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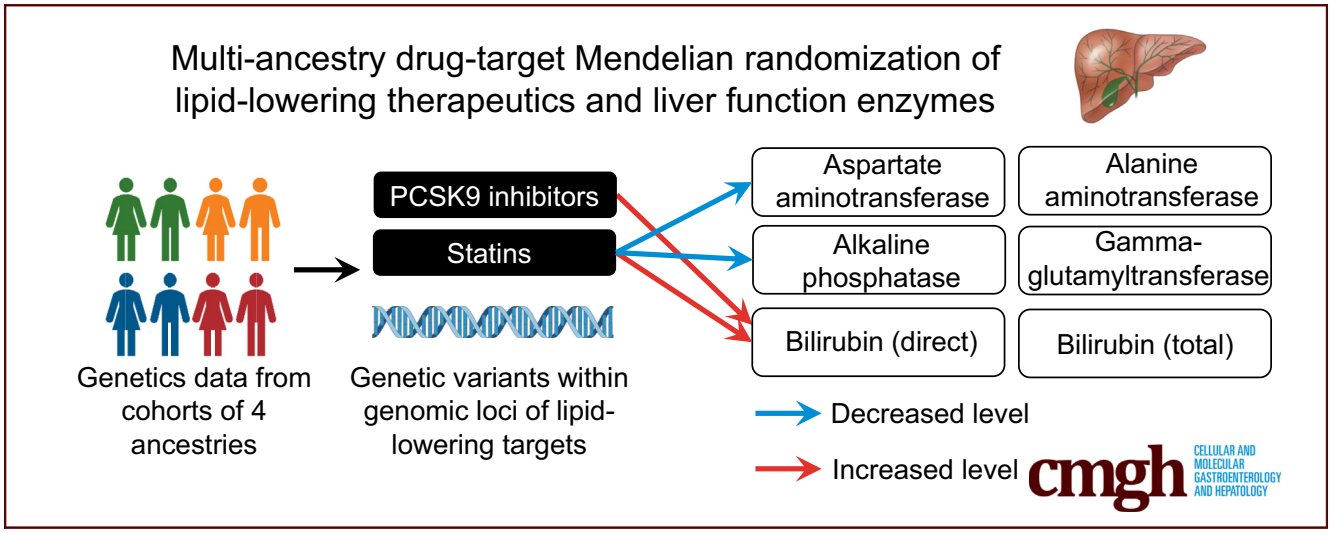
CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Assessing the Impact of PCSK9 and HMGCR Inhibition on Liver Function: Drug-Target Mendelian Randomization Analyses in Four Ancestries

Daniel B. Rosoff,[1,2,*] Andrew S. Bell,[1,*] Josephin Wagner,[1] Lucas A. Mavromatis,[1] Ali Hamandi,[1] Lauren Park,[1] Jeesun Jung,[1] and Falk W. Lohoff[1]

[1]Section on Clinical Genomics and Experimental Therapeutics, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, Maryland; and [2]Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, United Kingdom



SUMMARY

Using large population genetics data derived from participants across 4 diverse ancestries and drug-target Mendelian randomization methods, we find generally neutral relationships of lipid-lowering therapeutic targets and liver enzyme levels, suggesting safe long-term hepatologic profile of these therapeutics.

BACKGROUND & AIMS: Observational studies have linked lipid-lowering drug targets pro-protein convertase subtilisin/kexin 9 (PCSK9) and HMG-CoA reductase (HMGCR) with adverse liver outcomes; however, liver disease incidence varies across diverse populations, and the long-term hepatic impact of these lipid-lowering drugs among non-white Europeans remains largely unknown.

METHODS: We use single nucleotide polymorphisms (SNPs) in *PCSK9* and *HMGCR* loci from genome-wide association study data of low-density lipoprotein cholesterol in 4 populations (East Asian [EAS], South Asian [SAS], African [AFR], and European [EUR]) to perform drug-target Mendelian randomization investigating relationships between PCSK9 and HMGCR inhibition and alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), and bilirubin.

RESULTS: Analyses of PCSK9 instruments, including functional variants R46L and E670G, failed to find evidence for relationships of low-density lipoprotein cholesterol lowering via PCSK9 variants and adverse effects on ALT, AST, GGT, or ALP among the cohorts. PCSK9 inhibition was associated with increased direct bilirubin levels in EUR ($\beta = 0.089$; P value $= 5.69 \times 10^{-6}$) and, nominally, in AFR ($\beta = 0.181$; P value $= .044$). HMGCR inhibition was associated with reduced AST in SAS ($\beta = -0.705$; P value $= .005$) and, nominally, reduced AST in EAS ($\beta = -0.096$; P value $= .03$), reduced ALP in EUR ($\beta = -2.078$; P value $= .014$), and increased direct bilirubin in EUR ($\beta = 0.071$; P value $= .032$). Sensitivity analyses using genetic instruments derived from circulating PCSK9 protein levels, tissue-specific *PCSK9* expression, and *HMGCR* expression were in alignment, strengthening causal inference.

CONCLUSIONS: We did not find ALT, AST, GGT, or ALP associated with genetically proxied PCSK9 and HMGCR inhibition across ancestries. We identified possible relationships in several ancestries between PCSK9 and increased direct and total bilirubin and between HMGCR and reduced AST. These findings support long-term safety profiles and low hepatotoxic risk of PCSK9 and HMGCR inhibition in diverse populations.

