# Genetics and life course epidemiology of cardiometabolic disease: towards personalized medicine

Ibi, D.

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

**Ibi. Apo A-V as a potential therapeutic target for CAD prevention**

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## ABSTRACT

**Background and objectives:** Triglyceride (TG)-lowering *LPL* variants in combination with genetic low-density lipoprotein cholesterol (LDL-C)-lowering are associated with reduced coronary artery disease (CAD) risk. Genetic variation in the *APOA5* gene encoding apolipoprotein AV (apo A-V) strongly affects TG levels, but the potential clinical impact and underlying mechanisms relative to LPL are yet to be resolved. Here, we aimed to study the effects of *APOA5* genetic variation on CAD risk and plasma lipoproteins, separately and in combination with variation in *LPL* and LDL-C through factorial genetic association analyses.

**Methods:** Using factorial analyses in 309,780 European-ancestry participants from the UK Biobank, we evaluated the effects of genetically-influenced lower TG levels via *APOA5* and/or *LPL* with or without a background of genetically-influenced lower LDL-C levels on CAD risk. Next, we associated the genetically-influenced lower TG levels via *APOA5* and *LPL* with over 100 lipoprotein measures in a combined sample from the Netherlands Epidemiology of Obesity study (N=4,838) and the Oxford BioBank (N=6,999).

**Results:** Exposure to genetically-influenced lower TG levels via *APOA5* on top of exposure to genetically-influenced lower TG via *LPL* and genetically-influenced lower LDL-C levels provided the largest reduction in CAD risk (OR: 0.78 (0.73–0.82)). Compared to genetically-influenced lower TG via *LPL*, genetically-influenced lower TG via *APOA5* had similar and independent effects on the lipoprotein profile, but notably larger effect sizes.

**Conclusion:** Our results suggest that lower TG via *APOA5* have additional beneficial effects on CAD risk and the lipoprotein profile, which make apo A-V a potential novel therapeutic target for CAD prevention.

## INTRODUCTION

Current guidelines for coronary artery disease (CAD) prevention focus on statins as the first-line treatment aimed at reducing low-density lipoprotein cholesterol (LDL-C). However, statins reduce cardiovascular risk by only approximately 20-30% (1,2). In addition to LDL-C, elevated levels of triglycerides (TG) and TG-rich lipoproteins (TRLs) have emerged as independent and causal risk factors for CAD (3–5).

Numerous genes have been linked to TG metabolism, among which *LPL*, which encodes the lipoprotein lipase (LPL), has been shown to be play a major role (6,7). In addition to *LPL*, *APOA5*, encoding apolipoprotein A-V (apo A-V), is an important determinant of plasma TG levels (8–10). Apo A-V is mainly expressed in the liver and is present on and is exchanged between TRLs and high-density lipoprotein cholesterol (HDL-C) (11,12). Despite its low plasma concentration (≈150 ng/mL) compared with other apolipoproteins, apo A-V appears to be a potent regulator of circulating TG levels (13). *In-vivo* experiments found that mice overexpressing human *APOA5* had 66% lower plasma TG levels compared with controls, primarily due to a lower TG content in VLDL particles (14,15). Reciprocally, *APOA5* knockout mice had a four-fold increase in plasma TG levels (14), and resembled apo A-V deficient patients exhibiting type V familial hyperlipoproteinemia (10,11). Furthermore, genome-wide association studies have identified rare and common variants in the *APOA5* locus to be associated with 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. (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 (Supplementary figure 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 aged 40 to 70 years from across the United Kingdom. For the present study, we restricted the analyses to the UK Biobank participants who reported to be of European ethnicity, were unrelated (based on the availability of kinship data), and were present in the full release imputed genotyped datasets (N=309,780).

For the second aim, we used individual-level genetic data including 11,837 participants from a combined cross-sectional cohort of the Netherlands Epidemiology of Obesity (NEO) and the Oxford BioBank (OBB) study to perform genetic association analyses in 2 × 2 factorial design. The NEO study is a population-based prospective cohort study of 6,671 men and women aged 45 to 65 years. For the present study, we excluded participants with lipid-lowering drug use ($n$ = 906) and/or missing data on genotype ($n$ = 927). Therefore, the present study population consisted of 4,838 NEO participants. The OBB is a population-based cohort of 7,185 randomly selected healthy participants aged 30 to 50 years from Oxfordshire (UK). Individuals with a history of myocardial infarction, diabetes mellitus, heart failure, untreated malignancy, other ongoing systemic diseases or ongoing pregnancy were not eligible for study inclusion. Participants with lipid-lowering drug use and missing genotype data were excluded, which resulted in a total of 6,999 participants included for the current study.

