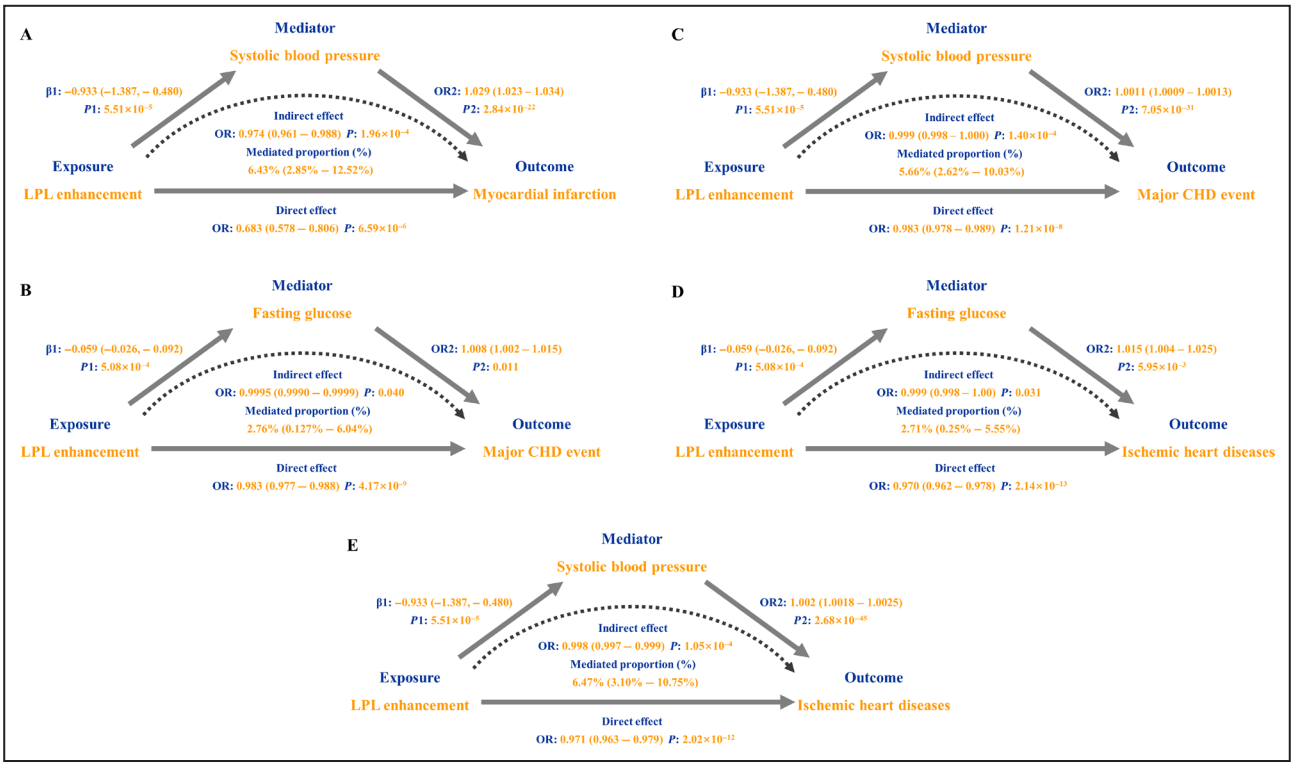


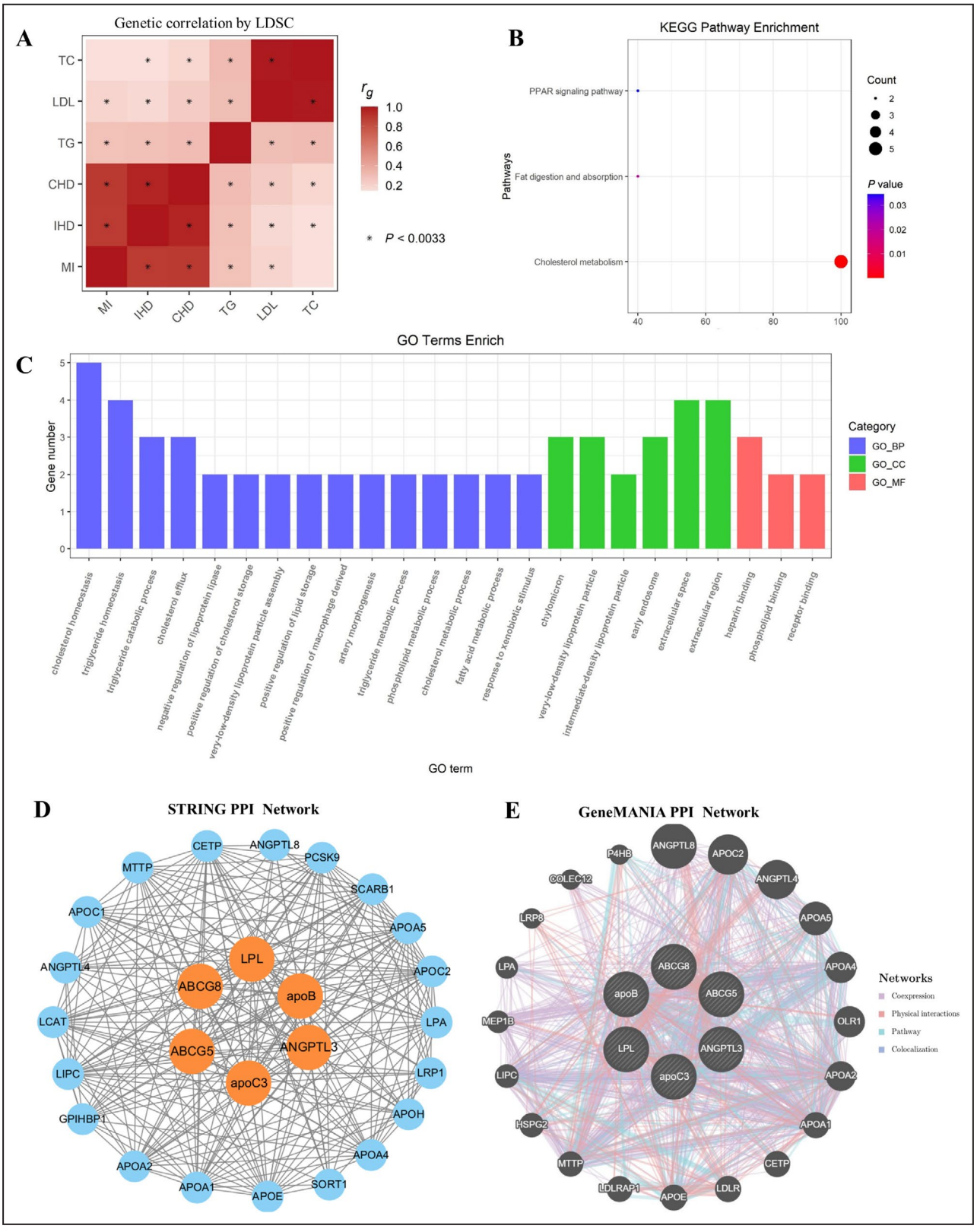
Figure 5. Mediation analysis detecting the effect of LPL on myocardial infarction (n=171 875), major CHD event (n=361 194), and ischemic heart diseases (n=361 194) via potential mediators under a 2-step MR analysis framework.
"Direct effect" suggests the effect of LPL on myocardial infarction, major CHD event, and ischemic heart diseases after adjusting for the mediator (systolic blood pressure (n=757 601) [**A, C, E**] or fasting glucose (n=200 622) [**B, D**]). "Indirect effect" suggests the effect of LPL on myocardial infarction, major CHD event, and ischemic heart diseases through the mediator (systolic blood pressure [**A, C, E**] or fasting glucose [**B, D**]). CHD indicates coronary heart disease; LPL, lipoprotein lipase; MR, Mendelian randomization; OR, odds ratio.

proportion, 2.71% [95% CI, 0.25%–5.55%]; Figure 5D). Additionally, systolic blood pressure also mediated the association between LPL enhancement and both ischemic heart disease and CHD, with direct effects quantified in subsequent analyses (Figure 5C, 5E).

