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Published in:
European Journal of Preventive Cardiology

DOI (link to publication from Publisher):
10.1093/eurjpc/zwac219

Publication date:
2022

Document Version
Publisher's PDF, also known as Version of record

ESC European Society of Cardiology

European Journal of Preventive Cardiology (2022) **29**, 2374–2385
https://doi.org/10.1093/eurjpc/zwac219

FULL RESEARCH PAPER
Coronary artery disease

Cholesterol not particle concentration mediates the atherogenic risk conferred by apolipoprotein B particles: a Mendelian randomization analysis

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Received 10 July 2022; revised 23 August 2022; editorial decision 9 September 2022; accepted 19 September 2022; online publish-ahead-of-print 20 September 2022

See the editorial comment for this article 'Apolipoprotein B versus non-high-density lipoprotein cholesterol: is the debate really over?', by V. Bittner, https://doi.org/10.1093/eurjpc/zwac261.

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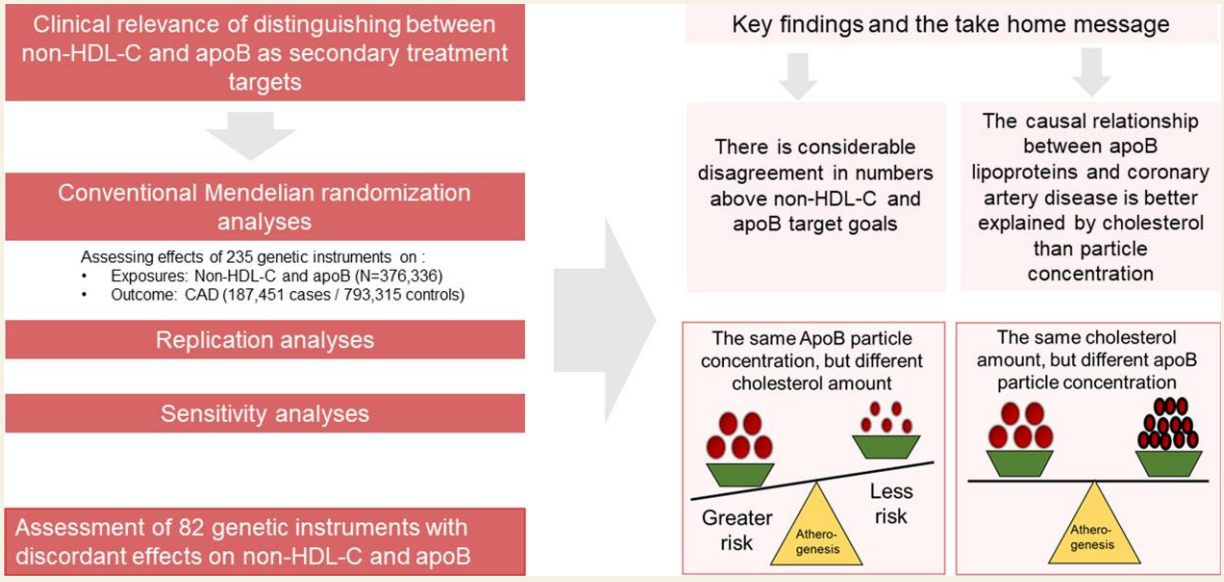
Background and aims The causal contribution of apolipoprotein B (apoB) particles to coronary artery disease (CAD) is established. We examined whether this atherogenic contribution is better reflected by non-high-density lipoprotein cholesterol (non-HDL-C) or apoB particle concentration.

Method and results We performed Mendelian randomization (MR) analysis using 235 variants as genetic instruments; testing the relationship between their effects on the exposures, non-HDL-C and apoB, and on the outcome CAD using weighted regression. Variant effect estimates on the exposures came from the UK Biobank ($N = 376\,336$) and on the outcome from a meta-analysis of five CAD datasets (187 451 cases and 793 315 controls). Subsequently, we carried out sensitivity and replication analyses.

In univariate MR analysis, both exposures associated with CAD ($\beta_{\text{non-HDL-C}} = 0.40$, $P = 2.8 \times 10^{-48}$ and $\beta_{\text{apoB}} = 0.38$, $P = 1.3 \times 10^{-44}$). Adding effects on non-HDL-C into a model that already included those on apoB significantly improved the genetically predicted CAD effects ($P = 3.9 \times 10^{-5}$), while adding apoB into the model including non-HDL-C did not ($P = 0.69$). Thirty-five per cent (82/235) of the variants used as genetic instruments had discordant effects on the exposures, associating with non-HDL-C/apoB ratio at $P < 2.1 \times 10^{-4}$ (0.05/235). Fifty-one variants associated at genome-wide significance.

Conclusion Many sequence variants have discordant effects on non-HDL-C and apoB. These variants allowed us to show that the causal mechanism underlying the relationship between apolipoprotein B particles and CAD is more associated with non-HDL-C than apoB particle concentration.

Graphical Abstract



Keywords Apolipoprotein B • Non-HDL cholesterol • Coronary artery disease • Mendelian randomization

Introduction

Apolipoprotein B (apoB) containing particles, including low-density lipoproteins (LDL), are primary drivers in the pathogenesis of atherosclerotic cardiovascular disease (ASCVD).[1] However, many aspects of the pathophysiological mechanisms underlying this relationship are still uncertain. This includes the relative atherogenic contribution of the apoB particle count vs. their cholesterol content, the product of which yields non-high-density lipoprotein cholesterol (non-HDL-C) concentration. Currently, the cornerstone of ASCVD prevention and treatment is pharmacological reduction of LDL-C.

The European Society of Cardiology/European Atherosclerosis Society (ESC/EAS) 2019 guidelines for the management of dyslipidemias[2] recommend measuring non-HDL-C or apoB as secondary treatment targets in patients with high triglyceride levels, obesity, metabolic syndrome, or diabetes mellitus, since LDL-C alone may underestimate cardiovascular risk in these patient groups. The guidelines prefer apoB measurements to non-HDL-C.[2] Furthermore, consensus-based documents[1,3] endorse apoB particle concentration as the principal lipid driver of ASCVD.

Recent Mendelian randomization (MR) studies evaluating the causal contribution of lipid traits on ASCVD analysed LDL-C, HDL-C,

triglycerides, and apolipoproteins,[4,5] but not non-HDL-C that includes cholesterol from triglyceride-rich lipoprotein particles. Conversely, our previously reported MR study that assessed the causal contribution of the standard lipid measures, including non-HDL-C, to CAD, did not examine apoB.[6] All these studies support that apoB lipoproteins, either measured as particle concentration (apoB),[4,5] or as cholesterol concentration (non-HDL-C),[6] capture more of the atherogenic risk than LDL-C, and can explain the effect of triglycerides and HDL-C, on CAD risk. However, no previous MR study has directly compared apoB and non-HDL-C as predictors of ASCVD.