All included studies received ethical approval by their respective medical ethics committees (NEO was approved by the medical ethics committee of the Leiden University Medical Center (LUMC), 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 (MREC)), 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 Supplementary table 1.

## 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, Supplementary table 2) that comprise most of the variation in the *APOA5* locus, are in linkage equilibrium ($R^2$=0.003), 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 (GLGC) (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, Supplementary table 2), which were weighted by their effect on TG levels in the analyses from GLGC (23). Based on the calculated GRS for *APOA5* and *LPL*, we divided the study population based on the median values of the two GRS resulting in 4 different study groups based on genetically-influenced apo A-V and LPL activity (2x2 factorial design, Supplementary figure 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 *($p < 5 \times 10^{-8}$)* previously identified in relation to LDL-C levels (188 577 individuals; 15 SNPs, Supplementary table 2) (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 $5 \times 10^{-8}$. 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 (Supplementary figure 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 (ICD) (24). CAD was defined as: angina pectoris (I20), myocardial infarction (I21 and I22), and acute and chronic ischemic heart disease (I24 and I25).

### *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 nuclear magnetic resonance (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 (MRC IEU) 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 (CVs) 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 Supplementary figure 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) (Supplementary figure S2); 3) 2 × 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 (Supplementary figure 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 to correct for possible population stratification within the separate study samples. In addition, we included in the regression model an additive interaction term by using a product term between the continuous *LPL* and *APOA5* genetic scores to test whether they had additive effects on the NMR-based metabolomics measures. Finally, these analyses were also performed for replication purposes using non-fasting NMR-based metabolomics measures in the UK Biobank cohort.

All analyses in the NEO and OBB cohort were adjusted for multiple testing, dividing the alpha by 37, as this was the number of independent metabolic measures in our study. The number of independent biomarkers was determined using the method by Li and Ji (27). Associations were considered to be statistically significant in case the *p* value was below $1.35 \times 10^{-3}$ (i.e. 0.05/37). All results for the NEO cohort were based on analyses weighted towards the reference BMI distribution of the general Dutch population, and therefore apply to a population-based study without oversampling of individuals with overweight or obesity. A more detailed description of the weighting can be found elsewhere (28).

Finally, the separate results from the NEO and the 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 12.0 (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/).

# RESULTS

## 2 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) versus 56.2 (8.0) years, respectively) and a higher BMI (29.0 (5.0) and 27.2 (4.7) kg/m2 for cases and controls, respectively). In addition, the case group consisted of more male participants compared to the control group (66 versus 43 %, respectively).

**Table 1** Characteristics of the UK Biobank total study population , as well as stratified in cases and controls

| Characteristics | Total | Cases | Controls |
|---|---|---|---|
| Number of participants | 309,780 | 36,391 | 273,389 |
| Age at inclusion, years | 56.8 (8.0) | 61.1 (6.4) | 56.2 (8.0) |
| Sex, % men | 46 | 66 | 43 |
| BMI (kg/m2) | 27.4 (4.8) | 29.0 (5.0) | 27.2 (4.7) |
| *Fasting serum concentrations (mmol/L)* | | | |
| TG (median (IQR)) | 1.49 (1.1) | 1.72 (1.25) | 1.46 (1.08) |
| Total cholesterol | 5.71 (1.14) | 5.25 (1.28) | 5.77 (1.11) |
| LDL-cholesterol | 3.47(0.87) | 3.27 (0.97) | 3.61 (0.85) |
| HDL-cholesterol | 1.45 (0.38) | 1.30 (0.35) | 1.47 (0.38) |
| LDL-C GRS (median (IQR)) | 0.41 (0.30) | 0.39 (0.30) | 0.41 (0.30) |
| [a]*LPL* GRS (median (IQR)) | 0.09 (0.20) | 0.09 (0.20) | 0.09 (0.23) |
| *APOA5* GRS (median (IQR)) | 0.86 (0.00) | 0.86 (0.00) | 0.86 (0.00) |

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.