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Because new lipid-lowering therapeutics, such as proprotein convertase subtilisin/kexin type 9 (*PCSK9*) monoclonal antibody inhibitors (alirocumab and evolocumab)[1] and the recently approved small interfering RNA (siRNA) inhibitor of hepatic PCSK9, inclisiran,[2] are now approved for the treatment of hypercholesterolemia and coronary heart disease,[3] it is important to evaluate the long-term side effects of lipid-lowering via inhibition of PCSK9, especially in the liver, where preclinical and observational data have suggested relationships between PCSK9 and liver function. There has been a degree of concern about potential hepatotoxic effects of PCSK9 inhibitors, owing in part to the results of some preclinical studies of PCSK9 inhibitors identifying potential adverse associations with liver damage[4–6] and reports of elevated liver enzymes (liver function tests [LFTs]) in a small percentage of patients taking statins.[7–9] However, causal inference from preclinical and observational studies is challenging because of the unknown generalizability of results observed in preclinical animal models to human patients[10] and potential biases such as reverse causation and confounding in observational data.[11]

Because of the recency of PCSK9 inhibitors' approval by the Food and Drug Administration, long-term data from patients taking these medications are not available in the quantity necessary to definitively determine whether PCSK9 inhibition significantly affects liver function; these data are important for assessing the risk such a study may impose upon participants. This is especially relevant in light of the recency of approval of the siRNA inclisiran, which has a much longer duration of action than monoclonal antibody inhibitors, and ongoing clinical trials for CRISPR-mediated *PCSK9* base editors, which induce a knockout genome and effectively permanently disable PCSK9.[12,13]

Although several genetic investigations proxying pharmacologic PCSK9 inhibition via variants in the PCSK9 locus have suggested neutral hepatic side profiles,[14–20] they were performed by using data derived from participants of primarily white European ancestry; meanwhile, liver disease prevalence and severity are known to vary widely between ancestral populations.[21] Furthermore, caution is required in extrapolating genetic risk estimates from studies of European-based populations to non-European populations because of the differences in population structure, linkage disequilibrium (LD), and other factors.[22] As a result, and despite the importance of understanding ancestry-linked adverse outcome profiles of medications that are prescribed globally, the nature of the relationships between PCSK9 inhibition and liver function in non-European populations cohorts remains unexplored.

More generally, there exists a need to improve race/ancestry representation in both genetics-based studies and randomized controlled trials (RCTs) across all disciplines.[22–28] For example, in the more than 20,500 RCTs performed in the United States between 2000 and 2020, only 43% reported any race/ancestry data.[29] Among studies reporting race/ancestry data, non-white participants have been underrepresented, with white participants of European ancestry comprising almost 80% of all enrollees compared with only 10% for those of African ancestry and only 1% for participants of Asian ancestry (East Asian and South Asian combined).[29] There are important clinical, research, and public health consequences of this European bias in genetics and RCTs, including limited potential for extrapolation of the primarily European findings to ancestrally diverse populations, which reduces the ability to translate genetics-based research into clinical and public health policy, contributes to public health inequalities, and underscores the need to investigate across diverse populations the potential impact of long-term PCSK9 inhibition on liver function.[22]

Therefore, we leveraged summary-level genome-wide association study (GWAS) data from large genomics consortia derived from participants of East Asian (EAS), South Asian (SAS), African (AFR), and European (EUR) ancestries to evaluate the impact of long-term low-density lipoprotein cholesterol (LDL-C) lowering using PCSK9 variants as instrumental variables.[30–32] To perform the multi-ancestry comparison, we used drug-target Mendelian randomization (MR), which uses single nucleotide polymorphisms (SNPs) located in the genomic locus of the drug target (here PCSK9 and 3-hydroxy-3-methylglutaryl coenzyme A reductase [HMGCR]) to proxy, using population-level genetics data, the pharmacologic inhibition of the target[33]; drug-target MR has previously been used to proxy the cardiovascular efficacy and investigate possible side effects of lipid-lowering therapies.[15,17,34–36] Because statins remain the most prescribed lipid-lowering drug class worldwide,[37,38] we also perform drug-target MR of LDL-C lowering by the statin drug target HMGCR[15] for comparison. We also supplement PCSK9 and HMGCR instruments constructed using LDL-C levels (the primary physiological response to pharmacologic PCSK9i and HMGCRi) by leveraging recently released GWAS data on circulating PCSK9 protein levels,[39] liver *PCSK9* gene expression data,[40] and whole blood HMGCR expression data.[41] Quantitative

***Authors share co-first authorship.**

Abbreviations used in this paper: **AFR, African; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CVD, cardiovascular disease; EAS, East Asian; eQTL, expression quantitative trait loci; EUR, European; GGT, gamma-glutamyl transferase; GWAS, genome-wide association study; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; IVW, inverse variance weighted; LD, linkage disequilibrium; LDL-C, low-density lipoprotein cholesterol; LFT, liver function test; MR, Mendelian randomization; NAFLD, nonalcoholic fatty liver disease; PCSK9, proprotein convertase subtilisin/kexin type 9; pQTL, protein quantitative trait loci; RCT, randomized controlled trial; SAS, South Asian; siRNA, small interfering RNA; SNP, single nucleotide polymorphism.**

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trait loci (QTL) analyses were performed only in EUR because of the lack of available non-EUR QTL data. Together, these results will inform our understanding of the long-term safety profile of PCSK9 inhibition in these populations, which may reduce ongoing health disparities due, in part, to the underrepresentation of non-European ancestry populations in RCTs and genetics studies.[22,26,42–44]

Results

Instrument Strength

F-statistics for genetic variants comprising the *PCSK9* and *HMGCR* drug-target instruments in each population were strong (average F-statistic >30) (Supplementary Tables 1–3), suggesting that the ancestry-specific drug-target instruments are unlikely to be subject to weak instrument bias.[45] For the PCSK9 drug-target instruments, average F-statistics were approximately 380, 120, 40, and 62 for the EUR, AFR, SAS, and EAS cohorts, respectively. F-statistics for the PCSK9 instruments composed solely of functional variants were also strong. For the HMGCR drug-target instruments, average F-statistics were approximately 305, 46, 63, and 74, respectively. QTL instruments were similarly strong with the PCSK9 protein quantitative trait loci (pQTL) average F-statistic = 90.5 (replication PCSK9 instrument F-statistic = 74.7), PCSK9 liver expression quantitative trait loci (eQTL) instrument average F-statistic = 24.9, and HMGCR whole blood expression average F-statistic = 117.1.

