Apolipoprotein A-V is a potential target for treating coronary artery disease: evidence from genetic and metabolomic analyses

Dorina Ibi1,2,*, Manon Boot1, Martijn E. T. Dollé2, J. Wouter Jukema3,4, Frits R. Rosendaal1, Constantinos Christodoulides6, Matt J. Neville6,7, Robert Koivula6, Patrick C. N. Rensen8,9, Fredrik Karpe6,7, Raymond Noordam10, and Ko Willems van Dijk1,8,9

1Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands; 2Department of Public Health and Primary Care, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands; 3Department of Cardiology, Leiden University Medical Center, Leiden, The Netherlands; 4Netherlands Heart Institute, Utrecht, The Netherlands; 5Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands; 6Oxford Centre for Diabetes, Endocrinology and Metabolism, Radcliffe Department of Medicine, University of Oxford, Oxford, United Kingdom; 7NIHR Oxford Biomedical Research Centre, Oxford University Hospitals Foundation Trust, Oxford, United Kingdom; 8Division Endocrinology, Department of Internal Medicine, 9Einthoven Laboratory for Experimental Vascular Medicine, and 10Division of Gerontology and Geriatrics, Department of Internal Medicine, Leiden University Medical Center, Leiden, The Netherlands

Abstract Triglyceride (TG)-lowering LPL variants in combination with genetic LDL-C-lowering variants are associated with reduced risk of coronary artery disease (CAD). Genetic variation in the APOA5 gene encoding apolipoprotein A-V also strongly affects TG levels, but the potential clinical impact and underlying mechanisms are yet to be resolved. Here, we aimed to study the effects of APOA5 genetic variation on CAD risk and plasma lipoproteins through factorial genetic association analyses. Using data from 309,780 European-ancestry participants from the UK Biobank, we evaluated the effects of lower TG levels as a result of genetic variation in APOA5 and/or LPL on CAD risk with or without a background of reduced LDL-C. Next, we compared lower TG levels via APOA5 and LPL variation with over 100 lipoprotein measurements in a combined sample from the Netherlands Epidemiology of Obesity study (N = 4,838) and the Oxford Biobank (N = 6,999). We found that lower TG levels due to combined APOA5 and LPL variation and genetically-influenced lower LDL-C levels afforded the largest reduction in CAD risk (odds ratio: 0.78 (0.73–0.82)). Compared to patients with genetically-influenced lower TG via LPL, genetically-influenced lower TG via APOA5 had similar and independent, but notably larger, effects on the lipoprotein profile. Our results suggest that lower TG levels as a result of APOA5 variation have strong beneficial effects on CAD risk and the lipoprotein profile, which suggest apo A-V may be a potential novel therapeutic target for CAD prevention.

Supplementary Key words cardiovascular disease • lipoproteins • triglycerides • lipid-lowering therapy • hyperlipidemia • LDL.

*For correspondence: Dorina Ibi, dibi@lumc.nl.
TG levels (8, 9, 16). Despite playing a crucial role in TG metabolism, the precise mechanism(s) through which apo A-V regulates TG levels remain under debate. Most evidence suggests that apo A-V enhances LPL-dependent TG lipolysis, either directly or indirectly (17, 18). Other hypotheses suggest that apo A-V regulates hepatic VLDL production (18) or facilitates the recognition of VLDL particles by members of the LDL receptor family and heparan sulfate proteoglycans (19, 20), thereby enhancing the clearance of these particles from the circulation.

Previously, factorial Mendelian Randomization analyses showed that genetically-influenced lower plasma TG levels via LPL have additional beneficial effects on reducing CAD risk on top of genetically-influenced lower LDL-C (21). As an important TG regulator, apo A-V could therefore be an interesting additional therapeutic target for CAD prevention. In the present study, we aimed to study APOA5 genetic variation in relation to CAD, as well as the detailed lipoprotein profile, separately and in combination with variation in LPL and LDL-C-lowering through factorial genetic analyses in multiple cohorts.