BMI, body mass index; GRS, genetic risk score; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; TG, triglycerides

[a]Due to unavailability of rs301, the *LPL* GRS for the UK BioBank was calculated based on five variants (rs268, rs326, rs328 and rs10096633) versus the six variants (rs268, rs301, rs326, rs328 and rs10096633) used in the NEO and OBB cohorts.

Characteristics of the NEO study population (N=4,838) and OBB cohort (N=6,999), as well as of the combined population are summarized in Table 2. Compared to participants from NEO, OBB participants had a lower mean age (41.6 (5.9) versus 55.5 (6.0) years, respectively), but a similar mean BMI (25.8 (4.6) and 26.0 (4.3) kg/m$^2$ for OBB and NEO, respectively). Levels of TG, total cholesterol, LDL-C and HDL-C were higher in the NEO cohort compared to the OBB cohort.

**Table 2** Characteristics of the NEO and the OBB cohort, as well as their combination

| Characteristics | NEO [a] | OBB | Total[b] |
|---|---|---|---|
| Number of participants | 4,838 | 6,999 | 11,837 |
| Age (years) | 55.5 (6.0) | 41.6 (5.9) | 47.3 (5.9) |
| Men (%) | 42 | 44 | 43 |
| BMI (kg/m$^2$) | 26.0 (4.3) | 25.8 (4.6) | 25.9 (4.5) |
| *Fasting serum concentrations (mmol/L)* | | | |
| TG (median (IQR)) | 0.99 (0.71) | 0.93 (0.65) | 0.95 (0.67) |
| Total cholesterol | 5.80 (1.01) | 5.18 (1.01) | 5.43 (1.01) |
| LDL-cholesterol | 3.66 (0.94) | 3.22 (1.26) | 3.40 (1.13) |
| HDL-cholesterol | 1.60 (0.47) | 1.38 (0.42) | 1.47 (0.44) |
| *APOA5* GRS (median (IQR)) | 0.86 (0.00) | 0.86 (0.00) | 0.86 (0.00) |
| *LPL* GRS (median (IQR)) | 0.48 (0.24) | 0.48 (0.24) | 0.48(0.24) |

Values are mean (SD), unless otherwise specified. GRS unit is in SD.
BMI, body mass index; GRS, genetic risk score; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; TG, triglycerides.
[a]In NEO, results are based on analyses weighted towards the reference BMI distribution of the general Dutch population.
[b]The total represents averaged results from the individual analyses in NEO and OBB cohort

## 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 GRS are shown in Supplementary table 3. Results from factorial genetic analyses with CAD in the UK Biobank are presented in Figure 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) versus 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 RR 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-influenced lower LDL-C levels, genetically-influenced 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) versus 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-influenced lower LDL-C (OR (95% CI): (0.78 (0.73;0.82)).

| Genotype Category | Proxy for | TG levels (mmol/L) | LDL-C levels (mmol/L) | OR(95% CI) | Cases/Controls |
|---|---|---|---|---|---|
| *Single instrument genetic analyses* | | | | | |
| Higher LDL-C only | Reference | 1.48(1.10) | 3.46 (0.83) | 1 | 19,097/135,683 |
| Lower LDL-C only | LDL-C-lowering therapy only | 1.50(1.12) | 3.68 (0.89) | 0.88(0.86-0.90) | 17,294 /137,506 |
| Higher TG via *LPL* only | Reference | 1.42(1.04) | 3.56 (0.86) | 1 | 26,091/192,940 |
| Lower TG via *LPL* only | LPL-enhancing therapy only | 1.52(1.13) | 3.57(0.87) | 0.94(0.91-0.97) | 103,00/80,449 |
| Higher TG via *APOA5*only | Reference | 1.44(1.05) | 3.46 (0.83) | 1 | 8,586/62,388 |
| Lower TG via *APOA5*only | Apo A-V-enhancing therapy only | 1.67(1.29) | 3.61 (0.89) | 0.95(0.92-0.97) | 27,805/211,001 |
| *2x2 genetic analyses* | | | | | |
| Higher TG via *LPL*and higher LDL-C | Reference | 1.40(1.03) | 3.45 (0.82) | 1 | 13,711/96,022 |
| Lower TG via *LPL*and lower LDL-C | LPL-enhancing therapy and LDL-C-lowering therapy | 1.54(1.15) | 3.56(0.86) | 0.83(0.80-0.86) | 4,914/40,588 |
| Higher TG via *APOA5*and higher LDL-C | Reference | 1.43(1.04) | 3.44 (0.82) | 1 | 4,502/30,753 |
| Lower TG via *APOA5*and lower LDL-C | Apo A-V-enhancing therapy and LDL-C-lowering therapy | 1.68(1.30) | 3.72(0.92) | 0.83(0.79-0.86) | 13,210/105,871 |
| Higher TG via both *LPL* and *APOA5* | Reference | 1.38(0.98) | 3.55 (0.85) | 1 | 6,168/44,071 |
| Lower TG via both *LPL* and *APOA5* | LPL- and Apo A-V-enhacning therapy | 1.70(1.31) | 3.62 (0.89) | 0.89(0.85-0.93) | 7,882/62,132 |
| *2x2x2 genetic analyses* | | | | | |
| Higher TG via both *APOA5* and *LPL* and higher LDL-C | Reference | 1.36(0.98) | 3.43 (0.81) | 1 | 3,252/21,762 |
| Lower TG via both *APOA5* and *LPL* and lower LDL-C | LPL- and Apo A-V-enhacning therapy and LDL-C-lowering therapy | 1.71(1.33) | 3.73 (0.92) | 0.78(0.73-0.82) | 3,746/31,262 |