Other MR studies have leveraged lipid measures quantified with nuclear magnetic resonance (NMR) spectroscopy.[5,7] These studies did not distinguish between apoB particle concentration and non-HDL-C in ASCVD, i.e. non-HDL-C was excluded from the analyses.[7]

Here, we performed an MR study to assess the relative contribution of non-HDL-C and apoB particle concentration to the development of coronary artery disease (CAD), using vast resources of genetic information on these traits.

Methods

Blood lipid measurements

For the main analyses, exposure measurements were based on data from the UK Biobank (accessed under application # 56270). Non-HDL-C (total cholesterol minus HDL-C) and apoB were assayed using the Beckman Coulter AU5800 analytical platform. In this platform, $apoB_{100}$ is measured by a turbidimetric method, based on the formation of apoB-antibody complex, detected as a light scatter that is directly proportional to the concentration of apoB. Lipid measurements from 376 336 self-reported white British individuals (birth year range: 1934–1970) with similar genetic ancestry based on principal component analysis and who had available measurements of both apoB and non-HDL-C were used. We further created a variable based on the ratio between levels of the two traits in blood (non-HDL-C/apoB ratio) for the same samples.

We tested the apoB-associated genetic instruments selected for our MR analysis for association with triglycerides ($N = 411\,799$) and LDL-C ($N = 369\,350$) in white British individuals from the UK Biobank.

For replication analyses, variant associations with non-HDL-C, apoB, and non-HDL-C/apoB ratio, assayed using Roche/Hitachi Cobas 6000 analyzer, were assessed in Icelandic samples ($N = 24\,043$). In this platform, $apoB_{(100\text{ and }48\text{ isoforms})}$ is measured by a turbidimetric method.

Variant associations with non-HDL-C/apoB ratio were further validated in patient samples measured at baseline in the FOURIER trial, a randomized, double-blind, placebo-controlled, multinational clinical trial. All patients eligible for participation in the trial had clinically evident ASCVD and were treated with statins at baseline.[8] For the purpose of our study, variant associations with non-HDL-C/apoB ratios were examined in participants of European origin, defined with principal component analyses, who also were genotyped ($N = 11\,728$).

Lipid measurements from the three studies were adjusted for sex, year of birth, age at measurement, measurement centre (in the UK Biobank), and principal components, a measure of genetic ancestry, by including those variables as explanatory variables in a multiple regression model with the lipid values as outcome. This was done for males and females separately and the residuals were then combined and standardized using inverse rank-normalization. The reason for including both year of birth and age at measurement as explanatory variables is that both are significantly correlated with the lipid measurements in the regression analysis, although the association with year of birth is much less than with age at measurement. In the UK Biobank and Icelandic studies, lipid levels were additionally adjusted for lipid lowering medication, by dividing total cholesterol and ApoB levels by 0.8 for individuals taking lipid lowering medication (see ref.[9]). Of the participants with lipid measurements from the UK Biobank ($N = 376\,336$) and Iceland ($N = 24\,043$), 4.0 and 9.3% had CAD, respectively. In the UK Biobank data, the mean non-HDL-C was 4.26 mmol/L and each standard deviation (SD) corresponds to 1.06 mmol/L; mean ApoB was 1.03 g/L and each SD corresponds to 0.24 g/L. In the Icelandic data, the mean non-HDL-C was 3.82 mmol/L (SD = 1.10) and mean apoB was 1.06 g/L (SD = 0.26). In the baseline data from FOURIER (genotyped subset) the mean non-HDL-C was 3.25 mmol/L (SD = 0.89) and mean apoB was 0.86 g/L (SD = 0.21).

We counted the instances where common treatment target goals for non-HDL-C and apoB disagreed in 26 851 FOURIER participants with available raw lipid measures at baseline.

Coronary artery disease datasets

We obtained variant association results with the outcome from a meta-analysis of five CAD datasets excluding data from the UK Biobank, including 187 451 cases and 793 315 controls; from Iceland (43 684 cases/306 935 controls), Denmark (38 581 cases/165 423 controls), CARDIoGRAMplusC4D (60 801 cases/123 504 controls), Finngen (31 640 cases/152 665 controls), and the United States (12 745 cases/44 788 controls). Additional analyses included 47 292 CAD cases and 383 322 controls from the UK Biobank. Details about each of the CAD datasets are provided in the Supplementary material online, *Supplementary methods*.

Type 2 diabetes with CAD

The relative contribution of apoB and non-HDL-C in CAD development was assessed in 82 410 type 2 diabetes (T2D) patients. The associations of genetic instruments with CAD among these patients were obtained from a meta-analysis of four datasets including 33 002 cases and 49 408 controls. The four datasets were from Iceland [6106 cases with mean year of birth YOB 1938 (SD = 13 years), 67.5% males, and 7896 controls with mean YOB 1944 (SD = 13 years), 50.3% males], UK Biobank [11 401 cases with mean YOB 1946 (SD = 6 years), 70.0% males, and 22 028 controls with mean YOB 1949 (SD = 7 years), 57.0% males], Denmark [11 348 cases with mean YOB 1943 (SD = 11 years), 66.8% males, and 12 342 controls with mean YOB 1943 (SD = 10 years), 55.6% males], and United States [4147 cases with mean YOB 1942 (SD = 12 years), 70.1% males, and 7142 controls with mean YOB 1952 (SD = 13 years), 46.5% males]. Case definition in all studies included cases with T2D who are also diagnosed with CAD. Controls included patients with type 2 diabetes who had not been diagnosed with CAD. T2D diagnosis was based on ICD10 codes E11, and on self-reported diabetes (Iceland and UK Biobank), on the use of oral diabetes medication, or on at least two measures of HbA1C > 6.5% (Iceland). Where available, known MODY (Iceland) or T1D cases (Iceland and UK Biobank) were excluded.

Selection of genetic instruments

We based our analysis on 255 sequence variants recently reported to associate with apoB[4] (see Supplementary material online, *Table S1*). Since the association with non-HDL-C was not reported for these variants, we assessed their association with non-HDL-C and apoB in our dataset. Out

of 255 apoB variants, we used proxies ($R^2 > 0.85$ with the index variant) for 8 of them to reduce missing outcome data (see Supplementary material online, *Table S2* and *Figure S1*). Of these seven variants had $R^2 > 0.92$. We had missing association results for five variants (rs57754494, rs377181093, rs576573069, rs560238897, and rs12990177) for which we did not find proxies.