MATERIALS AND METHODS

Study design and population

In this study, we aimed to: (1) assess the clinical relevance of genetically-influenced lower TG levels via APOA5 and/or LPL variants on top of genetically-influenced lower LDL-C on CAD risk and (2) investigate the mechanisms of apo A-V relative to LPL by estimating the individual and combined associations with metabolomic measures of genetically-influenced lower TG via APOA5 and genetically-influenced lower TG via LPL.

For the first aim, we performed single instrument and factorial genetic association analyses (supplemental Fig. S1–S3) using individual-level data from 309,780 CAD cases and controls in the UK Biobank. The UK Biobank cohort is a prospective general population cohort of 502,628 participants with a median age of 65 years. For the present study, we excluded participants with lipid-lowering drug use (n = 906) and/or missing data on genotype (n = 957). Therefore, the present study population consisted of 4,838 NEO participants. The OBB is a population-based cohort of 7,185 randomly selected individuals aged 40–65 years. For the present study, we included participants with lipid-lowering drug use (n = 927). Therefore, the present study population consisted of 6,999 participants included for the present study.

All included studies received ethical approval by their respective medical ethics committees (NEO was approved by the Leiden University Medical Center, OBB was approved by the Oxfordshire Clinical Research Ethics Committee (08/H0606/107+5), and UK Biobank was approved by the North-West Multi-center Research Ethics Committee), and all participants gave their written informed consent. The studies conformed to the principles outlined in the Declaration of Helsinki. A more detailed description of the included studies, their designs, and the genotyping platforms is provided in supplementary methods and supplemental Table S1.

Genetic instruments and genotype groups

In NEO, OBB, and UK Biobank, we calculated weighted genetic scores for both APOA5 and LPL using TG-lowering alleles. For the APOA5 genetic score, we used two variants (rs662799 and rs3135506; Extended Methods, supplemental Table S2) that comprise most of the variation in the APOA5 locus, are in linkage equilibrium (R^2=0.005), and are strongly associated with TG levels (22). Weights for the GRS calculation were derived from the genome-wide association study on TG levels from the Global Lipids Genetics Consortium (23). Likewise, the LPL genetic score was constructed using variants associated independently with TG levels that were mapped to the LPL gene (rs268, rs301, rs326, rs328, and rs10096633; Extended Methods, supplemental Table S2), which were weighted by their effect on TG levels in the analyses from Global Lipids Genetics Consortium (23). Based on the calculated GRS for APOA5 and LPL, we divided the population based study on the median values of the two GRS resulting in 4 different study groups based on genetically-influenced apo A-V and LPL activity (2 × 2 factorial design, supplemental Fig. S2): (1) reference group (higher TG through APOA5 and LPL), (2) lower TG through LPL only, (3) lower TG through APOA5 only, and (4) lower TG through both APOA5 and LPL.

In UK Biobank, in addition to the APOA5 and LPL genetic scores, we calculated a genetic LDL-C score by extracting from published genome-wide association studies in which the UK Biobank did not contribute the independent lead variants (P < 5 × 10^-8) previously identified in relation to LDL-C levels (188,577 individuals; 15 SNPs, supplemental Table S2) (23). Using the beta estimates of the independent lead variants, we calculated weighted LDL-C genetic risk scores (GRS) per participant. To limit bias by pleiotropy, we did not allow overlap in independent lead variants between LDL-C and the other lipid traits (notably HDL-C and TG) based on a p-value cut-off of 5 × 10^-8. Next, based on the weighted GRS of LDL-C, LPL, and APOA5, we stratified the study population into different groups based on the median values of the three GRS (supplemental Fig. S3).

Study outcomes

Cardiovascular disease outcomes. In UK Biobank, the clinical outcome was CAD. Information on incident CAD was collected through information from the data provided by the NHS record systems. Diagnoses were coded according to the International Classification of Diseases (24). CAD was defined as: angina pectoris (I20), myocardial infarction (I21 and I22), and acute and chronic ischemic heart disease (I24 and I25).