PCSK9 and HMGCR Inhibition Across Ancestries

In our primary analyses, we failed to find evidence of associations between genetically lowered LDL-C levels via PCSK9 variants and gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), or alkaline phosphatase (ALP) levels in the EAS, EUR, AFR, or SAS cohorts (Figure 1, Supplementary Table 4). We did find that lowered LDL-C levels via PCSK9 variants were associated with increased direct bilirubin in the EUR cohorts ($\beta = 0.089$, $P = 5.69 \times 10^{-6}$) and nominally in the AFR cohorts ($\beta = 0.181$, $P = .044$); these results aligned in magnitude and direction in Single variable Mendelian randomization using more stringent LD thresholds, although with reduced precision (Supplementary Table 5). Lowered LDL-C levels via PCSK9 inhibition were also associated with decreased total bilirubin levels, but only nominally in the SAS cohorts ($\beta = -0.654$, $P = .034$). Estimates for ALT, AST, GGT, ALP, and total bilirubin from genetically proxied PCSK9i using instruments composed of the R46L and E670G functional variants were similarly null, whereas the EUR PCSK9 estimate on direct bilirubin using R46L was consistent with the others ($\beta = 0.061$, $P = .001$) (Supplementary Table 6). Corresponding R46L estimates on direct bilirubin in the other cohorts were null.

In contrast with the neutral results observed for genetically proxied PCSK9i, HMGCR analyses revealed potential ancestry-dependent effects of HMGCR inhibition on LFT outcomes (Figure 2, Supplementary Table 4). HMGCR inhibition was associated with lower AST in SAS ($\beta = -0.705$, $P = .005$) and, nominally, EAS ($\beta = -0.106$, $P = .001$) cohorts. HMGCR inhibition was associated, nominally, with ALP and direct bilirubin in the EUR cohort ($\beta = -2.078$, $P = .014$ and $\beta = -0.071$, $P = .032$, respectively). Estimates were generally consistent in magnitude and direction across complementary MR methods; MR Egger estimates are generally less precise than MR inverse variance weighted (IVW) and MR Maxlik. Corresponding HMGCR estimates from single variant instruments constructed using a more stringent LD clumping threshold generally aligned in magnitude and direction but with reduced precision (Supplementary Table 5). We failed to find evidence of associations between HMGCR inhibition and any LFT in African ancestry cohorts.

PCSK9 and HMGCR Protein Levels and Gene Expression

We further evaluated the impact of PCSK9i and HMGCRi on LFTs using pQTL and eQTL data sourced from EUR participants (Figure 3, Supplementary Tables 7 and 8). Instruments proxying lowered PCSK9 protein levels broadly reflected the primary analyses assessing LDL-C lowering via PCSK9 variants, ie, null estimates for ALT, AST, GGT, and ALP as well as an increase in direct bilirubin levels ($\beta = 0.030$, $P = 3.10 \times 10^{-5}$). These relationships were consistent with the PCSK9 protein instrument derived from an independent EUR cohort (Supplementary Table 7). By contrast, we observed MR estimates for reduced hepatic *PCSK9* expression and lowered ALT ($\beta = -0.238$, $P = .012$), AST ($\beta = -0.146$, $P = .046$), and direct bilirubin ($\beta = -0.014$, $P = .025$). MR estimates from the sensitivity analyses using PCSK9 QTL instruments constructed at more stringent LD thresholds aligned with the initial estimates (Supplementary Table 8). We failed to find evidence for MR relationships between *HMGCR* whole blood gene expression and any of the LFTs.

Discussion

In this study, we leveraged drug-target MR methods and GWAS data derived from participants of 4 ancestries to assess the impact of genetically lowered LDL-C levels by using variants in the *PCSK9* and *HMGCR* loci to proxy the long-term impact on liver enzymes of PCSK9 inhibition and statins, respectively. Broadly, our analyses yielded evidence that PCSK9 inhibition displays no pattern of ancestry-specific or overall associations with increased ALT, AST, GGT, ALP, or total bilirubin levels; HMGCR inhibition also showed no pattern.

We failed to find evidence of a genetic effect of PCSK9 inhibition on ALT, AST, GGT, and ALP for any group assessed in this study, suggesting that PCSK9 inhibitors do not adversely impact these levels in individuals of East Asian, South Asian, European, or African ancestry. We also found that lowered LDL-C via PCSK9 variants and lowered PCSK9 protein levels were linked with increased direct bilirubin, which aligns with recent observational data showing an inverse relationship between PCSK9 and bilirubin,[46,47] and whole exome results reporting that a variant within the

Population	Outcome	Beta (95% CI)	*P*
EUR	ALP	0.5530 (−0.728 to 1.834)	.398
	ALT	0.5830 (−0.111 to 1.277)	.099
	AST	−0.1300 (−0.641 to 0.380)	.617
	GGT	1.2280 (−0.614 to 3.070)	.191
	Bilirubin (direct)	0.0890 (0.051 to 0.127)	5.69e−06
	Bilirubin (total)	0.1010 (−0.094 to 0.296)	.309
AFR	ALP	−0.0270 (−0.186 to 0.132)	.74
	ALT	0.0200 (−0.151 to 0.191)	.822
	AST	−0.0003 (−0.192 to 0.191)	.997
	GGT	−0.0720 (−0.207 to 0.063)	.298
	Bilirubin (direct)	0.1810 (0.005 to 0.357)	.044
	Bilirubin (total)	−0.0280 (−0.197 to 0.142)	.75
EAS	ALP	−0.0160 (−0.120 to 0.088)	.764
	ALT	0.0120 (−0.078 to 0.103)	.789
	AST	−0.0100 (−0.100 to 0.080)	.83
	GGT	−0.0050 (−0.102 to 0.093)	.926
	Bilirubin (direct)	−0.3110 (−0.980 to 0.359)	.363
	Bilirubin (total)	−0.0150 (−0.117 to 0.087)	.778
SAS	ALP	0.1840 (−0.399 to 0.767)	.536
	ALT	0.2390 (−0.341 to 0.819)	.419
	AST	0.2250 (−0.368 to 0.819)	.456
	GGT	0.4880 (−0.052 to 1.028)	.077
	Bilirubin (direct)	0.1760 (−0.482 to 0.833)	.601
	Bilirubin (total)	−0.6570 (−1.266 to −0.048)	.034

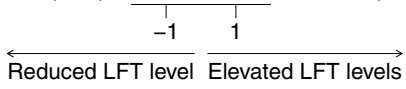

Figure 1. Drug-target MR results of genetically proxied PCSK9 inhibition on LFTs in cohorts of 4 ancestries. Results presented are the main drug-target MR estimates (either IVW or Wald ratio methods) for the genetically lowered LDL-C levels via variants in the *PCSK9* locus (1 standard deviation lower LDL-C levels). CI, confidence interval.