Bioinformatics Analysis

The genetic correlation between lipid traits and cardiometabolic conditions like ischemic heart disease, myocardial infarction, and CHD was investigated by employing LD score regression, indicating a moderate to high significant correlation, except for TC with myocardial infarction (Figure 6A). Gene Ontology enrichment and Kyoto Encyclopedia of Genes and Genomes enrichment analyses were employed to explore the interactions between genes and biological terms, as well as the relationships between genes and functional pathways. Notably, the pathways of most significance within the biological process category were related to cholesterol homeostasis. In the cellular component category, drug target genes were enriched in the lipoprotein particle. Key pathways identified by Kyoto Encyclopedia of Genes and Genomes enrichment analysis included metabolic pathways involving cholesterol, fat absorption, and activation of PPARs (peroxisome proliferator-activated receptors; Figure 6B). These drug target genes are primarily involved in cholesterol and fat metabolism, as well as the PPAR signaling pathways. Using the STRING database, we

Figure 6. Bioinformatics analysis for lipids and lipid drug targets.
A, LDSC evaluates genetic correlation between lipids and CHD, IHD, and MI. **B**, KEGG enrichment results for lipid drug targets. **C**, GO enrichment results for lipid drug targets. **D**, PPI network built with STRING. **E**, PPI network built with GeneMANIA. ABCG5 indicates ATP binding cassette subfamily G member 5; ABCG8, ATP binding cassette subfamily G member 8; ANGPTL3, angiopoietin-like 3; apoB, apolipoprotein B-100; apoC3, apolipoprotein C-III; CHD, coronary heart disease; GO, Gene Ontology; GO_BP, GO analysis for biological process; GO_CC, GO analysis for cellular component; GO_MF, GO analysis for molecular function; IHD, ischemic heart diseases; KEGG, Kyoto Encyclopedia of Genes and Genomes; LDL-C, low-density lipoprotein cholesterol; LDSC, linkage disequilibrium score regression; LPL, lipoprotein lipase; MI, myocardial infarction; PPAR, peroxisome proliferator-activated receptor; PPI, protein–protein interaction networks; TC, total cholesterol; and TG, triglyceride.



constructed a network of these 6 drug target genes that was then visualized in Cytoscape. Figure 6D illustrates the relationships between the 6 drug targets and 20 other proteins within a protein–protein interactions network. For the GeneMANIA-derived protein–protein interactions network (Figure 6E), besides the 6 primary drug targets, it includes 20 potentially interacting genes, exhibiting physical interactions (56.84%),

pathways (26.36%), coexpression (9.41%), and colocalization (7.39%). The functional analysis of this network aligns with earlier enrichment studies, highlighting the involvement of these genes in the assembly of active LPL and LIPC lipase complexes and in regulating lipid homeostasis and glycerolipid metabolism.

DISCUSSION

In this study, we have analyzed genetic variations linked to lipids such as LDL-C, triglycerides, and TC, along with lipid-targeted medications including *APOB*, *LPL*, *ANGPTL3*, *ABCG5/ABCG8*, and *APOC3* and a spectrum of cardiometabolic conditions encompassing myocardial infarction, heart failure, stroke, atrial fibrillation, type 2 diabetes, ischemic heart disease, hypertension, varicose veins, DVT and PE, and CHD using extensive GWAS data. Our MR analyses sought to elucidate the causal impacts of lipid concentrations, as well as lipid-drug targets on these cardiometabolic conditions and their mediators (birth weight, BMI, waist circumference adjusted for BMI, childhood BMI, waist-to-hip ratio adjusted for BMI, body fat percentage, mass, fat-free mass, fasting insulin, fasting glucose, 2-hour glucose, HOMA-IR, and systolic and diastolic blood pressure). The findings suggest that higher genetically proxied concentrations of LDL, triglycerides, and TC are causally linked to myocardial infarction, ischemic heart diseases, and CHD. Furthermore, our drug-target MR analysis indicated that an increase in LPL is causally linked with various outcomes encompassing myocardial infarction, atrial fibrillation, heart failure, ischemic heart diseases, hypertension, type 2 diabetes, CHD, HOMA-IR, fasting glucose, and systolic blood pressure; with apoB and apoC3 specifically linked to myocardial infarction and apoC3 also correlated with both systolic and diastolic blood pressure.

Public Health and Clinical Implications

Our research elucidates the significant influence of dyslipidemia on the incidence of cardiometabolic diseases, including hypertension, diabetes, and cardiovascular diseases, which pose serious public health challenges globally.[73] Evidence from prior studies underscores the intricate connection between dyslipidemia and a cluster of cardiometabolic risk factors such as glucose dysregulation and increased adiposity.[73] There is robust support for the effectiveness of lipid-lowering therapies in reducing the incidence of cardiometabolic events, especially among individuals with established cardiovascular conditions.[74] Information obtained from the Cholesterol Treatment Trialists' Collaboration indicates that every 1 mmol/L decrease in LDL (approximately 40 mg/dL) corresponds to a 20% decrease in cardiovascular disease events,[75] which has prompted a change in clinical guidelines toward more stringent lipid targets. The identification and validation of lipid targets are crucial for both understanding the pathogenesis of dyslipidemia and implementing effective interventions to reduce cardiometabolic disease prevalence. Our findings propose that LPL acts as a viable pharmacological target for managing myocardial infarction, ischemic heart diseases, and CHD. The lipid-lowering effect of LPL, confirmed through our bioinformatics analysis, underscores its role in mitigating cardiometabolic disease risks. Mediation analyses indicate that this protective benefit is partially mediated through the regulation of fasting glucose, and further investigation indicates that systolic blood pressure may also mediate the relationship between LPL activity and cardiometabolic outcomes. In summary, a more nuanced understanding of the relationships between dyslipidemia and cardiometabolic traits can inform clinical strategies, enhance individual health outcomes, and alleviate the economic burden on health care systems.