J. Lipid Res. (2022) 63(5) 100193
**NMR-based metabolomic profile.** In NEO and OBB, the primary outcomes were the fasting NMR-based metabolomic measures. In both cohorts, a high-throughput proton NMR-metabolomics platform (25) (Nightingale Health Ltd, Helsinki, Finland) was used to measure 159 metabolic measures (excluding ratios) at the Medical Research Council Integrative Epidemiology Unit at the University of Bristol, Bristol, United Kingdom, which were quantified by Nightingale library. This method provides lipoprotein subclass profiling with lipid concentrations within 14 lipoprotein subclasses. Details of the experimentation and applications of the NMR-metabolomics platform have been described previously (25), as well as representative coefficients of variations for the metabolic biomarkers (26).

In this study, we excluded all ratios, resulting in a final number of 145 NMR-derived metabolic measures present in both NEO and OBB cohort. Values below the detection limit were treated as missing. For all analyses, metabolic measures were inverse rank transformed to obtain normal distributions.

**Statistical analyses**

**Factorial genetic association analyses with CAD risk in the UK Biobank cohort.** We performed three types of genetic analyses on CAD cases and controls in the UK Biobank: 1, single instrument genetic analyses, where each dichotomized genetic score (LDL-C, LPL, and APOA5 GRS) was associated with CAD outcomes, assuming that the other alleles were randomly distributed in the other groups (supplemental Fig. S1); 2, 2 × 2 factorial genetic analyses resulting from three different combinations (LDL-C-lowering and lower TG via LPL alleles, LDL-C-lowering and lower TG via APOA5 alleles, and lower TG via both LPL and APOA5 alleles) (supplemental Fig. S2); 3, 2 × 2 factorial genetic analyses with the combination of the three genetic scores to assess the clinical relevance of lower TG via APOA5 and LPL variants on top of genetically-influenced lower LDL-C (supplemental Fig. S3).

Analyses in UK Biobank were performed in R (Version 3.6.1, the R Project, https://www.r-project.org/) using logistic regression adjusted for age, sex, and the first 10 principal components in unrelated individuals.

**Factorial genetic association analyses with NMR-metabolomics.** Using four “naturally randomized” subgroups based on LPL and APOA5 GRS, we performed linear regression analyses to estimate the associations with NMR-based metabolomic measures between groups using a 2 × 2 factorial design in NEO and OBB separately. These association analyses were adjusted for age, sex, and the first four genomic principal components in unrelated individuals. In stratified analyses, the number of cases and controls varies per genotype group. Values are mean (SD), unless otherwise specified. GRS unit is in SD.

**RESULTS**

**Population characteristics**

The UK Biobank study population investigated herein (Table 1) consisted of 309,780 participants (mean (SD): 56.8 (8.0) years of age at study inclusion), out of which 36,391 were CAD cases. Compared to the controls, the cases had a higher mean age (61.1 (6.4) vs. 56.2 (8.0) years, respectively) and a higher BMI (27.2 (4.7) vs. 26.0 (4.3) kg/m^2 for cases and controls, respectively). In addition, the case group consisted of more male participants than the control group (66 vs. 43%, respectively).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>309,780</td>
<td>36,391</td>
<td>273,389</td>
</tr>
<tr>
<td>Age at inclusion, years</td>
<td>56.8 (8.0)</td>
<td>61.1 (6.4)</td>
<td>56.2 (8.0)</td>
</tr>
<tr>
<td>Sex, % men</td>
<td>46</td>
<td>66</td>
<td>43</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>27.4 (4.8)</td>
<td>29.0 (5.0)</td>
<td>27.2 (4.7)</td>
</tr>
<tr>
<td>TG, total cholesterol, LDL-C, and HDL-C were higher in the NEO cohort than the OBB cohort.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In the NEO and OBB cohorts, the separate results from the NEO and OBB cohorts were meta-analyzed using the fixed-effect model of rmeta package in R. Linear regression analyses were carried out using STATA Statistical Software version 120 (Statacorp, College Station, Texas, USA) and R version 3.6.1 (The R Project, https://www.r-project.org/). The circular plots were designed using Python version 2.7.6 (Python Software Foundation, https://python.org/).
TABLE 2. Characteristics of the NEO and the OBB cohort, as well as their combination