PCSK9 locus, rs11591147, is associated with direct bilirubin.[48] Overall, the PCSK9 results are in line with a growing body of literature indicating that PCSK9 inhibitors are safe and effective overall and when assessed in specific population subgroups[17,20,36] and for long-term side effect profiles across many disease domains.[49–53] They also support the use of PCSK9 inhibitors in patients with comorbid nonalcoholic fatty liver disease (NAFLD) and cardiovascular diseases (CVDs), which is particularly important because of the increasing prevalence of NAFLD globally (eg, it is estimated that 25% of adults worldwide have NAFLD[54,55]) and the common comorbidity with CVDs.[56,57]

We also failed to identify evidence that genetically proxied HMGCR inhibition adversely impacts these outcomes overall; all findings returned either neutral or beneficial associations between genetically lowered LDL-C levels via variants in the *HMGCR* locus and LFTs, which may help alleviate the ongoing underutilization of statins due to concerns about statin use in patients with chronically elevated liver levels or liver disease.[58] In both the East Asian and South Asian population cohorts, HMGCR inhibition resulted in lowered AST; in the European population cohorts, HMGCR inhibition resulted in lower alkaline phosphatase; in the African cohorts, there were no significant impacts found. Ancestry thus may be responsible for some of the beneficial effects on liver outcomes reported in some studies of patients taking statins.[59,60] However, we stress that the differences between the magnitude of MR estimates and the lowered LFT levels corresponding to statins over periods may exist and may be due to the MR estimates reflecting long-term impacts of these drug targets.[61] Therefore, future studies are needed to further assess potential beneficial effects of HMGCR inhibition on LFT levels.

Our study made use of the ability of drug-target MR to leverage publicly available data to assess the impact of a modifiable exposure (ie, HMGCR and PCSK9 levels) on metabolic outcomes of interest in an ancestry-dependent manner. The alignment of primary and sensitivity tests for the outcomes assessed in this study improves causal inference and strengthens the validity of our genomic analytical model. Furthermore, our study was also able to make use of multi-ancestral data to perform parallel ancestry-specific MR analyses to perform an extensive genetics-based analysis of the risk for hepatotoxicity in a cohort of ancestrally diverse individuals taking PCSK9 inhibitors to date. Specific investigations into differential risk profiles for medications between individuals in various subpopulations are crucial to determine the most appropriate route of treatment for each individual. Furthermore, despite positive recent trends in RCT enrollment, data from a meta-analysis of more than 20,000 RCTs in the United States indicate that there remain substantial racial and ethnic gaps in study sample makeup,

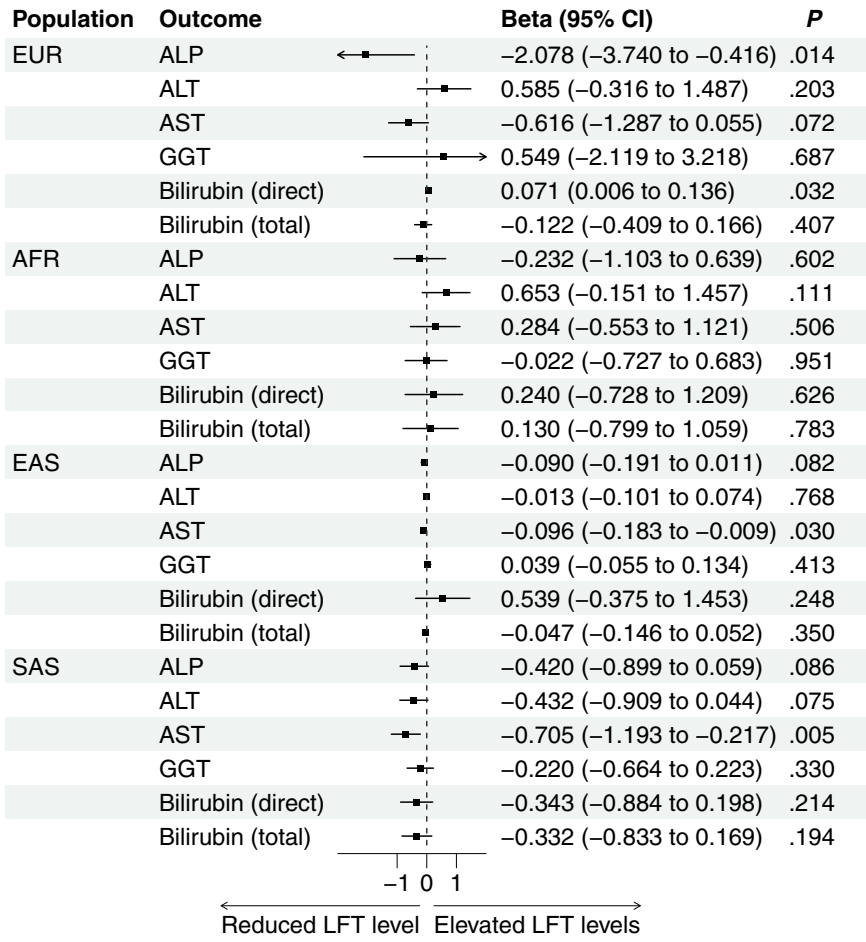

Figure 2. Drug-target MR results of genetically proxied HMGCR inhibition on LFTs in cohorts of 4 ancestries. Results presented are the main drug-target MR estimates (either IVW or Wald ratio methods) for the genetically lowered LDL-C levels via variants in the *HMGCR* locus (1 standard deviation lower LDL-C levels). CI, confidence interval.

and certain subpopulations remain underrepresented in recruitment and data reporting.[29] Until such time as more robust clinical data from diverse samples become available, genetic methods such as those used in the present study are important tools for addressing the lack of data about the safety profile of medications such as PCSK9 inhibitors for underrepresented populations.[26]