Assessing the validity of the genetic instruments

We performed a weighted regression analysis regressing the effects of the 250 variants on CAD on the effects on each exposure, including an intercept term in the model. This test has been referred to as MR-Egger.[10] For both exposures, the relationship with CAD had an intercept that deviated significantly from zero, indicating bias by pleiotropy, i.e. the association with CAD is unlikely only explained by the tested exposure (see Supplementary material online, *Table S3*). Thus, we removed 15 variants with CAD causal effect ($\beta_{CAD}/\beta_{exposure}$) that deviated more than 4 standard deviations (estimated using a robust estimator) from the mean, indicating that the causal effect on CAD is likely driven through other mechanisms than the tested exposures (see Supplementary material online, *Table S1* and *Figure S2*). These included several known CAD variants[11] (e.g. at the chromosome 9p21 and *LPA* loci), with relatively small effects on the exposures. The remaining 235 genetic instruments were used for the main MR analyses.

Mendelian randomization analyses

For the main and replication analyses we used a two-sample MR approach, testing associations of the 235 genetic instruments with exposure and outcome traits in non-overlapping populations.[12] Associations with the exposures were tested in measurements from the UK Biobank ($N = 376\,336$) and Iceland ($N = 24\,043$), in the main and replication analyses, respectively. We applied weighted linear regression without an intercept term, referred to as inverse-variance-weighted (IVW) method. The regression was performed on the allele that has a positive effect on the exposure and the inverse of the square of the standard error of the variant-outcome associations was used as weights. The same approach was used in multivariate analyses including variant effects on both exposures in the model. ANOVA *F*-test was used to compare the full model including both exposures, with the nested model including only one of the exposures.

Sensitivity analyses

To evaluate if the method of adjustments for lipid lowering medication might have introduced bias, influencing our results in a way that one of the two lipid trait is inappropriately prioritized, we repeated the MR analyses using variant association results for the two lipid traits from a subgroup not taking lipid lowering medications. We also assessed whether results were dependent on 10 variants at chromosome 19 *APOE/APOC1/APOC2* locus with large effects on apoB and non-HDL-C (see Supplementary material online, *Table S1*). Several other analyses can be considered as sensitivity analyses. This includes the analysis using effect estimates on the exposures from Icelandic samples ($N = 24\,043$), measured with assays from a different manufacturer, and variant effects on the outcome from the UK Biobank (47 292 CAD cases and 383 322 controls), as well as analyses that specifically used a subset of the genetic instruments that also associate with triglycerides.

Other statistical analyses

We assessed the relationships between blood lipid levels, and between variant effects on blood lipids with linear regression and weighted linear regression, respectively. Using the 82 variants associating with non-HDL-C/apoB ratio, we assessed the relationship between apoB and non-HDL-C including an interaction term, allowing the genetic relationships between non-HDL-C and apoB to differ between variants with main-effects on non-HDL-C and apoB. ANOVA *F*-test was used to compare this model to the nested model, without the interaction term.

For determining thresholds for genome-wide significance of associations with non-HDL-C/apoB ratio, we grouped variants according to functional annotations when correcting for multiple testing, yielding *P*-value thresholds ranging from 3.9×10^{-10} for lowest impact to 1.3×10^{-7} for highest impact variants.[13]

Genotyping

Information on genetic measurements is provided in the Supplementary material online, *Supplementary methods*.

Results

Clinical relevance of distinguishing between non-HDL-C and apoB as secondary biomarkers

Given the high correlation between non-HDL-C and apoB, it could be argued that distinguishing between the two biomarkers is not clinically relevant. Thus, we assessed available raw baseline lipid values from the FOURIER trial ($N = 26\,851$), in which all participants had clinically evident ASCVD and were treated with statins at baseline. We examined instances in which commonly used treatment target goals for LDL-C had been reached, but not the comparable treatment targets for non-HDL-C or apoB.[2] As shown in *Table 1*, there is considerable disagreement in numbers above target goals depending on which secondary biomarker is considered.

Disagreement in numbers over non-HDL-C and apoB target goals is further observed among individuals with triglycerides ≥4.5 mmol/L, i.e. in cases when LDL-C calculation using the Friedewald formula is not valid. Instances where treatment target goals have been reached for non-HDL-C but not apoB are relatively rare, whereas the reverse is much more common (*Table 1*).

ApoB and non-HDL-C genetic instruments and their relationship

Figure 1 shows the relationships between apoB and non-HDL-C blood levels and genetic instruments. Each SD unit of genetically elevated apoB associates with 0.87 [95% confidence interval (CI): 0.84, 0.90] SD genetically elevated non-HDL-C (*Figure 1*). Converted into the original scale, 1 g/L change in apoB associates with 3.86 mmol/L change in non-HDL-C.

Main Mendelian randomization analyses and replication

In univariate MR analyses, both exposures, non-HDL-C and apoB, associated with the risk of CAD, in a dose-dependent manner (*Table 2*). The performance of the genetic instruments in predicting the effects on CAD were better for non-HDL-C ($R^2 = 0.60$) than apoB ($R^2 = 0.57$) (*Table 1*). Adding variant effects on non-HDL-C into a model that already included those on apoB significantly

Table 1 The disagreement in numbers over treatment goals for apoB and non-HDL-C in FOURIER trial participants at baseline ($N = 26\,851$)

LDL-C treatment goal[a]	Treatment goals for apoB and non-HDL-C[a]	N	P
A) Participants under LDL-C treatment goals			
<2.6 mmol/L	apoB > 1.0 g/L	672 (3.7%)	5.6×10^{-114}
$N = 18\,056$	non-HDL-C > 3.4 mmol/L	1752 (9.7%)	
	apoB < 1.0 g/L and non-HDL-C > 3.4 mmol/L	1126 (6.2%)	1.3×10^{-84}
	apoB > 1 g/L and non-HDL-C < 3.4 mmol/L	120 (0.7%)	
<1.8 mmol/L	apoB > 0.8 g/L	478 (12.6%)	7.6×10^{-83}
$N = 3799$	non-HDL-C > 2.6 mmol/L	1172 (30.9%)	
	apoB < 0.8 g/L and non-HDL-C > 2.6 mmol/L	626 (16.5%)	1.8×10^{-130}
	apoB > 0.8 g/L and non-HDL-C < 2.6 mmol/L	30 (0.8%)	
<1.4 mmol/L	apoB > 0.65 g/L	261 (39.4%)	9.1×10^{-3}
$N = 663$	non-HDL-C > 2.2 mmol/L	309 (46.6%)	
	apoB > 0.65 g/L and non-HDL-C > 2.2 mmol/L	58 (8.7%)	2.0×10^{-6}
	apoB > 0.65 g/L and non-HDL-C > 2.2 mmol/L	17 (2.6%)	
Triglycerides > 4.5 mmol/L	**Treatment goals for apoB and non-HDL-C[a]**	**N**	**P**
B) Participants with hypertriglyceridemia			
	apoB > 1.0 g/L	256 (62.3%)	1.4×10^{-21}
$N = 411$	non-HDL-C > 3.4 mmol/L	373 (90.8%)	
	apoB < 1.0 g/L and non-HDL-C > 3.4 mmol/L	116 (28.2%)	5.2×10^{-30}
	apoB > 1 g/L and non-HDL-C < 3.4 mmol/L	1 (0.2%)	
	apoB > 0.8 g/L	371 (90.3%)	2.6×10^{-10}
$N = 411$	non-HDL-C > 2.6 mmol/L	411 (100%)	
	apoB < 0.8 g/L and non-HDL-C > 2.6 mmol/L	32 (7.8%)	2.3×10^{-8}
	apoB > 0.8 g/L and non-HDL-C < 2.6 mmol/L	0 (0%)	
	apoB > 0.65 g/L	406 (98.8%)	0.073
$N = 411$	non-HDL-C > 2.2 mmol/L	411 (100%)	
	apoB < 0.65 g/L and non-HDL-C > 2.2 mmol/L	5 (1.2%)	0.073
	apoB > 0.65 g/L and non-HDL-C < 2.2 mmol/L	0 (0%)	