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>NEO</th>
<th>OBB</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>4,838</td>
<td>6,999</td>
<td>11,837</td>
</tr>
<tr>
<td>Age (years)</td>
<td>55.5 (6.0)</td>
<td>41.6 (5.9)</td>
<td>47.3 (5.9)</td>
</tr>
<tr>
<td>Men (%)</td>
<td>42</td>
<td>44</td>
<td>43</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.0 (4.3)</td>
<td>25.8 (4.6)</td>
<td>25.9 (4.3)</td>
</tr>
<tr>
<td>Fasting serum concentrations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG (median (IQR))</td>
<td>0.99 (0.71)</td>
<td>0.93 (0.65)</td>
<td>0.95 (0.67)</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>5.80 (1.01)</td>
<td>5.18 (1.01)</td>
<td>5.43 (1.01)</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>3.66 (0.94)</td>
<td>3.22 (1.26)</td>
<td>3.40 (1.13)</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>1.60 (0.47)</td>
<td>1.38 (0.42)</td>
<td>1.47 (0.44)</td>
</tr>
<tr>
<td>APOA5 GRS (median (IQR))</td>
<td>0.86 (0.00)</td>
<td>0.86 (0.00)</td>
<td>0.86 (0.00)</td>
</tr>
<tr>
<td>LPL GRS (median (IQR))</td>
<td>0.48 (0.24)</td>
<td>0.48 (0.24)</td>
<td>0.48 (0.24)</td>
</tr>
</tbody>
</table>

Values are mean (SD), unless otherwise specified. GRS unit is in SD.

GRS, genetic risk score; IQR, interquartile range; NEO, Netherlands Epidemiology of Obesity; OBB, Oxford Biobank.

The characteristics of the UK Biobank cohort stratified by the dichotomized LDL and APOA5 GRSs are shown in supplemental Table S4. Compared with the reference group (genetically-influenced higher TG via both LPL and APOA5), lower genetically-influenced TG levels via LPL only were associated with altered levels of eight metabolomic measures (particularly higher levels of medium-sized HDL subparticles; Figure 2 and supplemental Table S5) and lower genetically-influenced TG levels via APOA5 only were associated with changed levels of 81 metabolomic measures (particularly lower levels of all sizes of VLDL subparticles; Figure 3 and supplemental Table S5). Despite these observed differences, in general, the effects of the APOA5 and LDL genetic scores on the metabolomic measures showed a moderate overlap $R^2 = 0.68$ (supplemental Fig. S4).

Compared to the same reference group, lower genetically-influenced TG levels via both LDL and APOA5 are associated with the same CAD risk as the genetically lower LDL-C (OR (95% CI): 0.83 (0.79;0.86) vs. 0.83 (0.80;0.86), respectively). The most beneficial effect on CAD risk was observed when genetically-influenced lower TG via both LPL and APOA5 were combined with genetically-lower LDL-C (OR (95% CI): 0.78 (0.73;0.82)).