This study took advantage of the statistical benefits of leveraging complementary MR methods incorporating genetic matrices of the underlying LD structure between drug-target instrument variants to improve instrument precision, an important determinant of the potential for causal inference in MR.[11] Using complementary MR methods, heterogeneity tests, and alternate instruments—and observing consistent MR estimates across methods—further strengthens causal inference and allows a more robust and meaningful interpretation of MR estimates.[11,62,63]

However, our study is limited by both the constraints of our source data and the limitations inherent to statistical bioinformatic techniques such as MR. Specifically, although our study analyzed data from 4 ancestral populations, the combined sample does not include individuals from the many other ancestral groups comprising the total population of those taking statins. In addition, the nature of the GWAS data did not allow for evaluation of any potential country-to-country heterogeneity within shared nominal ancestral background (eg, individuals classified as "White European" from either the United Kingdom versus France) or ethnolinguistic variation within a single ancestry group. For example, there are 5 African ethnolinguistic divisions; however, African populations in the United States and United Kingdom are primarily from only one of these divisions (ie, Niger-Congo speakers),[26,64] and as of 2022, more than 90% of these continental ethnolinguistic divisions are still not represented in genetic biobanks, creating challenges when generalizing data from U.S. or European study participants of African descent to Africans living in Africa.[26,64] Similarly, because of the lack of sex-stratified data for non-European LFTs, we were unable to assess potential differences between men and women.

Furthermore, although MR is useful in that it allows for causal inference without requiring resource-intensive clinical investigations, clinically meaningful inference necessitates triangulating study designs,[65] which, in addition to supporting and extending these findings, would help elucidate potential repurposing opportunities for PCSK9 inhibitors and statins. For example, preclinical and epigenomic studies have suggested that PCSK9 inhibition

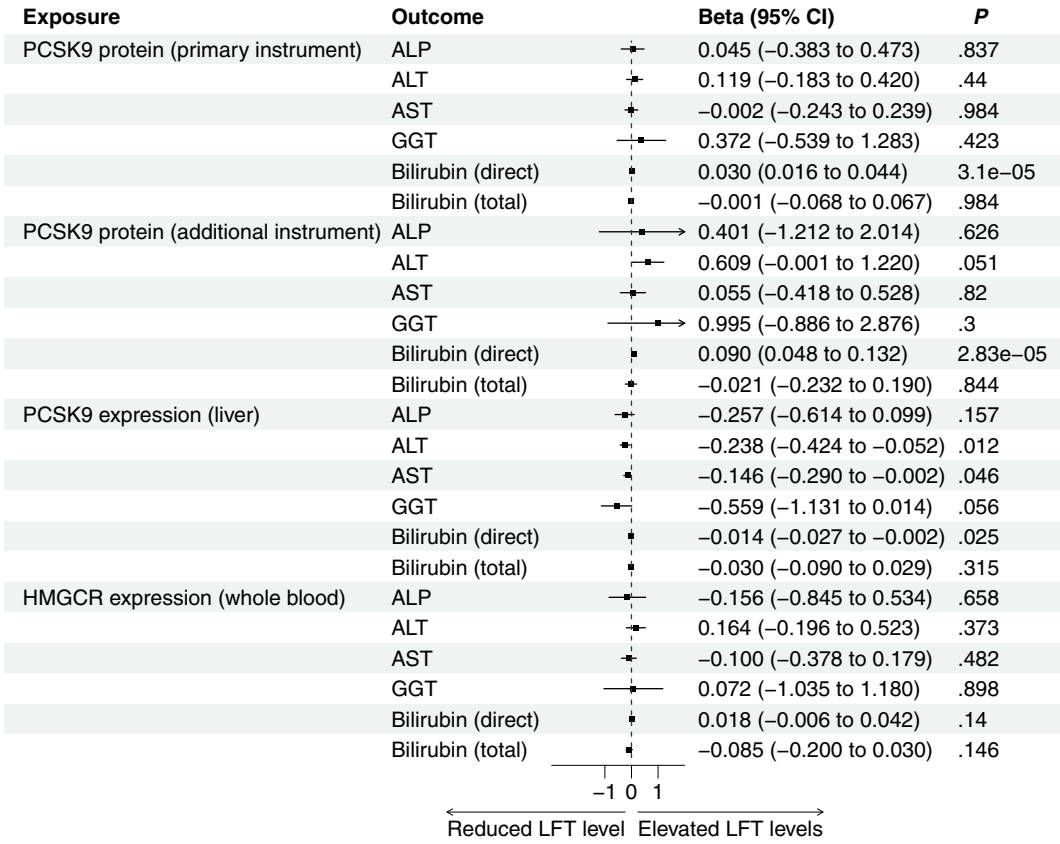

Figure 3. Drug-target MR results of genetically proxied PCSK9 and HMGCR inhibition on LFTs using QTL instruments. Results presented are the main drug-target MR estimates (either IVW or Wald ratio methods) for the genetically lowered circulating PCSK9 protein levels, liver *PCSK9* expression, and whole blood *HMGCR* expression cholesterol. Exposure and outcome data are derived from participants of European ancestry. CI, confidence interval.

may be a potential therapeutic for alcoholic liver disease.[66,67] Although improving racial and ethnic diversity in genetics-based studies is needed for genetic techniques such as MR to meaningfully assess differential disease risk and drug response patterns between clinical subpopulations, long-term RCTs with diverse participants are needed to definitively conclude that PCSK9 inhibition is free from hepatotoxic or other adverse outcomes. Other study limitations are inherent to the drug-target MR framework. For example, we were unable to evaluate potential off-target effects and pathways of PCSK9 and HMGCR inhibition, including any effects occurring through mechanisms outside their respective lipid-lowering pathways.[18] In addition, because the 2021 GLGC meta-analysis incorporated LDL-C data from UK Biobank participants, there exists some potential for bias due to sample overlap in our analyses[68,69]; however, it has been recently shown that 2-sample MR may be used in single samples when the data are derived from large biobanks such as the UK Biobank,[70] and bias would likely be minimal because of the strength of the PCSK9 and HMGCR instruments.[68,69]

Conclusions

Our study provides evidence that genetic PCSK9 inhibition displays no ancestry-mediated associations with ALT, AST, GGT, ALP, or total bilirubin levels but might be linked with increased direct bilirubin. We also show genetic HMGCR inhibition may beneficially impact AST. These results are in line with those of previous studies reporting an association between statin use and lowered LFTs and add to a growing body of literature demonstrating that PCSK9 inhibitors are safe and effective medications across patient populations. Although replication with data from more countries, additional populations, and new age groups is necessary, these findings should help to ameliorate concerns over adverse hepatotoxic effects that may contribute to underuse of PCSK9 inhibitors for patients with hypercholesterolemia or who are at risk for CVD.