Comparison With Related Studies

Observational data have consistently demonstrated there is a connection between elevated levels of LDL and a heightened risk of CHD and stroke mortality. Our study reinforces this link by establishing a causal connection between CHD and elevated LDL levels, supported by multiple genetic instruments. In contrast, an analysis of more than 14 000 individuals included in the National Health and Nutrition Examination Survey spanning 2 decades identified a higher likelihood of death from any cause in people with LDL levels <70 mg/dL, compared with those with levels ranging from 100 to 129.9 mg/dL, after accounting for demographic variables and concurrent health conditions.[76] However, such observational findings could be influenced by confounding factors, including unadjusted subclinical conditions. Additionally, a breadth of evidence from observational,[77] genetic,[19,78] interventional[79] studies supports the causality of LDL-C in the pathogenesis of CHD. Recent genetic research further implicates triglycerides[80,81] as a causal factor in cardiometabolic diseases. A synthesis of data from many studies uniformly demonstrated a direct causal association between increased LDL concentrations and a heightened risk of cardiometabolic diseases.[82,83] Particularly in European populations, MR studies have consistently shown that genetic variations linked with decreased LDL levels are correlated to a reduced risk of cardiometabolic conditions, offering a solid foundation for targeted preventive strategies.[84,85] These studies also noted a dose-dependent correlation between LDL levels and the risk of cardiometabolic diseases, assessing the impact of each LDL associated genetic variant.[83] Compared with these studies, our research used >200

genetic instruments to provide robust evidence that lipids—including LDL, TC, and triglycerides—not only LDL, have causal effects on a variety of cardiometabolic diseases, each with distinct impacts. It is crucial to note that total cholesterol comprises LDL and HDL, plus a small contribution from very LDL. The interpretation of its causal association with CHD is primarily influenced by LDL, as it contains the majority of the cholesterol found in plasma.

Current advancements in therapeutic development are marked by several promising treatments aimed at significantly reducing triglycerides concentrations.[86,87] This direction is partly informed by genetic findings that rare loss-of-function variations within the *LPL* gene are correlated with elevated triglycerides concentrations and an increased risk of cardiometabolic diseases. Similarly, mutations in *APOC3* and *ANGPTL3*, which influence *LPL* expression, are also associated with variations in triglycerides concentrations and the susceptibility to cardiometabolic disorders.[20,88–90] Despite these genetic associations, it remains uncertain whether interventions targeting the LPL pathway to reduce plasma triglycerides levels will concretely diminish the risk of such diseases. Our research suggested that targeting the *LPL* gene to lower triglycerides levels could significantly reduce the risk of several cardiometabolic diseases, including ischemic heart disease, myocardial infarction, atrial fibrillation, heart failure, type 2 diabetes, hypertension, and CHD. Gene expression analyses in various tissues and colocalization studies provided robust evidence that targeting LPL has causal effects on myocardial infarction, ischemic heart diseases, and CHD. Moreover, drugs that lower triglycerides by targeting LPL were observed to be correlated not only with decreased cardiometabolic risk but also with lower HOMA-IR, fasting glucose levels, and systolic blood pressure. These findings align with previous studies.[10,72,91] Mediation analysis indicates that the protective effects of LPL activation against ischemic heart disease, myocardial infarction and CHD are partially mediated by reductions in fasting glucose and systolic blood pressure, suggesting these factors as potential mediators in the amelioration of cardiometabolic conditions. Recent MR analyses offered evidence that reducing type 2 diabetes risk through pathways related to LPL is viable.[91] Additionally, our research identified apoC3 as a factor causally linked with myocardial infarction and blood pressure, marking it as a significant risk marker for cardiometabolic diseases because of its pivotal role in lipid metabolism.[92] High apoC3 levels result in increased triglycerides concentrations through an accumulation of VLDL[3] and chylomicrons. The literature supports our findings that apoC3 affects the risk of cardiometabolic diseases.[90,93,94]

Explanations and Implications

LDL is recognized as an apoB-containing, cholesterol-rich lipoprotein, playing a critical role in cholesterol delivery to the arterial intima. The permeability of the arterial wall facilitates the entry of several lipoproteins, compounded by elevated lipoprotein levels in plasma and increased blood pressure.[95] Within the subendothelial space, macrophage-derived foam cells phagocytose oxidized, aggregated, or otherwise modified LDL, contributing to atherosclerosis and, subsequently, cardiometabolic diseases. ApoB-containing lipoproteins, primarily consisting of triglycerides and cholesterol, are metabolized by LPL, which hydrolyzes triglycerides from these particles, transforming triglyceride-rich very LDL into triglyceride-depleted LDL particles that are cleared from the plasma via hepatic LDL receptors. All lipoproteins containing apoB and measuring <70 nm in diameter, such as remnants of triglyceride-rich very LDL and LDL particles, have the potential to permeate the endothelial barrier and potentially accumulate within arterial walls,[96] where their lipid content can induce inflammatory responses and promote atherosclerotic plaque formation.[21]

Triglyceride levels are largely influenced by LPL activity, which is associated with the surface of microvascular endothelial cells.[97] Genetic variations that reduce LPL activity can increase triglyceride-rich lipoprotein particle levels,[97] Conversely, studies focusing on genetics have consistently shown heightened cardiometabolic events in individuals carrying specific genetic variants that enhance the action of LPL inhibitors, such as apoC3 and ANGPTL3. ANGPTL3,[20,88–90] mainly synthesized in the liver, primarily functions to suppress LPL and endothelial lipase, affecting the breakdown of phospholipids in lipoproteins, especially within HDL particles.[98] Our bioinformatics analysis has identified key genes primarily involved in cholesterol metabolism, fat digestion, and absorption. Although various medications, including omega-3 fatty acids, thiazolidinediones, fibrates, and metformin, can modulate pharmacological activity affecting LPL, they do not primarily target LPL activation. Given the pivotal function of LPL in lipid processing, there is increasing interest in developing novel therapeutics that directly activate LPL, such as compounds 50F10 and C10d.[99] Our findings not only associate LPL activation with a reduction in cardiometabolic disease risks but also link it to decreased fasting glucose levels and systolic blood pressure, underscoring its potential in therapeutic strategies. Given the pivotal role of LPL in improving cardiometabolic health, our investigation, alongside previous pharmacological and genetic studies,[72,85,100] underscored a substantial clinical necessity for the development of drugs that enhance LPL activity.

Strengths and Limitations

Several notable strengths are evident in this study: It includes a substantial cohort of cardiometabolic disease cases and uses dual independent data sets for validating outcomes. Colocalization and mediation analyses add analytical rigor. The implementation of MR minimizes residual confounding and reduces the risk of false negatives in extensive GWAS. Previous work established robust genetic statistics and used MR methodologies to ascertain causal relationships between risk factors and chronic diseases, further reducing confounding effects.[101–110] To the best of our knowledge, this study represents a pioneering effort to integrate MR analysis with colocalization and mediation analysis. This approach aims to elucidate the causal relationships between genetically proxied lipids and the targets of lipid-modifying pharmaceuticals, using genetic summary data and eQTL data. The large pool of SNVs used as instrumental variables enhances the statistical power of our interpretations, although some weak associations may be overlooked. Genetic variants used to simulate drug effects allows for causal inferences with minimal confounding from traditional epidemiological factors, akin to random allocation in randomized controlled trials.[111] Prior research supports that MR design can refine the design and interpretation of clinical trials, particularly those involving lipid-modifying agents.[112] Design of positive controls and diverse sensitivity assessments within the drug-target MR framework lend additional credibility to our findings.