**Figure 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.
Confidence interval; CAD, coronary artery disease; LDL-C, low-density lipoprotein cholesterol, OR, odds ratio; TG, triglycerides

## Factorial Analyses with NMR-based metabolomic measures

The characteristics of the combined population of NEO and OBB cohorts stratified by the dichotomized *LPL* and *APOA5* GRS are shown in Supplementary table 4. 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 8 metabolomic measures (particularly higher levels of medium-sized HDL sub-particles; Figure 2 and Supplementary table 5), 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 sub-particles; Figure 3 and Supplementary table 5). Despite these observed differences, in general the effects of the *APOA5* and *LPL* genetic scores on the metabolomic measures showed a moderate overlap $R^2 = 0.68$; Supplementary figure S4).

Compared to the same reference group, lower genetically-influenced TG levels via both *LPL* and *APOA5* were associated with altered levels of 86 metabolomic measures (Figure 4 and Supplementary table 5). 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 sub-particles and most LDL sub-particles, as well as a lower average VLDL particle size (VLDLD: beta (SE) = -0.30 (0.03), $p = 2.3 \times 10^{-23}$). 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 sub-particles,

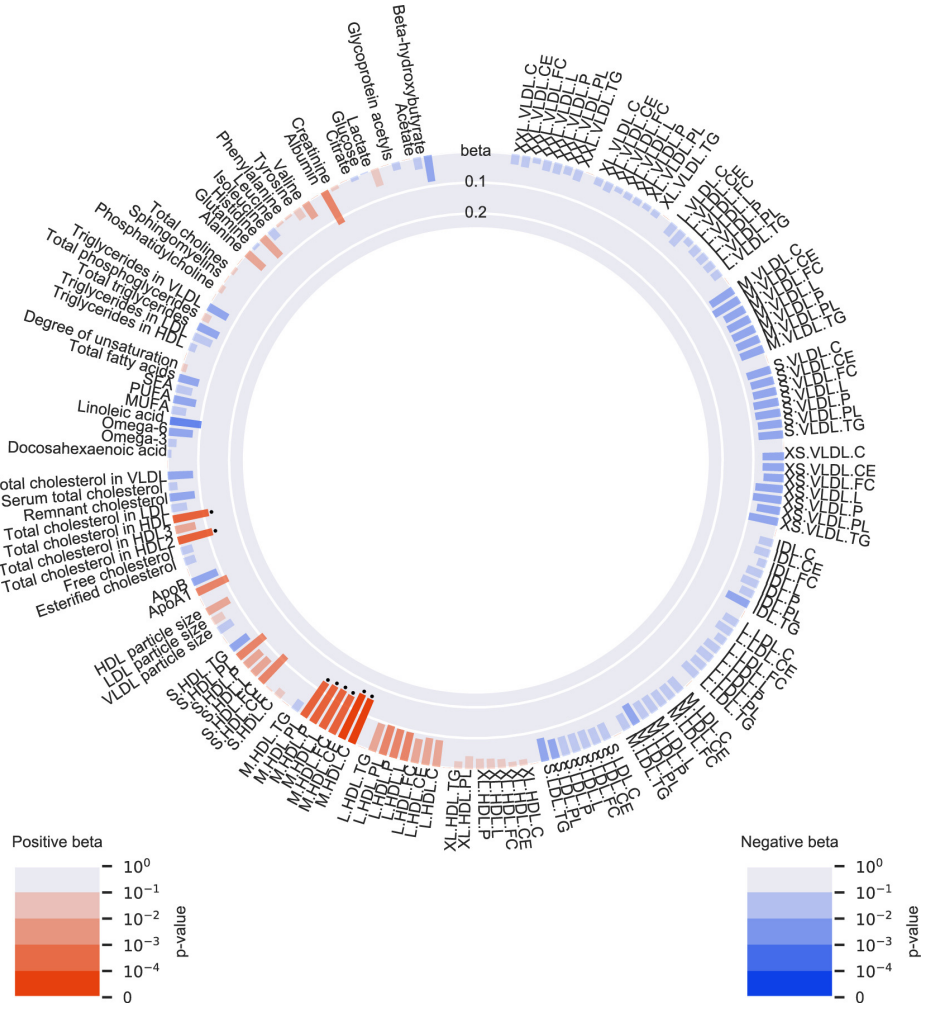


**Figure 2: Associations of the group with genetically-influenced lower TG levels via *LPL* with 145 NMR-based metabolomic measures in 2 × 2 factorial analyses, in the Netherlands Epidemiology of Obesity (NEO) study (n = 4,838) and in the Oxford Biobank (OBB) cohort (n=6,999).** Group with genetically- influenced lower TG levels via *LPL* compared with the reference group (genetically-influenced higher TG levels via both *LPL* and *APOA5*). Bar heights represent the magnitude of the beta coefficient from linear regression, which is expressed in standard deviation (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 x $10^{-3}$ is regarded statistical significant, as represented by the black dots.

HDL-C and ApoA1 were higher (ApoA1: beta (SE) = 0.12 (0.03), $p$ = 2.2 x $10^{-04}$). 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 x $10^{-17}$) and several free FAs (omega-3, omega-6, monounsaturated FAs, polyunsaturated FAs and short-