Methods

We used the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guideline (Supplementary Table 9) to guide this study structure. The GWAS studies contributing the summary-level statistics used in this study each have the relevant institutional review board approval from their respective countries, in accordance with the Declaration of Helsinki, and all participants provided informed consent. A summary graphic detailing the experimental procedure used in this study can be found in Figure 4.

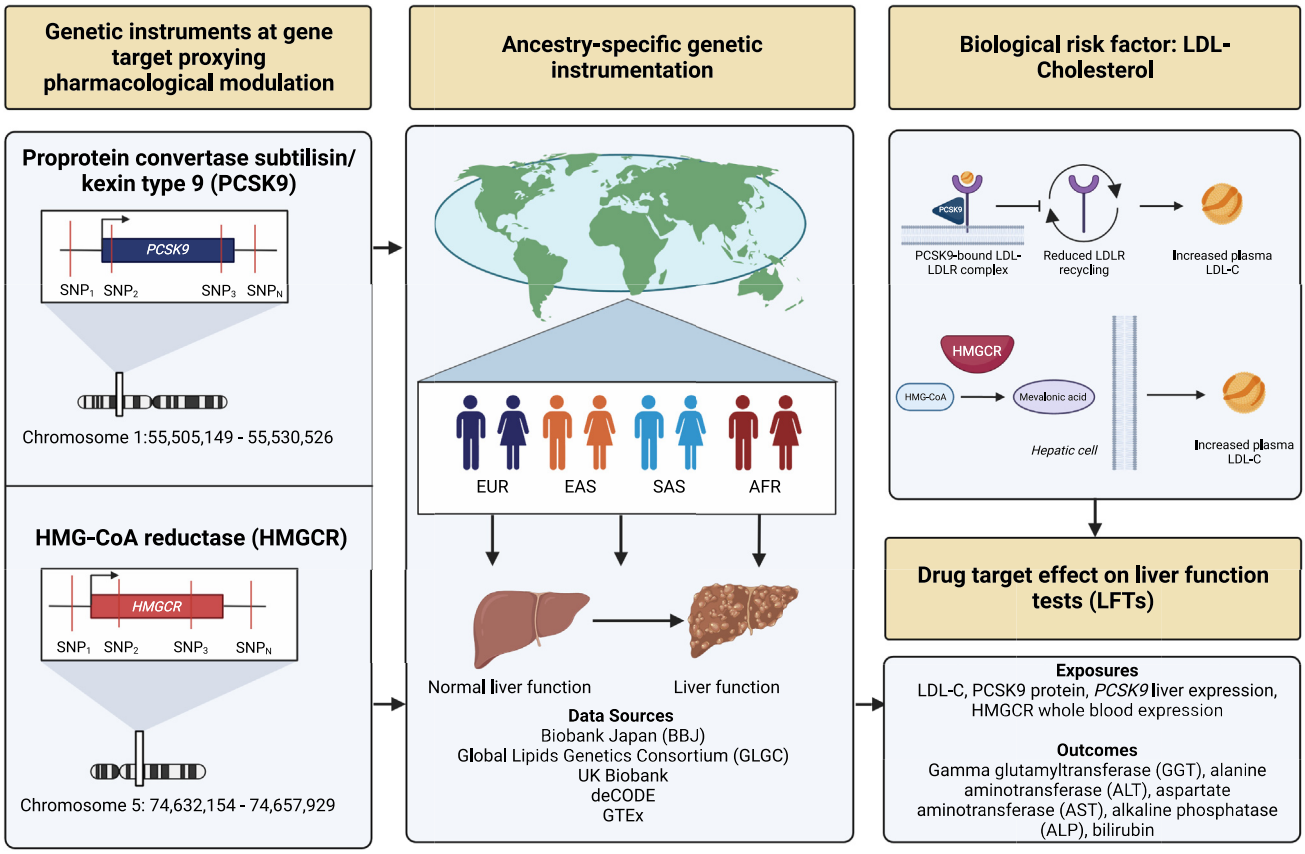

Figure 4. Study overview. The figures outline data sources, drug-target instrument selection, and analysis plan. First, we obtained ancestry-specific summary-level GWAS data of circulating LDL cholesterol levels from the 2021 Global Lipid Genetics Consortium (GLGC) meta-analysis GWAS for EUR, EAS, SAS, and AFR populations. We obtained corresponding ancestry-specific LFTs from the corresponding populations. Next, we constructed genetic instruments for *PCSK9* and *HMGCR* by extracting variants (ie, common SNPs) associated with LDL-C levels (*P* value $<5 \times 10^{-8}$) at genomic loci within ±100 kilobase window of the gene start and end coordinates (see panel describing mechanism of actions for PCSK9 and HMGCR). We included drug-target instruments using LD clumping threshold of LD $r^2 \leq 0.2$. We also included as sensitivity analyses drug-target instruments composed of variants clumped at LD $r^2 \leq 0.001$. In addition, for the PCSK9 analyses, we included additional drug-target instruments composed of previously identified functional variants (R46L, E670G). For European data, we also constructed drug-target instruments for circulating PCSK9 protein levels, *PCSK9* liver expression, and *HMGCR* whole blood expression using QTLs. Before performing the MR analyses, we harmonized the drug-target exposure data with the LFT enzymes in each population. We used several MR methods, including the IVW random-effects method accounting for the correlation between the genetic variants, for 2+ SNP instruments, and for single SNP instruments, the Wald ratio, as main methods. For the drug-target instruments composed of variants clumped at LD $r^2 \leq 0.001$, we used additional methods, including MR EGGER, MR Maximum likelihood, Weighted median, Weighted mode, and Simple mode MR.