**Factorial genetic association analyses with CAD risk**

The characteristics of the UK Biobank cohort stratified by genotype group based on the LPL, APOA5, and LDL-C GRSs are shown in supplemental Table S3. Results from factorial genetic analyses with CAD in the UK Biobank are presented in Fig. 1. The group with lower TG via APOA5 and groups with lower TG via LPL had a similar reduced odds ratio for CAD risk (OR (95% CI): 0.95 (0.92;0.97) vs. 0.94 (0.91;0.97), respectively). In addition, the effects of the genetic scores on CAD were also additive based on the comparison between the sum of the individual effects (LPL: OR=0.94; APOA5: OR=0.95) and the effect of both scores combined (both LPL and APOA5: OR=0.89). Based on an approximation of the OR with the risk ratio when the outcome incidence is <10%, the sum of the risk reduction of the individual LPL and APOA5 scores translated into 9%, which was similar to the risk reduction in the group with both genetic exposures (11%). When combined with genetically-lower LDL-C levels, genetically-lower TG via APOA5 were associated with the same CAD risk as the genetically lower TG via LPL (OR (95% CI):0.83 (0.79;0.86) vs. 0.83 (0.80;0.86), respectively).

![Fig. 1. Associations of genotype group with Coronary Artery Disease in the UK Biobank cohort. Values are mean (SD) for LDL-C levels and median (IQR) for TG levels. GRS unit is in SD, CI, Confidence interval; OR, odds ratio; GRS, genetic risk scores.](image-url)
APOA5 were associated with altered levels of 86 metabolomic measures (Fig. 4 and supplemental Table S5). Overall, the effects of these associations showed an additive pattern of the individual associations of genetically-influenced lower TG levels via APOA5 and genetically-influenced lower TG levels via LPL but no evidence for an interaction between these scores ($p$ for interaction $>1.35 \times 10^{-3}$). More specifically, the group with genetically-influenced lower TG levels via both APOA5 and LPL was associated with lower levels of all VLDL subparticles and most LDL subparticles, as well as a lower average VLDL particle size (VLDLD: beta (SE) = $-0.30 (0.03)$, $p = 2.3 \times 10^{-22}$). In line with these results, levels of apolipoprotein B (apoB), total serum cholesterol, cholesterol in VLDL (VLDL-C), and cholesterol in LDL (LDL-C) were also lower (apoB: beta (SE) = $-0.28 (0.03)$, $p = 3.6 \times 10^{-19}$), whereas most HDL subparticles, HDL-C, and ApoA1 were higher (ApoA1: beta (SE) = $0.12 (0.03)$, $p = 2.2 \times 10^{-19}$). In addition, genetically-influenced lower TG levels via both LPL and APOA5 were associated with lower levels of total FAs (beta (SE) = $-0.27 (0.06)$, $p = 9.4 \times 10^{-17}$) and several free FAs (omega-3, omega-6, monounsaturated FAs, polyunsaturated FAs, and short-chain FAs) and with a higher degree of unsaturation. Replication analyses in the UK Biobank cohort confirmed these observations, despite the fact that the metabolomics measurements were done irrespective of fasting status, which likely increased the variability of the measurements (supplemental Fig. S5).

**DISCUSSION**

In this study, exposure to genetically-influenced lower TG levels via APOA5 had additional beneficial effects on CAD risk on top of genetically-influenced lower TG levels via LPL and genetically-influenced lower LDL-C levels. This was further supported by the independent and additive beneficial effects on the lipoprotein profile, of the genetically-influenced lower TG via APOA5 on top of genetically-influenced lower TG via LPL. Therefore, our data suggests that pharmacological TG-lowering therapy via APOA5 may have additional beneficial effects on the lipoprotein profile.
and CAD risk on top of LPL-enhancement therapy as well as LDL-C-lowering therapy.

Previously, it was reported that genetically-influenced lower TG levels through \textit{LPL} and \textit{APOA5} with 145 NMR-based metabolomic measures in 2 × 2 factorial analyses, in the Netherlands Epidemiology of Obesity (NEO) study (n = 4,838) and in the Oxford Biobank (OBB) cohort (n=6,999). Group with genetically-influenced lower TG levels via both \textit{LPL} and \textit{APOA5} compared with the reference group (genetically-influenced higher TG levels via both \textit{LPL} and \textit{APOA5}). Bar heights represent the magnitude of the beta coefficient from linear regression, which is expressed in SD units. Red bars indicate positive betas and blue bars indicate negative betas. The transparency of the bars indicates the level of statistical significance. A \( p < 1.35 \times 10^{-3} \) is regarded statistical significant, as represented by the black dots.