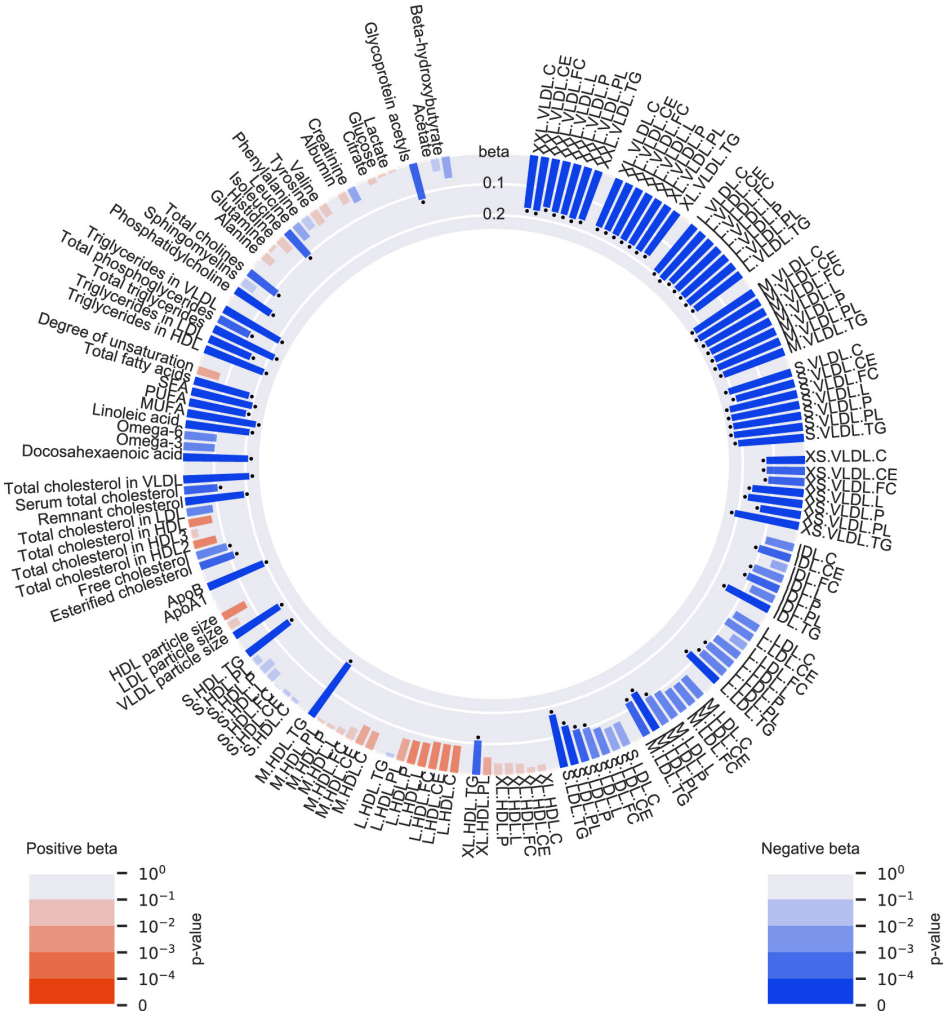


**Figure 3: Associations of the group with genetically-influenced lower TG levels via *APOA5* with 145 NMR-based metabolomic measures in 2 × 2 factorial analyses, in the Netherlands Epidemiology of Obesity (NEO) study (n = 4,838) and in the Oxford Biobank (OBB) cohort (n=6,999).** Group with genetically-influenced lower TG levels via *APOA5* compared with the reference group (genetically-influenced higher TG levels via both *LPL* and *APOA5*). Bar heights represent the magnitude of the beta coefficient from linear regression, which is expressed in standard deviation (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 x $10^{-3}$ is regarded statistical significant, as represented by the black dots.

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 (Supplementary figure S5).