[a]Treatment target goals are from the ESC/EAS 2019 guidelines for management of dyslipidemia (ref.2). *P*-value is given for the difference in proportions.

improved the genetically predicted CAD ($P = 3.9 \times 10^{-5}$), while adding apoB into the model that already included non-HDL-C did not ($P = 0.69$) (*Table 2A*). These results were consistent in all five CAD datasets (Iceland, Denmark, Finngen, CARDIoGRAMplusC4D, and the United States), and further in the UK Biobank using one-sample MR approach testing associations of exposures and outcome in the same population (see Supplementary material online, *Table S4*).

In addition, the results were validated in fully independent two-sample MR analysis using effect estimates for the exposures derived from Icelandic samples ($N = 24\,043$) and the effects for CAD from the UK Biobank (47 292 cases and 383 322 controls) (*Table 2B*).

Sensitivity analyses

We performed sensitivity analysis obtaining the variant effect estimates on non-HDL-C and apoB from individuals that are not receiving lipid lowering treatment. The results show slightly less effects on CAD than observed in the main MR analysis, most likely due to removal of individuals with genetic susceptibility to elevated levels of apoB-lipoproteins. However, consistent with the main MR analysis, the sensitivity analysis prioritized non-HDL-C, showing that our results were not dependent on the lipid-lowering treatment adjustment (see Supplementary material online, *Table S5*).

Furthermore, results from sensitivity analysis, excluding influential variants at the *APOE/APOC1/APOC2* locus, were consistent with the main analysis (see Supplementary material online, *Table S5*).

The atherogenic contribution of non-HDL-C and ApoB in the context of altered triglyceride metabolism and type 2 diabetes

We performed three additional MR analyses to address whether the relative contribution of genetically elevated apoB and non-HDL-C to atherogenesis differed either in the context of genetic mechanisms affecting triglyceride concentrations, or among people with type 2 diabetes (T2D) (see Supplementary material online, *Table S6*). First,

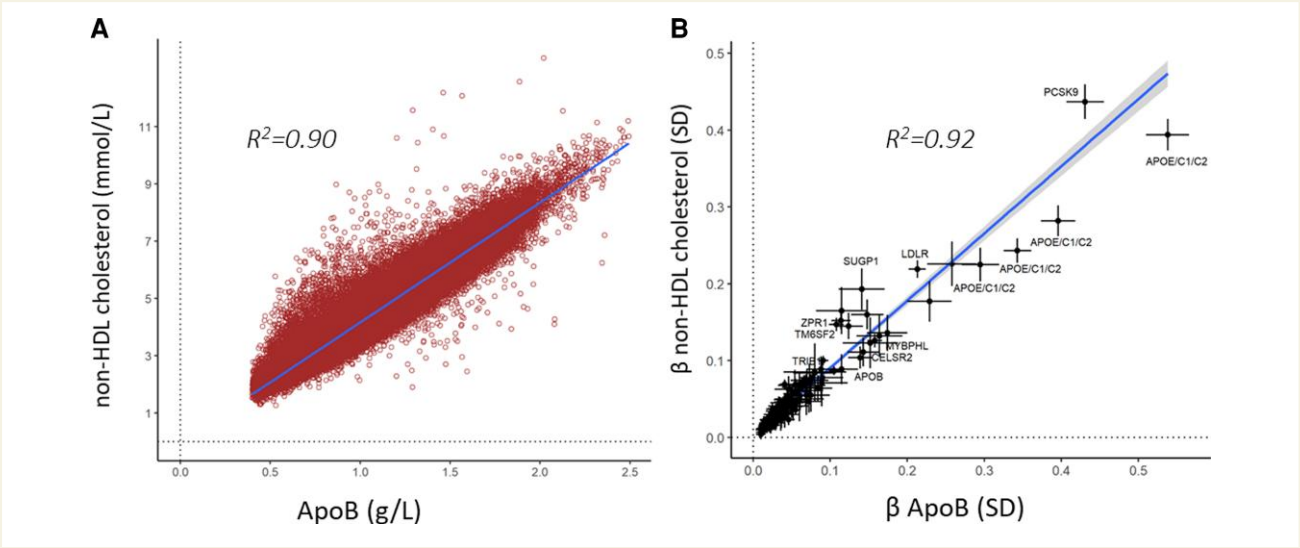


Figure 1 (Panel *A*) The relationship between blood levels of non-HDL-C and ApoB. (Panel *B*) The relationship between effects (β) on non-HDL-C and ApoB for the variants selected as instrumental variables ($N = 235$). SD, standard deviation units. Each 1 SD genetically elevated ApoB associates with 0.87 (95% CI: 0.84, 0.90) SD increase in non-HDL-C. The crosses show 95% CIs. The blue lines are the best-fitting lines, using weighted regression in panel *B*.

we repeated the main MR analysis examining the effects of apoB and non-HDL-C on the effects on CAD after restricting the 235 genetic instruments to 103 that also associate with triglycerides at $P < 2.1 \times 10^{-4}$ ($P < 0.05/235$; see Supplementary material online, *Table S1*). Then, we specifically assessed the relative contribution of the exposures in CAD development among T2D patients (33 002 T2D cases with CAD, and 49 408 controls with T2D but not CAD), both using all 235 genetic instruments, and only the 103 that also associate with triglycerides. The results from all three analyses were consistent with the main analysis indicating residual association of non-HDL-C with CAD, after accounting for apoB, but no association of apoB with CAD after accounting for non-HDL-C (see Supplementary material online, *Table S6*).