Previously, association studies of \textit{APOA5} variants with lipoprotein subparticles have been performed, although mostly with a less extensive metabolomics panel and limited cohort size. These studies showed the strongest associations of \textit{APOA5} variants with chylomicrons and large VLDLs (32–35), which is in line with the strong associations of lower TG via \textit{APOA5} observed in our study. Guardiola \textit{et al.} showed that the rare TG-increasing alleles the \textit{APOA5} variants used in our study, notably rs3135506 and rs662799, were associated with an atherogenic lipoprotein profile (34). Similarly, in our study, we showed that the TG-lowering alleles of rs3135506 and rs662799 had a lowering effect on the atherogenic TRLs, including mostly VLDL subparticles. In addition, lower TG levels via \textit{APOA5} were associated with lower levels of glycoprotein acetyls, a biomarker for inflammation (36), suggesting that \textit{APOA5} may also play a role in atherogenesis by affecting inflammation. Sarwar \textit{et al.} (33) reported no effect of \textit{APOA5} on LDL, which is partially in concordance with our study, where we showed lower levels of only some of the LDL subparticles.

To our knowledge, the present study is the first showing the effects of lower TG via \textit{APOA5} on an extensive NMR-metabolomic panel and its comparison with lower TG via \textit{LPL}. Overall, the effect sizes of the associations of the \textit{APOA5} alleles were stronger than those of the \textit{LPL} alleles. Nevertheless, the directionality and pattern of these effects largely overlapped. In general, genetically-influenced lower TG levels via \textit{APOA5} were predominantly associated with lower levels of VLDL subparticles and a smaller VLDL particle size and a lower number of particles, as indicated by apoB levels. Total cholesterol and total TG levels were lower in both, as well as total FAs. These associations could be due to enhanced TG hydrolysis, which is further confirmed by the higher levels of HLD subparticles and HDL particle size that result due to increased availability of surface components of TG-rich particles (37). However, these increasing effects on HDL subparticles...
were higher in the group with genetically-influenced lower TG via LPL than the group with genetically-influenced lower TG via APOA5. Except for the HDL subparticles, overall, the effect sizes of the associations with APOA5 were larger than the effect sizes of the associations with LPL. Whether these effects are additional to LPL-dependent TG hydrolysis via other mechanisms, we cannot conclude based on the present findings. In addition to LPL-dependent TG hydrolysis, a role for apo A-V in hepatic VLDL production has been suggested by previous studies in mice (18). In addition to LPL-dependent TG hydrolysis and hepatic VLDL production, studies have shown that apo A-V also facilitates the recognition of TG-rich VLDL particles by the LDL receptor and heparan sulfate proteoglycans, thereby enhancing clearance of these particles (20). These potential other functions of apo A-V, we could not identify nor exclude with our present study design and need to be investigated in future studies. Nevertheless, from these results, we can conclude that LPL and APOA5 are most likely associated with clinical outcomes via the same intermediates.