Data Sources

To construct our genetic instruments proxying long-term LDL-C lowering via *PCSK9* and *HMGCR* loci, we used 2021 Global Lipid Genetics Consortium meta-analyses in each population: AFR ($N \leq 94{,}623$), EAS ($N \leq 82{,}587$), SAS ($N \leq 40{,}472$), and EUR ($N \leq 1{,}320{,}016$).[71] We included 4 LFTs: ALT, AST, ALP, GGT, and bilirubin. We obtained LFT data for EUR, AFR, and SAS cohorts that were sourced from the Pan UK Biobank Project,[72] whereas outcome data for EAS individuals were sourced from the Biobank Japan Project[72] (Table 1, Supplementary Table 10).

PCSK9 and HMGCR Instruments

Because reduction of LDL-C levels is the primary biomarker measured to assess the physiological response to pharmacologic inhibition of PCSK9 and statin therapies,[73,74] we used GWAS data on LDL-C levels measured in the 4 populations. We extracted genetic variants within 100 kb of the genomic loci for PCSK9 and HMGCR boundaries associated with LDL-C levels at *P* value $<5 \times 10^{-8}$ (conventional genome-wide significance) to proxy the primary physiological response pharmacologic inhibition of these targets.[73] We clumped the *PCSK9* and *HMGCR* variants at LD $r^2 < 0.2$ (using a 250 kb window, and the AFR, EAS, SAS, and EUR 1000 Phase 3 Genomes Project reference populations).[75] In addition, we constructed additional instruments using more stringent LD clumping threshold of 0.001 and also PCSK9 instruments using functional variants at the loci R46L (rs505151)[76] and E670G (rs11591147) as sensitivity analyses.[77] See Supplementary Tables 1–3 for

Table 1. Study Data Sources

Phenotype	Population	Year	Cohort	Sample size
LDL-C (SD mg/dL)	European	2021	UKB	1,231,262
	African/African American	2021	UKB	94,623
	East Asian	2021	BBJ	82,587
	South Asian	2021	UKB	40,472
PCSK9 pQTL primary	European	2021	Icelandic Cancer Project, deCODE	35,362
PCSK9 pQTL replication	European	2021	LIFE-Heart, LIFE-Adult, LURIC, CAP and TwinGene	12,271
PCSK9 liver eQTL	European	2022	GTEx	178
HMGCR eQTL	European	2021	eQTLGen Consortium	31,684
Gamma-glutamyl transferase (U/L))	European	2018	UKB	344,104
	African/African American	2020	UKB	6212
	East Asian	2019	BBJ	118,309
	South Asian	2020	UKB	8422
Alkaline phosphatase (U/L)	European	2018	UKB	344,392
	African/African American	2020	UKB	6216
	East Asian	2019	BBJ	105,030
	South Asian	2020	UKB	8422
Alanine aminotransferase (U/L)	European	2018	UKB	344,136
	African/African American	2020	UKB	6214
	East Asian	2019	BBJ	134,182
	South Asian	2020	UKB	8407
Aspartate aminotransferase (U/L)	European	2018	UKB	342,990
	African/African American	2020	UKB	6165
	East Asian	2019	BBJ	134,154
	South Asian	2020	UKB	8395
Direct bilirubin (μmol/L)	European	2018	UKB	342,990
	African/African American	2020	UKB	6180
	East Asian	2020	UKB	2159
	South Asian	2020	UKB	6961
Total bilirubin (μmol/L)	European	2018	UKB	342,990
	African/African American	2020	UKB	6176
	East Asian	2019	BBJ	110,207
	South Asian	2020	UKB	8395

BBJ, Biobank Japan; LIFE, Leipzig Research Centre for Civilization Diseases; LURIC, The LUdwigshafen RIsk and Cardiovascular Health; SD, standard deviation; UKB,UK Biobank.

additional information about the PCSK9 and HMGCR instruments.

In the EUR analyses, we supplemented the PCSK9 and HMGCR instruments constructed using circulating LDL-C levels with available pQTL and eQTL data (Supplementary Table 3). We constructed instruments for circulating PCSK9 levels from pQTLs in participants in the deCODE cohort (N = 35,559).[39] We used a second PCSK9 protein instrument derived from a separate study providing PCSK9 pQTLs in 12,271 participants.[78] Liver *PCSK9* expression data were derived from Genotype-Tissue Expression version 8 liver tissue (N = 178),[40] and HMGCR whole blood gene expression was derived from the eQTLGen Consortia (N = 31,684).[41] Cis-QTL variants were extracted and clumped as with the PCSK9 instrument derived from LDL-C data (ie, variants within ±100 kb clumped at LD r^2 <0.2 using a 250 kb window). PCSK9 protein levels were measured in normalized protein units.[39] The eQTL data are measured in transcripts per million.[79,80] The pQTL and eQTL data for circulating PCSK9 and eQTL data were not available for non-EUR population cohorts. Therefore, these analyses are limited to the EUR LFTs.

Statistical Analysis

Drug-target MR relies on the same core assumptions as MR to evaluate experimental validity. The 3 major assumptions of MR (Figure 5) are (1) the relevance assumption, that MR instruments are associated with the exposure; (2) the exchangeability assumption, there must not be pathways other than via the exposure of interest through