Table 2 Instrumental variable associations of non-HDL-C and ApoB with coronary artery disease

	Coronary artery disease meta-analysis (187 451 cases/793 315 controls)		
A) Exposure ($N = 376\,336$; UK Biobank)	**β**	**SE**	***P***
Univariate model			
β non-HDL-C	0.40	0.022	2.8×10^{-48}
β ApoB	0.38	0.022	1.3×10^{-44}
Multivariate model			
β non-HDL-C	0.44	0.11	3.9×10^{-5}
β ApoB	−0.040	0.10	0.69
	Coronary artery disease UK biobank (47 292 cases/383 322 controls)		
B) Exposure ($N = 24\,043$; Iceland)	**β**	**SE**	***P***
Univariate model			
β non-HDL-C	0.40	0.027	1.0×10^{-35}
β ApoB	0.38	0.026	4.0×10^{-34}
Multivariate model			
β non-HDL-C	0.37	0.14	6.8×10^{-3}
β ApoB	0.031	0.13	0.81

Variants with discordant effects on the exposures

For further assessment, we explored whether we could leverage variants with discordant effects (i.e. with lower correlation) on non-HDL-C and apoB.

First, to identify such variants, we created a variable for 376 336 UK Biobank individuals based on the ratio between levels of the two traits in blood, the non-HDL-C/apoB ratio. Variants that associate with this ratio are considered to have discordant effects on the two exposures contributing to a deviation from the mean non-HDL-C/apoB ratio in the population.

Eighty-two of the 235 (35%) reported apoB variants used in our MR analyses associated with the ratio at Bonferroni corrected $P < 2.1 \times 10^{-4}$ (see Supplementary material online, *Table S7*). In two smaller blood lipid datasets, from Iceland ($N = 24\,043$) and the FOURIER trial ($N = 11\,728$), the association of 39 and 31 variants with non-HDL-C/apoB ratio replicated at $P < 0.05$, respectively (see Supplementary material online, *Table S7*). In both replication datasets, one additional variant (rs3764261 at the *CETP* locus) associated with the non-HDL-C/apoB ratio with directional effect inconsistent with that in the UK Biobank (see Supplementary material online, *Table S6*), potentially explained by different

Table 3 Variants with discordant effects on non-HDL-C and apoB

						Meta-analysis ($N = 412\,107$) (UK Biobank + Iceland + FOURIER) non-HDL-C/apoB ratio			
Chr.	Position	rsName	MAF (%)	EA/non-EA	Locus genes	β	95% CI	*P*	Main-effect
1	26695422	rs114165349	2.371	C/G	*ARID1A*	−0.059	(−0.076, −0.042)	1.8×10^{-11}	ApoB
1	55039974	rs11591147	1.779	G/T	*PCSK9*	0.111	(−0.131, −0.091)	1.8×10^{-27}	non-HDL-C
1	62511636	rs11207977	35.414	C/T	*DOCK7,ANGPTL3*	0.095	(−0.100, −0.089)	3.4×10^{-256}	non-HDL-C
1	109264661	rs6657811	13.033	A/T	*CELSR2*	−0.086	(0.078, 0.094)	1.5×10^{-105}	ApoB
1	109288646	rs6689611	1.241	G/A	*MYBPHL*	−0.082	(0.058, 0.106)	2.1×10^{-11}	ApoB
1	109297547	rs76186504	2.537	C/T	*MYBPHL*	−0.078	(0.062, 0.094)	6.9×10^{-21}	ApoB
1	230160042	rs10127775	39.291	!/T	*GALNT2*	−0.03	(−0.035, −0.024)	2.1×10^{-28}	ApoB
2	20993943	rs62122481	38.158	A/C	*APOB*	−0.041	(−0.046, −0.035)	2.6×10^{-49}	ApoB
2	21049906	rs60403635	4.374	!/C	*APOB*	−0.082	(0.069, 0.095)	7.2×10^{-36}	ApoB
2	43845437	rs4299376	32.436	G/T	*ABCG8*	0.041	(0.035, 0.047)	3.6×10^{-47}	non-HDL-C
3	12285932	rs13076933	25.88	T/G	*PPARG*	0.018	(−0.024, −0.012)	1.0×10^{-9}	non-HDL-C
4	68507689	rs9884390	23.718	C/T	*UGT2B17*	0.021	(0.015, 0.027)	8.6×10^{-12}	non-HDL-C
5	52799190	rs116734477	4.15	C/T	*ITGA1,PELO*	−0.052	(0.039, 0.065)	6.4×10^{-15}	ApoB
5	75360714	rs12916	39.966	C/T	*HMGCR*	0.042	(0.037, 0.048)	6.2×10^{-57}	non-HDL-C
5	156964617	rs6874202	36.438	C/T	*TIMD4*	0.033	(−0.039, −0.028)	3.9×10^{-34}	non-HDL-C
6	31138682	rs1265097	7.875	A/C	*PSORS1C1*	0.031	(0.021, 0.040)	5.0×10^{-10}	non-HDL-C
6	32444611	rs4935356	15.94	A/!	*HLA-DRA*	0.032	(0.024, 0.039)	6.8×10^{-18}	non-HDL-C
6	32633511	rs72848251	18.895	A/G	*HLA-DQA1*	0.035	(0.029, 0.042)	4.6×10^{-26}	non-HDL-C
6	42951484	rs9471975	41.725	!/C	*CNPY3-GNMT*	−0.025	(−0.030, −0.020)	6.2×10^{-21}	ApoB
7	44542732	rs2073547	18.273	G/A	*NPC1L1*	0.031	(0.025, 0.038)	1.7×10^{-20}	non-HDL-C
8	18415371	rs1495741	22.013	G/A	*NAT2*	0.038	(0.032, 0.044)	9.4×10^{-33}	non-HDL-C
8	58479765	rs9297994	33.55	G/A	*CYP7A1*	0.029	(0.024, 0.035)	5.9×10^{-26}	non-HDL-C
8	115655312	rs2737263	28.441	!/T	*TRPS1*	0.021	(−0.027, −0.015)	5.8×10^{-13}	non-HDL-C
8	125487789	rs28601761	42.041	C/G	*TRIB1*	0.033	(−0.038, −0.028)	3.0×10^{-35}	non-HDL-C
9	15305380	rs581080	17.967	C/G	*TTC39B*	−0.022	(0.015, 0.028)	1.6×10^{-10}	ApoB
9	133279427	rs635634	18.415	T/C	*ABO*	0.053	(0.046, 0.059)	2.5×10^{-53}	non-HDL-C
10	102415892	rs79931565	6.882	G/A	*FBXL15*	−0.044	(−0.054, −0.034)	6.0×10^{-17}	ApoB
11	61820833	rs174564	35.032	A/G	*FADS1*	−0.048	(0.042, 0.053)	1.6×10^{-66}	ApoB
11	116778201	rs964184	13.24	G/C	*ZPR1*	0.127	(0.120, 0.135)	9.5×10^{-237}	non-HDL-C
11	116843577	rs141469619	1.125	G/!	*SIK3*	0.135	(0.110, 0.160)	1.0×10^{-26}	non-HDL-C
12	120988675	rs1169292	30.791	T/C	*HNF1A*	0.017	(0.011, 0.022)	9.7×10^{-9}	non-HDL-C
13	32385062	rs2238162	47.561	C/T	*BRCA2*	−0.017	(−0.022, −0.011)	3.2×10^{-10}	ApoB
13	113849020	rs6602909	32.444	C/!	*GAS6*	0.021	(0.015, 0.026)	4.2×10^{-14}	non-HDL-C
15	101528840	rs4965894	40.281	T/C	*PCSK6*	−0.019	(0.013, 0.024)	6.6×10^{-12}	ApoB
16	72067626	rs34042070	18.534	G/C	*HPR*	0.025	(0.019, 0.032)	3.1×10^{-14}	non-HDL-C
17	28367840	rs704	47.492	A/G	*VTN*	−0.016	(−0.021, −0.011)	1.2×10^{-9}	ApoB
17	66214462	rs1801689	2.988	C/A	*APOH*	−0.047	(−0.063, −0.032)	1.7×10^{-9}	ApoB
17	69085137	rs77542162	2.304	G/A	*ABCA6*	0.063	(0.045, 0.080)	1.4×10^{-12}	non-HDL-C
19	11076648	rs143020224	11.842	!/G	*LDLR*	0.053	(−0.061, −0.045)	3.8×10^{-37}	non-HDL-C
19	19277691	rs8107974	7.608	!/T	*TM6SF2*	0.138	(−0.148, −0.128)	2.1×10^{-168}	non-HDL-C
19	19285807	rs188247550	1.367	!/T	*SUGP1*	0.175	(−0.198, −0.152)	2.5×10^{-50}	non-HDL-C
19	44631381	rs62119267	2.43	A/C	*IGSF23*	−0.242	(0.225, 0.260)	5.1×10^{-170}	ApoB
19	44748549	rs531660643	2.396	!/T	*BCL3*	−0.305	(0.288, 0.323)	6.2×10^{-256}	ApoB
19	44792629	rs113330691	3.558	G/A	*CBLC*	−0.228	(0.214, 0.243)	4.4×10^{-219}	ApoB
19	44843409	rs148601586	1.441	G/!	*NECTIN2*	−0.103	(−0.125, −0.081)	8.9×10^{-20}	ApoB
19	44915229	rs12691088	2.335	A/!	*APOC1*	−0.145	(−0.162, −0.129)	5.8×10^{-66}	ApoB
19	45791965	rs73045960	1.611	A/G	*RSPH6A*	−0.082	(0.061, 0.102)	5.2×10^{-15}	ApoB
19	48702888	rs516316	48.850	C/G	*FUT2*	0.014	(0.009, 0.019)	2.2×10^{-8}	non-HDL-C