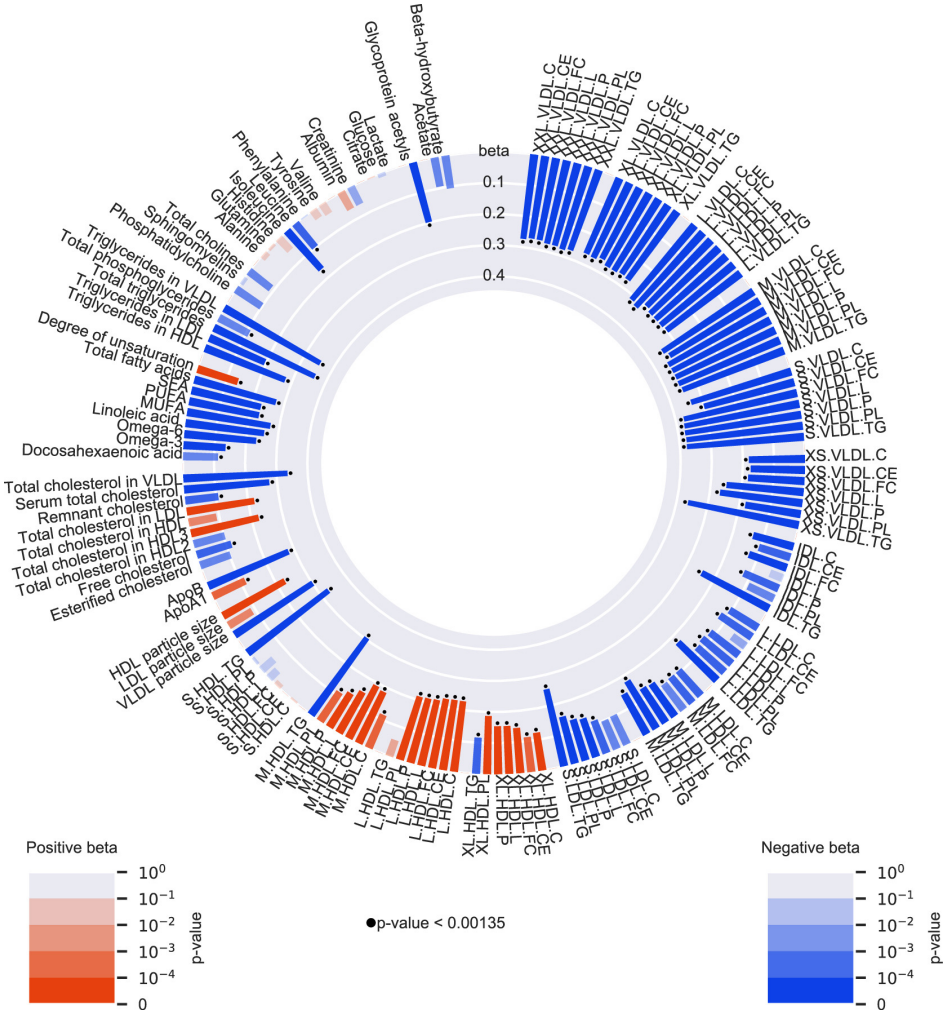


**Figure 4: Associations of the group with genetically-influenced lower TG levels via both *LPL* and *APOA5* with 145 NMR-based metabolomic measures in 2 × 2 factorial analyses, in the Netherlands Epidemiology of Obesity (NEO) study (n = 4,838) and in the Oxford Biobank (OBB) cohort (n=6,999).** Group with genetically-influenced lower TG levels via both *LPL* and *APOA5* compared with the reference group (genetically-influenced higher TG levels via both *LPL* and *APOA5*). Bar heights represent the magnitude of the beta coefficient from linear regression, which is expressed in standard deviation (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 x $10^{-3}$ is regarded statistical significant, as represented by the black dots.

## 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 through *LPL* have an additive lowering effect on CAD risk on top of genetically-influenced lower LDL-C (21,29), which were confirmed by the beneficial effects of this combination on the lipoprotein profile recently shown by our group (30). The results from the current study extend these findings by suggesting that genetically-influenced lower TG via *APOA5* have similar beneficial effects on CAD risk and the lipoprotein profile as genetically-influenced lower TG via *LPL*. Collectively, genetically-influenced lower TG through *APOA5* and genetically-influenced lower TG through *LPL* were associated with an additively improved lipoprotein profile and CAD risk. More importantly, exposure to genetically-influenced lower TG levels via *APOA5* gave an additional reduction in primary CAD risk on top of exposure to genetically-influenced lower TG via *LPL* and genetically-influenced lower LDL-C levels. Data from other MR studies have shown that particularly apoB may be the key trait accounting for the relationship between lipoproteins and CAD (29,31). Since in our study both the *APOA5* and *LPL* genetic scores were associated with lower levels of VLDL sub-particles and the LDL-C genetic score with lower levels of LDL sub-particles, these all translated to lower levels of apoB. Thus, the observed reduction in CAD might be explained by lower levels of apoB, which was indeed the lowest in the group with the three genetic exposures. Altogether, these data suggest that apo A-V might be an attractive therapeutic target for additional treatment to reduce CAD risk. This opens up a novel avenue for the development of potentially effective drugs in CAD prevention, which is of high importance given the residual risk that remains in patients already on statin therapy (1,2). One feasible approach, given the small size of the apo A-V (39 Kda), may be an *APOA5* expression construct targeted to muscle or liver.