The study also acknowledges several limitations. First, the inclusion of primarily individuals of European descent might restrict applicability across diverse ethnic populations. Second, despite extensive sensitivity analyses for validating MR assumptions, the independence of instruments from undetected confounding factors could not be directly assessed. Although germline instruments are less susceptible to confounders, minimizing population stratification is essential to address concerns about the violation of independence assumptions within a 2-sample MR framework.[113] Notably, sensitivity analyses help check for major violations of assumptions, but unless there is contrary evidence, they do not reliably identify all significant violations. Therefore, choosing an analysis method based solely on a test, when the power of that test is unknown, is not appropriate. If the power to detect specific violations cannot be determined, the confidence in the results should be greatly diminished. Additionally, testing to identify the final model is generally not a good approach. Tests should address scientific questions, not guide the choice of analysis method. Using robust methods as the primary approach helps avoid this issue. Therefore, multiple sensitivity analyses, including mode-based estimation and MR-PRESSO, should be used and reasonably interpreted with caution. Third, MR is constrained by the limitations of the originating data sources, such as the representativeness of the UK Biobank for the broader UK or European populations.[114] Fourth, confounding by LD could influence observed effects, though consistent findings across different LD thresholds, supported by colocalization analyses, suggest minimal influence. Fifth, the study focused solely on the on-target effects of specific drug targets without assessing potential off-target effects. Sixth, genetic variations illustrate how lifelong alterations in lipid levels affect cardiometabolic risks, contrasting with the shorter-term effects of lipid-lowering medications.[6] Seventh, the influence of environmental factors on genetic risks for cardiometabolic diseases might introduce bias into effect estimates.[6] Eighth, because lipid levels were considered the focal biomarkers, only the effects of lipid modification on cardiometabolic diseases were assessed, excluding other pharmacological impacts. Lastly, it is clinically pertinent to determine whether lipid-modifying therapeutic targets manifest consistent effects across different baseline lipid levels, a question that may be addressed by developing nonlinear methodologies for drug-target MR analyses.

CONCLUSIONS

In summary, our study elucidated the causal relationships between genetically proxied lipid levels and major cardiometabolic diseases such as myocardial infarction, ischemic heart diseases, and CHD. The results underscore LPL as a favorable candidate for targeted drug development, pivotal in the pathogenesis and progression of cardiometabolic disorders. The regulatory role of LPL in triglyceride metabolism, possibly mediated by factors like fasting glucose and systolic blood pressure, may be a part of the underlying mechanism. Genetic variations in *LPL* affect the expression of critical genes, suggesting potential biological mechanisms that could substantiate the observed causal effects and support our hypothesis. Additional research is necessary to validate these mechanisms thoroughly and evaluates the potential of LPL activators in both basic and clinical research settings for cardiometabolic diseases.

ARTICLE INFORMATION

Received September 13, 2024; accepted December 13, 2024.

Affiliations

Center for Non-Communicable Disease Management, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, China (L.F., J.M.); Department of Ultrasound, Children's Hospital

of the Capital Institute of Pediatrics, Beijing, China (Q.L.); Department of Epidemiology, Capital Institute of Pediatrics, Beijing, China (H.C., X.Z.); Child and Adolescent Chronic Disease Prevention and Control Department, Shenzhen Center for Chronic Disease Control, Shenzhen, China (J.X.); and Key Laboratory of Major Diseases in Children, Ministry of Education, Beijing, China (J.M.).

Acknowledgments

The authors would like to acknowledge the UK Biobank study and the summary statistics data provided by the Neale lab (http://www.nealelab.is/uk-biobank). They are grateful to the participants and investigators of the FinnGen study (https://www.finngen.fi/en) and other GWAS summary-level data accessed from https://www.ebi.ac.uk/gwas. The authors sincerely appreciate the contributions of all reviewers and editors, whose feedback greatly improved this study. Author contributions: Jie Mi and Liwan Fu initiated and planned the study. Liwan Fu, Qin Liu, Hong Cheng, and Xiaoyuan Zhao collected the data. Liwan Fuconducted the data analysis and interpretation. Liwan Fu, Jingfan Xiong, and Jie Mi prepared the initial draft of the article. Liwan Fu, Qin Liu, Hong Cheng, Xiaoyuan Zhao, Jingfan Xiong, and Jie Mi extensively reviewed and edited the article for significant intellectual contributions. Liwan Fu, Jingfan Xiong, and Jie Mi received funding for the project.

Sources of Funding

Financial support for this study was provided through grants awarded by the National Natural Science Foundation of China (82204063, 82373589), the Capital's Funds for Health Improvement and Research (grant no. Capital's Funds for Health Improvement and Research 2024-4-20911), and the Beijing Natural Science Foundation Program and Scientific Research Key Program of Beijing Municipal Commission of Education (KZ202010025039). Jie Mi and Jingfan Xiong were also supported by the Sanming Project of Medicine in Shenzhen (SZSM202311020).

Disclosures

None.