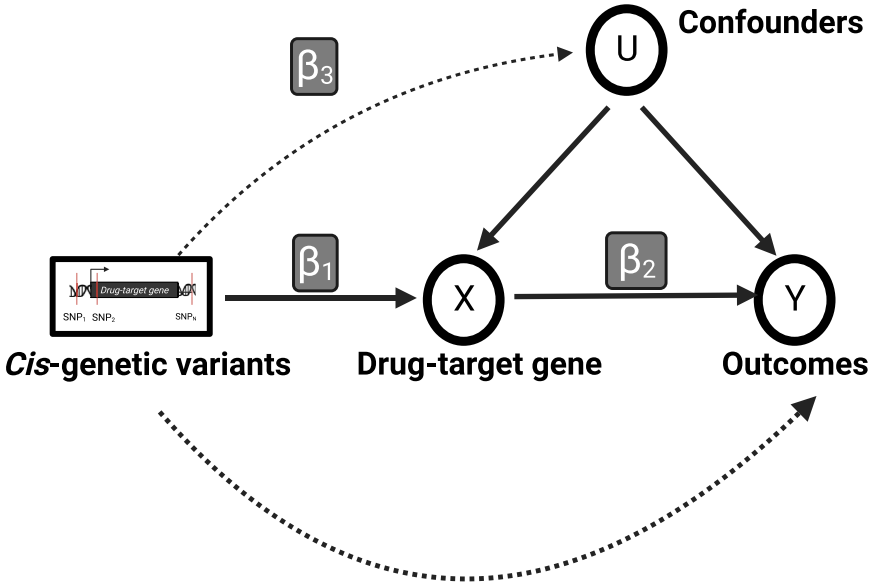

Figure 5. Mendelian randomization model and assumptions. B_2 is the genetic association of interest, estimated by $B_2 = B_1/ B_3$. B_1 and B_3 are the associations of the genetic variants with the exposure and the outcome. MR assumes that the genetic variants comprising the instrument for the exposure only impact the outcome of interest via the exposure and not directly, or via confounders (*dotted lines*).[11]

which the MR instruments impact the outcomes; and (3) the exclusion restriction, the instruments themselves must not affect the outcome through a mechanism independent of the exposure, and they cannot affect another trait with a downstream effect on the outcome of interest (ie, no horizontal pleiotropy).[11,81] To test the relevance assumption, we determined the strength of each ancestry-specific instruments by calculating F-statistics and R^2 values for each variant comprising the instruments,[11,45] retaining only instruments with sufficient calculated strength (ie, F-statistic >10, by convention). The use of complementary MR methods along with alternate instruments because sensitivity analyses both improve the study workflow and minimize deviation from the exchangeability and exclusion restriction assumptions. For the SAS PCSK9 instrument, the only instrument that contained a single variant, we used the Wald Ratio as the primary MR method.[82] For drug-target instruments with 2 or more variants, we performed IVW MR random-effects model as the primary analysis, with the MR Egger and Maximum Likelihood methods (also random-effects models)[82] as sensitivity analyses. LD between variants was accounted for by incorporating correlation matrices generated with the 1000 Genomes Phase 3 Project data to assess evidence for associations between PCSK9, HMGCR, and the outcomes and also evaluate potential violations of the MR assumptions.[83] Consistency of estimates across each of these MR methods would therefore suggest an unbiased estimate.[11,62,63] For the drug-target instruments constructed using >2 SNPs, we also used the MR Egger intercept test[84] and Cochran Q heterogeneity test[85] to assess heterogeneity and the MR Steiger test to evaluate the hypothesized causal direction between circulating LDL-C levels and outcomes.[63] All analyses were conducted by using the TwoSampleMR and MendelianRandomization packages[63,86] in R version 4.0.3. All authors for this study had access to the study data and reviewed and approved the final manuscript.

Interpretation of Results and Power Calculations

We report the MR estimates and corresponding 95% confidence intervals for the impact of LDL-C lowering via the *PCSK9* and *HMGCR* loci on LFTs. We aligned the direction of the estimates with the physiological impact of PCSK9 inhibitors and statins by transforming each MR estimate to correspond to a standard deviation-unit reduction in either PCSK9 or HMGCR. We also aligned the QTL-based analyses to align with reduced PCSK9 and HMGCR levels. For the primary instruments derived in LDL-C data, we annotate high confidence findings using a Bonferroni-corrected threshold *P* value <.00208 to account for multiple testing bias. We use a nominal threshold (*P* value <.05) for the exploratory QTL analyses.

Data Availability

All exposure instruments required to replicate the analyses are located in the Supplementary Tables. This study uses publicly available GWAS summary statistics. The European, African, and South Asian liver endpoint data are available from the Neale Lab repository (http://www.nealelab.is/uk-biobank). The East Asian data are available from the Biobank Japan Project (https://biobankjp.org/en/index.html). The GLGC LDL-C data are available from the GLGC downloads page (http://csg.sph.umich.edu/willer/public/glgc-lipids2021/). PCSK9 protein data from deCODE are available at https://www.decode.com/summarydata/. The replication PCSK9 protein data by Pott et al[78] are available at https://zenodo.org/record/5643551. Liver expression PCSK9 data from GTEx are available at https://www.gtexportal.org/home/gene/PCSK9, and whole blood HMGCR gene expression from the eQTLGen consortium is available at https://www.eqtlgen.org/. Figures 4 and 5 were generated by using https://www.biorender.com/.

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Correspondence
Address correspondence to: Falk W. Lohoff, MD, Section on Clinical Genomics and Experimental Therapeutics (CGET), National Institute on Alcohol Abuse and Alcoholism (NIAAA), National Institutes of Health, 10 Center Drive (10CRC/2-2352), Bethesda, Maryland 20892-1540. e-mail: falk.lohoff@nih.gov.

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CRediT Authorship Contributions

Daniel B. Rosoff, AB, ScB (Conceptualization: Lead; Data curation: Lead; Formal analysis: Lead; Investigation: Lead; Methodology: Lead; Validation: Equal; Visualization: Equal; Writing – original draft: Equal; Writing – review & editing: Equal)

Andrew S. Bell, BA (Conceptualization: Equal; Data curation: Equal; Formal analysis: Equal; Investigation: Equal; Methodology: Equal; Validation: Equal; Visualization: Equal; Writing – original draft: Lead; Writing – review & editing: Lead)

Josephin Wagner, MD, MS (Conceptualization: Supporting; Methodology: Supporting; Validation: Equal; Writing – review & editing: Equal)

Lucas A. Mavromatis, ScB (Conceptualization: Supporting; Methodology: Supporting; Validation: Equal; Writing – review & editing: Equal)

Ali Hamandi, BS (Conceptualization: Supporting; Methodology: Supporting; Visualization: Supporting; Writing – review & editing: Equal)

Lauren Park, BS (Conceptualization: Equal; Methodology: Equal; Validation: Supporting; Visualization: Equal)

Jeesun Jung, PhD (Conceptualization: Equal; Methodology: Equal; Validation: Equal)

Falk Lohoff, MD (Conceptualization: Equal; Funding acquisition: Lead; Methodology: Equal; Project administration: Lead; Resources: Lead; Supervision: Lead; Validation: Equal; Writing – review & editing: Equal)

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