Continued

Table 3 *Continued*

						Meta-analysis ($N = 412\,107$) (UK Biobank + Iceland + FOURIER) non-HDL-C/apoB ratio			
Chr.	Position	rsName	MAF (%)	EA/non-EA	Locus genes	β	95% CI	*P*	Main-effect
20	17864040	rs2618566	33.786	G/T	*SNX5*	−0.017	(−0.022, −0.011)	6.7×10^{-10}	ApoB
20	40551182	rs1883711	3.062	C/I	*MAFB*	0.082	(0.067, 0.097)	1.3×10^{-27}	non-HDL-C
20	44413724	rs1800961	3.136	C/T	*HNF4A*	0.064	(−0.079, −0.049)	2.7×10^{-17}	non-HDL-C
20	45923216	rs6073958	19.873	C/T	*PLTP*	−0.065	(−0.071, −0.058)	4.5×10^{-86}	ApoB

Shown are 52 variants associating with non-HDL-C/ApoB ratio at genome-wide significance. Of those rs79931565 at the *FBXL15* was excluded from the Mendelian randomization analyses, due to outlying effects on CAD. Chromosomal (Chr.) positions are for the NCBI reference sequence, build 38. Effect allele (EA) is the allele with positive effect on non-HDL-C and ApoB. Effects (β) on non-HDL-C/ApoB ratio are given in standard deviation units. CI, confidence interval.

measurement assays. In a meta-analysis of the 3 datasets, 51 of the 82 variants associated at genome-wide significance (*Table 3*).

As illustrated in *Figure 2* (panel *A*), the 82 variants that associate with the non-HDL-C/apoB ratio fall into two groups, i.e. those with discordantly greater effect, hereafter referred to as main-effects, on either non-HDL-C ($N = 47$) or apoB ($N = 35$) (see Supplementary material online, *Table S7*).

We compared the cholesterol amount associated with each unit increase in apoB between variant groups by regressing the variant effects on non-HDL-C on those on apoB (*Figure 2*, panel *A*). For variants with non-HDL-C main-effects, one SD change in apoB associates with 1.08 (95% CI: 1.02, 1.14) SD change in non-HDL-C. In contrast, for apoB main-effect variants, the respective number is 0.74 (95% CI: 0.72, 0.76) (*Figure 2*, panel *B*). Converted into the original scale, 1 g/L change in apoB associates with 4.80 and 3.28 mmol/L change in non-HDL-C, respectively. Neither estimate of cholesterol mass per apoB overlaps with the typical (overall average) amount (*Figure 1*, panel *B*).

Assessing whether CAD risk is proportional to non-HDL-C or apoB

The identification of two groups of variants, associated with different cholesterol mass per particle, provides the opportunity to assess in univariate MR models, whether the genetic effects on CAD are proportional to the effects on apoB or non-HDL-C. The genetic effect on CAD is expected to be proportional to the genetic effect on the causal exposure. The apoB effects of both non-HDL-C and apoB main-effect variants associated with their effect on CAD risk: the log(odds ratio (OR)) per SD change in apoB were 0.48 (95% CI: 0.37, 0.60) and 0.28 (95% CI: 0.19, 0.38), respectively. However, the increase in log(OR) per SD change in apoB was 71% greater for non-HDL-C main-effect variants than apoB main-effect variants, showing that CAD risk conferred by apoB is dependent on the associated cholesterol amount. In contrast, non-HDL-C effects did not associate differently with CAD risk between the main-effect groups ($P = 0.56$) (*Table 4*). Thus, the genetic effects on CAD risk are proportional to effects on non-HDL-C, but not to effects on apoB.