Supplemental Material

Data S1
Tables S1–S18

REFERENCES

1. Tsao CW, Aday AW, Almarzooq ZI, Anderson CAM, Arora P, Avery CL, Baker-Smith CM, Beaton AZ, Boehme AK, Buxton AE, et al. Heart disease and stroke Statistics-2023 update: a report from the American Heart Association. *Circulation*. 2023;147:e93–e621. doi: 10.1161/cir.0000000000001123
2. Vaduganathan M, Mensah GA, Turco JV, Fuster V, Roth GA. The global burden of cardiovascular diseases and risk: a Compass for future health. *J Am Coll Cardiol*. 2022;80:2361–2371. doi: 10.1016/j.jacc.2022.11.005
3. Michaeli DT, Michaeli JC, Albers S, Boch T, Michaeli T. Established and emerging lipid-lowering drugs for primary and secondary cardiovascular prevention. *Am J Cardiovasc Drugs*. 2023;23:477–495. doi: 10.1007/s40256-023-00594-5
4. Magnussen C, Ojeda FM, Leong DP, et al. Global effect of modifiable risk factors on cardiovascular disease and mortality. *N Engl J Med*. 2023;389:1273–1285. doi: 10.1056/NEJMoa2206916
5. Li S, Liu Z, Joseph P, Hu B, Yin L, Tse LA, Rangarajan S, Wang C, Wang Y, Islam S, et al. Modifiable risk factors associated with cardiovascular disease and mortality in China: a PURE substudy. *Eur Heart J*. 2022;43:2852–2863. doi: 10.1093/eurheartj/ehac268
6. Holmes MV, Richardson TG, Ference BA, Davies NM, Davey SG. Integrating genomics with biomarkers and therapeutic targets to invigorate cardiovascular drug development. *Nat Rev Cardiol*. 2021;18:435–453. doi: 10.1038/s41569-020-00493-1
7. Gao Y, Shah LM, Ding J, Martin SS. US trends in cholesterol screening, lipid levels, and lipid-lowering medication use in US adults, 1999 to 2018. *J Am Heart Assoc*. 2023;12:e028205. doi: 10.1161/JAHA.122.028205
8. Fulcher J, O'Connell R, Voysey M, et al. Efficacy and safety of LDL-lowering therapy among men and women: meta-analysis of individual data from 174,000 participants in 27 randomised trials. *Lancet*. 2015;385:1397–1405. doi: 10.1016/s0140-6736(14)61368-4
9. Fuentes AV, Pineda MD, Venkata KCN. Comprehension of top 200 prescribed drugs in the US as a resource for pharmacy teaching, training and practice. *Pharmacy (Basel)*. 2018;6:43. doi: 10.3390/pharmacy6020043
10. Richardson t, Leyden GM, Wang Q, Bell JA, Elsworth B, Davey Smith G, Holmes MV. Characterising metabolomic signatures of lipid-modifying therapies through drug target mendelian randomisation. *PLoS Biol*. 2022;20:e3001547. doi: 10.1371/journal.pbio.3001547
11. Walker VM, Davey Smith G, Davies NM, Martin RM. Mendelian randomization: a novel approach for the prediction of adverse drug events and drug repurposing opportunities. *Int J Epidemiol*. 2017;46:2078–2089. doi: 10.1093/ije/dyx207
12. Arsenault BJ. From the garden to the clinic: how Mendelian randomization is shaping up atherosclerotic cardiovascular disease prevention strategies. *Eur Heart J*. 2022;43:4447–4449. doi: 10.1093/eurheartj/ehac394
13. Schmidt AF, Finan C, Gordillo-Marañón M, Asselbergs FW, Freitag DF, Patel RS, Tyl B, Chopade S, Faraway R, Zwierzyna M, et al. Genetic drug target validation using Mendelian randomisation. *Nat Commun*. 2020;11:3255. doi: 10.1038/s41467-020-16969-0
14. Reay WR, Cairns MJ. Advancing the use of genome-wide association studies for drug repurposing. *Nat Rev Genet*. 2021;22:658–671. doi: 10.1038/s41576-021-00387-z
15. Cohen JC, Boerwinkle E, Mosley TH Jr, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N Engl J Med*. 2006;354:1264–1272. doi: 10.1056/NEJMoa054013
16. Williams DM, Finan C, Schmidt AF, Burgess S, Hingorani AD. Lipid lowering and Alzheimer disease risk: a mendelian randomization study. *Ann Neurol*. 2020;87:30–39. doi: 10.1002/ana.25642
17. Harrison SC, Holmes MV, Burgess S, Asselbergs FW, Jones GT, Baas AF, van't Hof FN, de Bakker PIW, Blankensteijn JD, Powell JT, et al. Genetic association of lipids and lipid drug targets with abdominal aortic aneurysm: a meta-analysis. *JAMA Cardiol*. 2018;3:26–33. doi: 10.1001/jamacardio.2017.4293
18. Ference BA, Robinson JG, Brook RD, Catapano AL, Chapman MJ, Neff DR, Voros S, Giugliano RP, Davey Smith G, Fazio S, et al. Variation in PCSK9 and HMGCR and risk of cardiovascular disease and diabetes. *N Engl J Med*. 2016;375:2144–2153. doi: 10.1056/NEJMoa1604304
19. Holmes MV, Asselbergs FW, Palmer TM, Drenos F, Lanktree MB, Nelson CP, Dale CE, Padmanabhan S, Finan C, Swerdlow DI, et al. Mendelian randomization of blood lipids for coronary heart disease. *Eur Heart J*. 2015;36:539–550. doi: 10.1093/eurheartj/eht571
20. Rosenson RS, Davidson MH, Hirsh BJ, Kathiresan S, Gaudet D. Genetics and causality of triglyceride-rich lipoproteins in atherosclerotic cardiovascular disease. *J Am Coll Cardiol*. 2014;64:2525–2540. doi: 10.1016/j.jacc.2014.09.042
21. Nordestgaard BG, Varbo A. Triglycerides and cardiovascular disease. *Lancet*. 2014;384:626–635. doi: 10.1016/s0140-6736(14)61177-6
22. Haase CL, Tybjærg-Hansen A, Grande P, Frikke-Schmidt R. Genetically elevated apolipoprotein A-I, high-density lipoprotein cholesterol levels, and risk of ischemic heart disease. *J Clin Endocrinol Metab*. 2010;95:E500–E510. doi: 10.1210/jc.2010-0450
23. Ferrières J, Lautsch D, Gitt AK, de Ferrari G, Toplak H, Elisaf M, Drexel H, Horack M, Baxter C, Ambegaonkar B, et al. Body mass index impacts the choice of lipid-lowering treatment with no correlation to blood cholesterol - findings from 52 916 patients in the Dyslipidemia International Study (DYSIS). *Diabetes Obes Metab*. 2018;20:2670–2674. doi: 10.1111/dom.13415
24. Eeg-Olofsson K, Cederholm J, Nilsson PM, Zethelius B, Svensson AM, Gudbjörnsdóttir S, Eliasson B. New aspects of HbA1c as a risk factor for cardiovascular diseases in type 2 diabetes: an observational study from the Swedish National Diabetes Register (NDR). *J Intern Med*. 2010;268:471–482. doi: 10.1111/j.1365-2796.2010.02265.x
25. Munafò MR, Davey SG. Robust research needs many lines of evidence. *Nature*. 2018;553:399–401. doi: 10.1038/d41586-018-01023-3
26. Skrivankova VW, Richmond RC, Woolf BAR, Yarmolinsky J, Davies NM, Swanson SA, VanderWeele TJ, Higgins JPT, Timpson NJ, Dimou N, et al. Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian randomization: the STROBE-MR statement. *JAMA*. 2021;326:1614–1621. doi: 10.1001/jama.2021.18236