Several assumptions and limitations of the genetic approach used in this study should be considered when interpreting the results of our study. Mendelian randomization assumes that genetic variants are associated with the outcome only through the exposure of interest so that the results cannot be violated by (directional) pleiotropy. To take this assumption into account, we chose APOA5 variants that are located within the APOA5 gene: rs3135506 in the second exon and rs662799 located 2kb upstream of the APOA5 gene. In addition, it has been previously found that rs3135506, also known as S19W, is a functional SNP that leads to an amino acid change, which subsequently leads to a 50% decrease in secretion, due to diminished translocation of apo A-V across the ER (38). Even though the effect of rs662799 on protein and functional level is less clear, rs662799 is in LD with rs2266788 (R^2=0.77), which has been associated with APOA5 gene expression (39). Although these data support our assumption that the observed effect on CAD via the APOA5 genetic score occurs through apo A-V, we cannot formally exclude the possibility that alternative variants in linkage with variants in our APOA5 GRS are the actual causative variants. Although the potential for such an alternative causative variant seems high given that APOA5 is part of the APOA1-C3-A4-A5 gene locus, such a variant remains to be identified. In addition, from the multitude of associations of the APOA5 genetic score with the NMR profile (Fig. 3), we cannot conclude that the effect on CAD is mediated through the effect of apo A-V on plasma TG. As such, this analysis is not a proper Mendelian randomization analysis testing the causative effect of TG on CAD. Similarly, the LPL genetic score comprised variants that were in or within 10 kb of the LPL gene itself and were either coding variants associated with LPL function or significant expression quantitative trait loci (40, 41). This makes it likely that the genetically-influenced lower TG via the LPL genetic score truly resulted through LPL. But similar to APOA5, the LPL GRS is associated with a multitude of metabolites in the NMR profile (Fig. 2). Furthermore, we attempted to minimize possible pleiotropic effects of the LDL-C genetic score by including variants associated with LDL-C only, hence without associations to other lipid traits. Another potential limitation of our study is the inclusion of only two variants in the APOA5 score, which in combination with a lower allele frequency could potentially lead to an underestimated effect estimate. Finally, our data are pertinent only to European populations, given that all the analyses in the NEO, OBB, and UK Biobank were performed in participants of European decent.

In summary, our study showed that genetically-influenced lower TG via APOA5 have additional beneficial effects on CAD risk and lipoprotein profile, which were independent from and comparable to the effects of genetically-influenced lower TG via LPL alleles. Altogether, these results indicate that apo A-V is a potential novel therapeutic target for CAD prevention to be explored in detail in future studies.

Data availability
Processed data for every figure described in the article are contained within the article and the supplementary materials. Because of consent issues, we cannot make the individual data of study participants available to other researchers for purposes of reproducing the results or replicating the procedure. 

Supplemental data
This article contains supplemental data (21–24, 28, 38, 42–48).

Acknowledgments
We express our gratitude to all individuals who participated in the Netherlands Epidemiology of Obesity study and Oxford Biobank. We are grateful for all participating general practitioners for inviting eligible participants. We furthermore thank P.R. van Beelen and all research nurses for collecting the data, P.J. Noordijk and her team for sample handling and storage, and I. de Jonge, MSc for data management of the NEO study. The authors also thank Alexander Blauw for writing the Python script to design the circular figures. The NEO study is supported by Leiden University Medical Center and by the Leiden University, Research Profile Area ‘Vascular and Regenerative Medicine’. The authors acknowledge the support from The Netherlands Cardiovascular Research Initiative: an initiative with support of the Dutch Heart Foundation (CVON2014-02 ENERGISe). The Oxford Biobank is supported by the NIHR Oxford BRC Obesity and Lifestyle theme.

Author contributions

Author ORCIDs
Dorina Ibi https://orcid.org/0000-0003-0908-4039
Raymond Noordam https://orcid.org/0000-0001-7801-809X
Ko Willems van Dijk https://orcid.org/0000-0002-2172-7394

Funding and additional information
R. N. was supported by an innovation grant from the Dutch Heart Foundation [grant number 2019T103]. F. K. is supported by British Heart Foundation. This research was partly conducted using data from the UK Biobank study under Application Number 56340 to R. N.

Conflict of interest
The authors declare that they have no conflicts of interest with the contents of this article.

Abbreviations
apo A-V, apolipoprotein A-V; apo B, apolipoprotein B; GRS, genetic risk scores; NEO, Netherlands Epidemiology of Obesity; OBB, Oxford Biobank.


REFERENCES