The important distinction between variant main-effects and their allelic effects on the non-HDL-C/apoB ratio is described in detail in *Figures S3 and S4*.

Variant main-effects and their association with triglycerides

Non-HDL-C or apoB are recommended as secondary biomarkers for risk assessment in hypertriglyceridemia, since calculated and directly measured LDL-C level may underestimate ASCVD risk associated with all apoB-containing lipoproteins.[2] Using available raw values, triglycerides had a closer correspondence to non-HDL-C than to apoB levels. The correlation (*r*) between triglycerides and non-HDL-C vs. triglyceride and apoB was 0.39 vs. 0.30 in the UK Biobank, 0.42 vs. 0.36 in Iceland, and 0.46 vs. 0.39 in the FOURIER trial at baseline.

We examined whether variants known to associate primarily with triglycerides,[14] mirroring changes in levels of triglyceride-rich lipoproteins, were more likely to belong to the non-HDL-C main-effect group rather than the apoB main-effect group. Consistent with observations examining raw values, a higher proportion or 61% of the non-HDL-C main-effect variants compared to 54% of the apoB main-effect variants, associated with triglycerides ($P < 2.1 \times 10^{-4}$; 0.05/235). For example, the variants at the *ZPR1/APOA5/APOA4/APOC3*, *LPL*, *DOCK1/ANGPTL3*, *TRIB1*, and *GCKR*, loci, all associate with non-HDL-C main-effects, whereas the variant at the *GALNT2* locus associates with apoB main-effect (*Table 3* and see Supplementary material online, *Table S7*). Out of the variants that did not associate with the non-HDL-C/apoB ratio, 36% associated with triglycerides at the same significance level, the variant at the *MLXIPL* locus being one example.

Discussion

Integrating large sets of genetic and phenotypic data demonstrates that the causative relationship between apolipoprotein B-containing lipoproteins and CAD is better explained by non-HDL-C than particle concentration.

While the relative causal contributions of non-HDL-C and apoB to ASCVD have not been reported before, epidemiologic studies have either not found convincing evidence for superior role of either variable in predictions of CAD,[15–17] or have reported stronger disease association of apoB than non-HDL-C.[18–21] However, observational epidemiological studies are inherently limited in their ability to establish causal associations as they are prone to various biases, residual

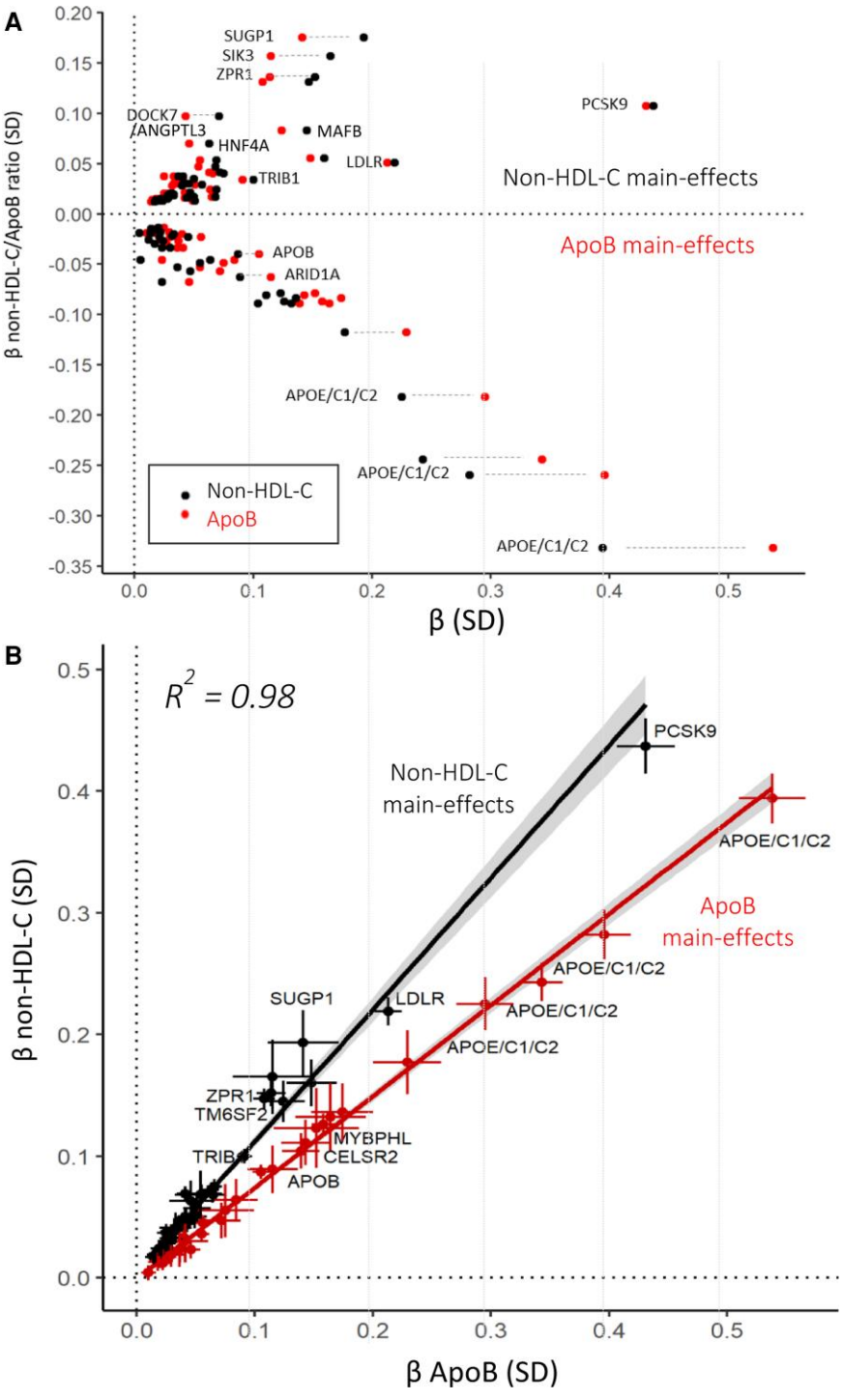


Figure 2 The relationship between variant effects (β) on non-HDL-C and apoB and the effect on non-HDL-C/apoB ratio is shown in panel *A*. The dashed lines indicate which dots belong to the same variant. The relationships between effects (β) on non-HDL-C and apoB for the variant groups with non-HDL-C and apoB main-effects are shown in panel *B*. The lines are the best-fitting lines using weighted regression. A regression model with an interaction term allowing the genetic relationships between non-HDL-C and apoB to differ between variant groups, provides a significant improvement in fitting the data compared with a model without the interaction term ($R^2 = 0.98$ and 90%, respectively).