27. Willer CJ, Schmidt EM, Sengupta S, Peloso GM, Gustafsson S, Kanoni S, Ganna A, Chen J, Buchkovich ML, Mora S, et al. Discovery and refinement of loci associated with lipid levels. *Nat Genet*. 2013;45:1274–1283. doi: 10.1038/ng.2797
28. Kurki MI, Karjalainen J, Palta P, Sipilä TP, Kristiansson K, Donner KM, Reeve MP, Laivuori H, Aavikko M, Kaunisto MA, et al. FinnGen provides genetic insights from a well-phenotyped isolated population. *Nature*. 2023;613:508–518. doi: 10.1038/s41586-022-05473-8
29. Shah S, Henry A, Roselli C, Lin H, Sveinbjörnsson G, Fatemifar G, Hedman ÅK, Wilk JB, Morley MP, Chaffin MD, et al. Genome-wide association and mendelian randomisation analysis provide insights into the pathogenesis of heart failure. *Nat Commun*. 2020;11:163. doi: 10.1038/s41467-019-13690-5
30. Xue A, Wu Y, Zhu Z, Zhang F, Kemper KE, Zheng Z, Yengo L, Lloyd-Jones LR, Sidorenko J, Wu Y, et al. Genome-wide association analyses identify 143 risk variants and putative regulatory mechanisms for type 2 diabetes. *Nat Commun*. 2018;9:2941. doi: 10.1038/s41467-018-04951-w
31. Nielsen JB, Thorolfsdottir RB, Fritsche LG, Zhou W, Skov MW, Graham SE, Herron TJ, McCarthy S, Schmidt EM, Sveinbjornsson G, et al. Biobank-driven genomic discovery yields new insight into atrial fibrillation biology. *Nat Genet*. 2018;50:1234–1239. doi: 10.1038/s41588-018-0171-3
32. Malik R, Chauhan G, Traylor M, Sargurupremraj M, Okada Y, Mishra A, Rutten-Jacobs L, Giese AK, van der Laan SW, Gretarsdottir S, et al. Multiancestry genome-wide association study of 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes. *Nat Genet*. 2018;50:524–537. doi: 10.1038/s41588-018-0058-3
33. Bycroft C, Freeman C, Petkova D, Band G, Elliott LT, Sharp K, Motyer A, Vukcevic D, Delaneau O, O'Connell J, et al. The UK Biobank resource with deep phenotyping and genomic data. *Nature*. 2018;562:203–209. doi: 10.1038/s41586-018-0579-z
34. Nikpay M, Goel A, Won HH, Hall LM, Willenborg C, Kanoni S, Saleheen D, Kyriakou T, Nelson CP, Hopewell JC, et al. A comprehensive 1,000 genomes-based genome-wide association meta-analysis of coronary artery disease. *Nat Genet*. 2015;47:1121–1130. doi: 10.1038/ng.3396
35. Chen J, Spracklen CN, Marenne G, Varshney A, Corbin LJ, Luan J', Willems SM, Wu Y, Zhang X, Horikoshi M, et al. The trans-ancestral genomic architecture of glycemic traits. *Nat Genet*. 2021;53:840–860. doi: 10.1038/s41588-021-00852-9
36. Vogelezang S, Bradfield JP, Ahluwalia TS, Curtin JA, Lakka TA, Grarup N, Scholz M, van der Most PJ, Monnereau C, Stergiakouli E, et al. Novel loci for childhood body mass index and shared heritability with adult cardiometabolic traits. *PLoS Genet*. 2020;16:e1008718. doi: 10.1371/journal.pgen.1008718
37. Horikoshi M, Beaumont RN, Day FR, et al. Genome-wide associations for birth weight and correlations with adult disease. *Nature*. 2016;538:248–252. doi: 10.1038/nature19806
38. Shungin D, Winkler TW, Croteau-Chonka DC, et al. New genetic loci link adipose and insulin biology to body fat distribution. *Nature*. 2015;518:187–196. doi: 10.1038/nature14132
39. Saxena R, Hivert M-F, Langenberg C, Tanaka T, Pankow JS, Vollenweider P, Lyssenko V, Bouatia-Naji N, Dupuis J, Jackson AU, et al. Genetic variation in GIPR influences the glucose and insulin responses to an oral glucose challenge. *Nat Genet*. 2010;42:142–148. doi: 10.1038/ng.521
40. Evangelou E, Warren HR, Mosen-Ansorena D, et al. Genetic analysis of over 1 million people identifies 535 new loci associated with blood pressure traits. *Nat Genet*. 2018;50:1412–1425. doi: 10.1038/s41588-018-0205-x
41. GTEx Consortium. The GTEx consortium atlas of genetic regulatory effects across human tissues. *Science*. 2020;369:1318–1330. doi: 10.1126/science.aaz1776
42. Liu B, Gloudemans MJ, Rao AS, Ingelsson E, Montgomery SB. Abundant associations with gene expression complicate GWAS follow-up. *Nat Genet*. 2019;51:768–769. doi: 10.1038/s41588-019-0404-0
43. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, Chapman MJ, de Backer GG, Delgado V, Ference BA, et al. 2019 ESC/EAS guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. *Eur Heart J*. 2020;41:111–188. doi: 10.1093/eurheartj/ehz455
44. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, Braun LT, de Ferranti S, Faiella-Tommasino J, Forman DE, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the Management of Blood Cholesterol: a report of the American College of Cardiology/American Heart Association task force on clinical practice guidelines. *Circulation*. 2019;139:e1082–e1143. doi: 10.1161/cir.0000000000000625
45. Borén J, Taskinen MR, Björnson E, Packard CJ. Metabolism of triglyceride-rich lipoproteins in health and dyslipidaemia. *Nat Rev Cardiol*. 2022;19:577–592. doi: 10.1038/s41569-022-00676-y
46. Ross S, D'Mello M, Anand SS, et al. Effect of bile acid sequestrants on the risk of cardiovascular events: a Mendelian randomization analysis. *Circ Cardiovasc Genet*. 2015;8:618–627. doi: 10.1161/circgenetics.114.000952
47. Ridker PM. LDL cholesterol: controversies and future therapeutic directions. *Lancet*. 2014;384:607–617. doi: 10.1016/s0140-6736(14)61009-6
48. Rosoff DB, Bell AS, Jung J, Wagner J, Mavromatis LA, Lohoff FW. Mendelian randomization study of PCSK9 and HMG-CoA reductase inhibition and cognitive function. *J Am Coll Cardiol*. 2022;80:653–662. doi: 10.1016/j.jacc.2022.05.041
49. Yarmolinsky J, Bull CJ, Vincent EE, Robinson J, Walther A, Smith GD, Lewis SJ, Relton CL, Martin RM. Association between genetically proxied inhibition of HMG-CoA reductase and epithelial ovarian cancer. *JAMA*. 2020;323:646–655. doi: 10.1001/jama.2020.0150
50. Emdin CA, Khera AV, Kathiresan S. Mendelian randomization. *JAMA*. 2017;318:1925–1926. doi: 10.1001/jama.2017.17219
51. Sanderson E. Multivariable Mendelian randomization and mediation. *Cold Spring Harb Perspect Med*. 2021;11:a038984. doi: 10.1101/cshperspect.a038984
52. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey SG. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med*. 2008;27:1133–1163. doi: 10.1002/sim.3034
53. Morrison J, Knoblauch N, Marcus JH, Stephens M, He X. Mendelian randomization accounting for correlated and uncorrelated pleiotropic effects using genome-wide summary statistics. *Nat Genet*. 2020;52:740–747. doi: 10.1038/s41588-020-0631-4
54. Bowden J, Del Greco MF, Minelli C, et al. A framework for the investigation of pleiotropy in two-sample summary data Mendelian randomization. *Stat Med*. 2017;36:1783–1802. doi: 10.1002/sim.7221
55. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol*. 2016;40:304–314. doi: 10.1002/gepi.21965
56. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol*. 2015;44:512–525. doi: 10.1093/ije/dyv080
57. Verbanck M, Chen C-Y, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from mendelian randomization between complex traits and diseases. *Nat Genet*. 2018;50:693–698. doi: 10.1038/s41588-018-0099-7
58. Hartwig FP, Davey Smith G, Bowden J. Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption. *Int J Epidemiol*. 2017;46:1985–1998. doi: 10.1093/ije/dyx102
59. Yavorska OO, Burgess S. MendelianRandomization: an R package for performing Mendelian randomization analyses using summarized data. *Int J Epidemiol*. 2017;46:1734–1739. doi: 10.1093/ije/dyx034
60. Giambartolomei C, Vukcevic D, Schadt EE, Franke L, Hingorani AD, Wallace C, Plagnol V. Bayesian test for colocalisation between pairs of genetic association studies using summary statistics. *PLoS Genet*. 2014;10:e1004383. doi: 10.1371/journal.pgen.1004383
61. Wallace C. Eliciting priors and relaxing the single causal variant assumption in colocalisation analyses. *PLoS Genet*. 2020;16:e1008720. doi: 10.1371/journal.pgen.1008720
62. Woolf B, Zagkos L, Gill D. TwoStepCisMR: a novel method and R package for attenuating bias in cis-Mendelian randomization analyses. *Gen*. 2022;13:1541. doi: 10.3390/genes13091541
63. Burgess S, Thompson SG. Interpreting findings from Mendelian randomization using the MR-Egger method. *Eur J Epidemiol*. 2017;32:377–389. doi: 10.1007/s10654-017-0255-x
64. Machiela MJ, Chanock SJ. LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. *Bioinformatics*. 2015;31:3555–3557. doi: 10.1093/bioinformatics/btv402

65. Wallace C. A more accurate method for colocalisation analysis allowing for multiple causal variants. *PLoS Genet*. 2021;17:e1009440. doi: 10.1371/journal.pgen.1009440
66. Bulik-Sullivan BK, Loh P-R, Finucane HK, et al. LD score regression distinguishes confounding from polygenicity in genome-wide association studies. *Nat Genet*. 2015;47:291–295. doi: 10.1038/ng.3211
67. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res*. 2000;28:27–30. doi: 10.1093/nar/28.1.27
68. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, et al. Gene ontology: tool for the unification of biology. *Nat Genet*. 2000;25:25–29. doi: 10.1038/75556
69. Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryary F, Hachilif R, Gable AL, Fang T, Doncheva NT, Pyysalo S, et al. The STRING database in 2023: protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Res*. 2023;51:D638–D646. doi: 10.1093/nar/gkac1000
70. Otasek D, Morris JH, Bouças J, Pico AR, Demchak B. Cytoscape automation: empowering workflow-based network analysis. *Genome Biol*. 2019;20:185. doi: 10.1186/s13059-019-1758-4
71. Warde-Farley D, Donaldson SL, Comes O, Zuberi K, Badrawi R, Chao P, Franz M, Grouios C, Kazi F, Lopes CT, et al. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. *Nucleic Acids Res*. 2010;38:W214–W220. doi: 10.1093/nar/gkq537
72. Wang Q, Oliver-Williams C, Raitakari OT, Viikari J, Lehtimäki T, Kähönen M, Järvelin MR, Salomaa V, Perola M, Danesh J, et al. Metabolic profiling of angiopoietin-like protein 3 and 4 inhibition: a drug-target Mendelian randomization analysis. *Eur Heart J*. 2021;42:1160–1169. doi: 10.1093/eurheartj/ehaa972
73. Guan B, Wang A, Xu H. Causal associations of remnant cholesterol with cardiometabolic diseases and risk factors: a mendelian randomization analysis. *Cardiovasc Diabetol*. 2023;22:207. doi: 10.1186/s12933-023-01927-z
74. Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) study group. Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels. *N Engl J Med*. 1998;339:1349–1357. doi: 10.1056/nejm199811053391902
75. Mihaylova B, Emberson J, Blackwell L, et al. The effects of lowering LDL cholesterol with statin therapy in people at low risk of vascular disease: meta-analysis of individual data from 27 randomised trials. *Lancet*. 2012;380:581–590. doi: 10.1016/s0140-6736(12)60367-5
76. Rong S, Li B, Chen L, Sun Y, du Y, Liu B, Robinson JG, Bao W. Association of low-Density Lipoprotein Cholesterol Levels with more than 20-year risk of cardiovascular and all-cause mortality in the general population. *J Am Heart Assoc*. 2022;11:e023690. doi: 10.1161/JAHA.121.023690
77. Di Angelantonio E, Sarwar N, Perry P, et al. Major lipids, apolipoproteins, and risk of vascular disease. *JAMA*. 2009;302:1993–2000. doi: 10.1001/jama.2009.1619
78. Ference BA, Yoo W, Alesh I, Mahajan N, Mirowska KK, Mewada A, Kahn J, Afonso L, Williams KA Sr, Flack JM. Effect of long-term exposure to lower low-density lipoprotein cholesterol beginning early in life on the risk of coronary heart disease: a mendelian randomization analysis. *J Am Coll Cardiol*. 2012;60:2631–2639. doi: 10.1016/j.jacc.2012.09.017
79. Silverman MG, Ference BA, Im K, Wiviott SD, Giugliano RP, Grundy SM, Braunwald E, Sabatine MS. Association between lowering LDL-C and cardiovascular risk reduction among different therapeutic interventions: a systematic review and meta-analysis. *JAMA*. 2016;316:1289–1297. doi: 10.1001/jama.2016.13985
80. White J, Swerdlow DI, Preiss D, Fairhurst-Hunter Z, Keating BJ, Asselbergs FW, Sattar N, Humphries SE, Hingorani AD, Holmes MV. Association of lipid fractions with Risks for coronary artery disease and diabetes. *JAMA Cardiol*. 2016;1:692–699. doi: 10.1001/jamacardio.2016.1884
81. Do R, Willer CJ, Schmidt EM, et al. Common variants associated with plasma triglycerides and risk for coronary artery disease. *Nat Genet*. 2013;45:1345–1352. doi: 10.1038/ng.2795
82. Borén J, Chapman MJ, Krauss RM, Packard CJ, Bentzon JF, Binder CJ, Daemen MJ, Demer LL, Hegele RA, Nicholls SJ, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease: pathophysiological, genetic, and therapeutic insights: a consensus statement from the European Atherosclerosis Society consensus panel. *Eur Heart J*. 2020;41:2313–2330. doi: 10.1093/eurheartj/ehz962
83. Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, Hegele RA, Krauss RM, Raal FJ, Schunkert H, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society consensus panel. *Eur Heart J*. 2017;38:2459–2472. doi: 10.1093/eurheartj/ehx144
84. Doi T, Langsted A, Nordestgaard BG. Lipoproteins, cholesterol, and atherosclerotic cardiovascular disease in east Asians and Europeans. *J Atheroscler Thromb*. 2023;30:1525–1546. doi: 10.5551/jat.RV22013
85. Ference BA, Kastelein JJP, Ray KK, Ginsberg HN, Chapman MJ, Packard CJ, Laufs U, Oliver-Williams C, Wood AM, Butterworth AS, et al. Association of Triglyceride-Lowering LPL variants and LDL-C-lowering LDLR variants with risk of coronary heart disease. *JAMA*. 2019;321:364–373. doi: 10.1001/jama.2018.20045
86. Dewey FE, Gusarova V, Dunbar RL, O'Dushlaine C, Schurmann C, Gottesman O, McCarthy S, van Hout CV, Bruse S, Dansky HM, et al. Genetic and pharmacologic inactivation of ANGPTL3 and cardiovascular disease. *N Engl J Med*. 2017;377:211–221. doi: 10.1056/NEJMoa1612790
87. Gaudet D, Alexander VJ, Baker BF, Brisson D, Tremblay K, Singleton W, Geary RS, Hughes SG, Viney NJ, Graham MJ, et al. Antisense inhibition of apolipoprotein C-III in patients with hypertriglyceridemia. *N Engl J Med*. 2015;373:438–447. doi: 10.1056/NEJMoa1400283
88. Stitziel NO, Khera AV, Wang X, Bierhals AJ, Vourakis AC, Sperry AE, Natarajan P, Klarin D, Emdin CA, Zekavat SM, et al. ANGPTL3 deficiency and protection against coronary artery disease. *J Am Coll Cardiol*. 2017;69:2054–2063. doi: 10.1016/j.jacc.2017.02.030
89. Khera AV, Won HH, Peloso GM, O'Dushlaine C, Liu D, Stitziel NO, Natarajan P, Nomura A, Emdin CA, Gupta N, et al. Association of Rare and Common Variation in the lipoprotein lipase gene with coronary artery disease. *JAMA*. 2017;317:937–946. doi: 10.1001/jama.2017.0972
90. Jørgensen AB, Frikke-Schmidt R, Nordestgaard BG, Tybjærg-Hansen A. Loss-of-function mutations in APOC3 and risk of ischemic vascular disease. *N Engl J Med*. 2014;371:32–41. doi: 10.1056/NEJMoa1308027
91. Lotta LA, Stewart ID, Sharp SJ, Day FR, Burgess S, Luan J', Bowker N, Cai L, Li C, Wittemans LBL, et al. Association of genetically enhanced lipoprotein lipase-mediated lipolysis and low-density lipoprotein cholesterol-lowering alleles with risk of coronary disease and type 2 diabetes. *JAMA Cardiol*. 2018;3:957–966. doi: 10.1001/jamacardio.2018.2866
92. Sacks FM. The crucial roles of apolipoproteins E and C-III in apoB lipoprotein metabolism in normolipidemia and hypertriglyceridemia. *Curr Opin Lipidol*. 2015;26:56–63. doi: 10.1097/mol.0000000000000146
93. Crosby J, Peloso GM, Auer PL, et al. Loss-of-function mutations in APOC3, triglycerides, and coronary disease. *N Engl J Med*. 2014;371:22–31. doi: 10.1056/NEJMoa1307095
94. Pollin TI, Damcott CM, Shen H, Ott SH, Shelton J, Horenstein RB, Post W, McLenithan JC, Bielak LF, Peyser PA, et al. A null mutation in human APOC3 confers a favorable plasma lipid profile and apparent cardioprotection. *Science*. 2008;322:1702–1705. doi: 10.1126/science.1161524
95. Nordestgaard BG, Nielsen LB. Atherosclerosis and arterial influx of lipoproteins. *Curr Opin Lipidol*. 1994;5:252–257. doi: 10.1097/00041433-199408000-00002
96. Borén J, Williams KJ. The central role of arterial retention of cholesterol-rich apolipoprotein-B-containing lipoproteins in the pathogenesis of atherosclerosis: a triumph of simplicity. *Curr Opin Lipidol*. 2016;27:473–483. doi: 10.1097/mol.0000000000000330
97. Tokgözoğlu L, Libby P. The dawn of a new era of targeted lipid-lowering therapies. *Eur Heart J*. 2022;43:3198–3208. doi: 10.1093/eurheartj/ehab841
98. Kersten S. Angiopoietin-like 3 in lipoprotein metabolism. *Nat Rev Endocrinol*. 2017;13:731–739. doi: 10.1038/nrendo.2017.119
99. Geldenhuys WJ, Lin L, Darvesh AS, Sadana P. Emerging strategies of targeting lipoprotein lipase for metabolic and cardiovascular diseases. *Drug Discov Today*. 2017;22:352–365. doi: 10.1016/j.drudis.2016.10.007
100. Li Z, Zhang B, Liu Q, Tao Z, Ding L, Guo B, Zhang E, Zhang H, Meng Z, Guo S, et al. Genetic association of lipids and lipid-lowering drug target genes with non-alcoholic fatty liver disease. *EBioMedicine*. 2023;90:104543. doi: 10.1016/j.ebiom.2023.104543