confounding, and reverse causation,[22] limitations that are largely avoided by the MR approach.[23] The distinction between causal and non-causal associations is important as it may improve choices of therapeutic targets that are put to the test in large and costly clinical trials. The failure of the HDL-C increasing CETP inhibitors to lower ASCVD risk in randomized clinical trials[24,25] demonstrates the

Table 4 The different effects on coronary artery disease conferred by genetically elevated ApoB

		Coronary artery disease meta-analysis (187 451 cases and 793 315 controls)			Coronary artery disease UK biobank (47 292 cases/383 322 controls)		
Genetic instruments used	**Predictor**	**β**	**SE**	***P***	**β**	**SE**	***P***
A	β ApoB	0.48	0.037	4.0×10^{-17}	0.46	0.045	1.1×10^{-13}
B	β ApoB	0.28	0.036	3.9×10^{-9}	0.27	0.036	1.6×10^{-8}
				P-het = 0.0069			*P-het = 0.014*
A	β non-HDL-C	0.42	0.033	1.0×10^{-16}	0.40	0.041	9.8×10^{-13}
B	β non-HDL-C	0.38	0.048	3.1×10^{-9}	0.37	0.047	5.4×10^{-9}
				P-het = 0.56			*P-het = 0.73*

Shown are results of univariate weighted linear regression analyses without an intercept term, assessing the relationship between variant effects on the exposures and those on the outcome, comparing results between two groups of genetic instruments, i.e. those associated with non-HDL-C main-effects (A) and ApoB main-effects (B). Effects (β) on coronary artery disease in logarithm ($\log_e$) of the odds ratio are given for each standard deviation increase in the exposure. SE, standard error.

relevance of this distinction. Despite the robust inverse association of HDL-C with risk of ASCVD in epidemiological studies, MR studies are consistent with results from randomized trials, indicating that HDL-C level does not directly affect the pathogenesis of CAD.[6]

Our study identifies a strong genetic contribution to the cholesterol amount carried by apoB particles, discovering associations between 51 sequence variants and non-HDL-C/apoB ratio at genome-wide significance. It is notable that the variant associations with non-HDL-C/apoB ratio were robust among statin-treated cases with established ASCVD from the FOURIER trial, as well in Icelandic data in which assays used for blood lipid measurements differed from those used for discovery.

The association with non-HDL-C/apoB ratio classifies variants associating with apoB into those with main-effects on non-HDL-C or apoB. Between the two variant groups, each unit increase in genetically elevated apoB corresponds to different increases in levels of non-HDL-C, resulting from the discordant associations on the two traits. We used these characteristics to explore whether the genetic effects on CAD risk are proportional to the effects on apoB or non-HDL-C. The results show that genetically elevated apoB predicts CAD with heterogeneous effect sizes depending on the non-HDL-C levels, whereas using genetically elevated non-HDL-C as the predictor, the effects on CAD risk are consistent between variant groups. These results indicate that for individuals with equal levels of non-HDL-C, the number of apoB particles it is carried on does not influence the development of CAD. The results also indicate that the clinical benefit of lipid lowering therapies may be expected to be proportional to the reduction in non-HDL-C, but not necessarily proportional to the reduction in apoB.

Our main-effect classification of the reported apoB variants[4] seems plausible, considering some of the apoB-associated candidate genes at the reported loci. For example, variants at the *APOB* locus have main-effects on apoB as expected, whereas variants at loci including the *HMGCR*, *ABCG5/8*, and *NPC1L1*, genes involved the synthesis and intestinal absorption of cholesterol, have the expected main-effects on non-HDL-C. The classification of apoB-associated variants provides new insights into the genetic influence on lipoprotein metabolism. For example, the results indicate that elevated triglycerides are more likely to associate with discordantly greater non-HDL-C than apoB, rather than the other way around.

Atherogenic dyslipidemia, the cluster of lipid and lipoprotein abnormalities, including elevated triglycerides, small cholesterol-depleted LDLs, and low HDL-C, is one of the major risk factors for ASCVD in people with type 2 diabetes and metabolic syndrome. Importantly, our genetic analyses do not indicate a different pathogenic contribution of atherogenic particles to CAD among T2D patients, or in the context of altered triglyceride metabolism.

Limitations. While our study addresses whether non-HDL-C apoB particle concentration weigh more in atherogenesis, it does not address the relative strength of non-HDL-C and apoB association with ASCVD in an epidemiological setting. Some epidemiological studies indicate that among people with the same non-HDL-C levels, the ones with higher apoB particle count would be at greater CAD risk.[21] However, our study indicates that higher observed risk would not be due to apoB particle concentration, but because of confounding with other risk factors.

Conclusion: Our MR analyses demonstrate that the contribution of apoB-containing particles to atherogenesis is better captured by non-HDL-C than apoB particle concentration. We show that apoB and non-HDL-C are not clinically equivalent. The results indicate that focusing on LDL-C and/or apoB lipid lowering treatment target goals[2] without considering elevated non-HDL-C levels, would not ensure adequate treatment for substantial proportion of patients. In contrast, the guidance of non-HDL-C target levels are expected to better capture risk related to apoB-containing particles.

Authorship

A.H., H.H., G.Thorl, and D.F.G., contributed to the conception or design of the work.

A.H., G.Thorl, A.S., L.S., G.S., V.T., E.B., V.S., S.G., H.H., J.S., I.O., J.J.T., A.A.R., J.G., M.S.O., A.C., R.L.J., J.D., M.T.B., K.N., K.K., L.N., R.B., C.E., O.B.P., K.B., S.B., D.B.D.S., H.B., S.R.O., P.S., D.O.A., G.Th., U.Th., D.F.G., K.S., and H.H. contributed to the acquisition, analysis, or interpretation of data for the work. A.H. drafted the manuscript. H.H., G.Thorl, D.F.G., A.S., E.B., G.Th., H.B., S.R.O., and K.S. critically revised the manuscript. All gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

Supplementary material

Supplementary material is available at *European Journal of Preventive Cardiology*.

Acknowledgements

We wish to express special gratitude to the participants in the studies from deCODE genetics, UK Biobank, Copenhagen Hospital Biobank, and the Danish Blood Donor Study. We also thank those who worked on generating these resources through data and sample collection, genotyping and analysis.

Funding

This work was supported by NordForsk through the funding to PM Heart, project number 90580. This work was funded by the Innovation Fund Denmark (IFD) under File No. 8114-00033B.

Conflict of interest: The following authors affiliated with deCODE genetics/Amgen, Inc., are employed by the company: A.H., G.Th., A.S., L.S., G.S., V.T., E.B., V.S., S.G., H.H., J.S., P.S., D.O.A., G.T., U.T., D.F.G., K.S, and H.H.

Data availability

The data used in this study are available in the article and in the online supplementary material.

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