101. Fu L, Wang Y, Hu YQ. Bi-directional causal effect between vitamin B12 and non-alcoholic fatty liver disease: inferring from large population data. *Front Nutr*. 2023;10:1015046. doi: 10.3389/fnut.2023.1015046
102. Fu L, Wang Y, Hu YQ. Association between homocysteine and nonalcoholic fatty liver disease: Mendelian randomisation study. *Eur J Clin Invest*. 2023;53:e13895. doi: 10.1111/eci.13895
103. Fu L, Cheng H, Gao L, Zhao X, Mi J. Genetically proxied vitamin B12 and homocysteine in relation to life course adiposity and body composition. *Diabetes Metab Syndr*. 2023;17:102883. doi: 10.1016/j.dsx.2023.102883
104. Fu L, Wang Y, Li T, Yang S, Hu YQ. A novel hierarchical clustering approach for joint analysis of multiple phenotypes uncovers obesity variants based on ARIC. *Front Genet*. 2022;13:791920. doi: 10.3389/fgene.2022.791920
105. Fu L, Wang Y, Hu YQ. Inferring causal effects of homocysteine and B-vitamin concentrations on bone mineral density and fractures: Mendelian randomization analyses. *Front Endocrinol*. 2022;13:1037546. doi: 10.3389/fendo.2022.1037546
106. Fu L, Wang Y, Hu YQ. Causal effects of B vitamins and homocysteine on obesity and musculoskeletal diseases: a Mendelian randomization study. *Front Nutr*. 2022;9:1048122. doi: 10.3389/fnut.2022.1048122
107. Fu L, Cheng H, Zhao X, Hou D, Xie X, Mi J. Distinct causal effects of body fat distribution on cardiometabolic traits among children: findings from the BCAMS study. *Nutr Metab Cardiovasc Dis*. 2022;32:1753–1765. doi: 10.1016/j.numecd.2022.03.030
108. Fu L, Wang Y, Li T, Hu YQ. A novel approach integrating hierarchical clustering and weighted combination for association study of multiple phenotypes and a genetic variant. *Front Genet*. 2021;12:654804. doi: 10.3389/fgene.2021.654804
109. Fu L, Cheng H, Xiong J, Xiao P, Shan X, Li Y, Li Y, Zhao X, Mi J. Mediating role of inflammatory biomarkers in the causal effect of body composition on glycaemic traits and type 2 diabetes. *Diabetes Obes Metab*. 2024;26:5444–5454. doi: 10.1111/dom.15923
110. Fu L, Cheng H, Xiong J, Xiao P, Shan X, Li Y, Li Y, Zhao X, Mi J. Effect of life course body composition on lipids and coronary atherosclerosis mediated by inflammatory biomarkers. *Free Radic Biol Med*. 2024;227:157–165. doi: 10.1016/j.freeradbiomed.2024.12.011
111. Gill D, Burgess S. The evolution of mendelian randomization for investigating drug effects. *PLoS Med*. 2022;19:e1003898. doi: 10.1371/journal.pmed.1003898
112. Sofat R, Hingorani AD, Smeeth L, Humphries SE, Talmud PJ, Cooper J, Shah T, Sandhu MS, Ricketts SL, Boekholdt SM, et al. Separating the mechanism-based and off-target actions of cholesteryl ester transfer protein inhibitors with CETP gene polymorphisms. *Circulation*. 2010;121:52–62. doi: 10.1161/circulationaha.109.865444
113. Yang Q, Sanderson E, Tilling K, Borges MC, Lawlor DA. Exploring and mitigating potential bias when genetic instrumental variables are associated with multiple non-exposure traits in mendelian randomization. *Eur J Epidemiol*. 2022;37:683–700. doi: 10.1007/s10654-022-00874-5
114. Fry A, Littlejohns TJ, Sudlow C, Doherty N, Adamska L, Sprosen T, Collins R, Allen NE. Comparison of sociodemographic and health-related characteristics of UK Biobank participants with those of the general population. *Am J Epidemiol*. 2017;186:1026–1034. doi: 10.1093/aje/kwx246