Previously, association studies of *APOA5* variants with lipoprotein sub-particles have been performed, although mostly with a less extensive metabolomics panel and limited cohort size. These studies showed the strongest associations of *APOA5* variants with chylomicrons and large VLDLs (32–35), which is in line with the strong associations of lower TG via *APOA5* observed in our study. Guardiola et al. showed that the rare TG-increasing alleles the *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

sub-particles. In addition, lower TG levels via *APOA5* were associated with lower levels of glycoprotein acetyls, a biomarker for inflammation (36), suggesting that *APOA5* may also play a role in atherogenesis by affecting inflammation. Sarwar et al. (33) reported no effect of *APOA5* on LDL, which is partially in concordance with our study, where we showed lower levels of only some of the LDL sub-particles.

To our knowledge, the present study is the first showing the effects of lower TG via *APOA5* on an extensive NMR-metabolomic panel, and its comparison with lower TG via *LPL*. Overall, the effect sizes of the associations of the *APOA5* alleles were stronger compared to those of the *LPL* alleles. Nevertheless, the directionality and pattern of these effects largely overlapped. In general, genetically-influenced lower TG levels via *APOA5* were predominantly associated with lower levels of VLDL sub-particles 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 fatty acids. These associations could be due to enhanced TG hydrolysis, which is further confirmed by the higher levels of HLD sub-particles and HDL particle size that result due to increased availability of surface components of TG-rich particles (37). However, these increasing effects on HDL sub-particles were higher in the group with genetically-influenced lower TG via *LPL* compared to the group with genetically-influenced lower TG via *APOA5*. Except for the HDL sub-particles, 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 up-

stream 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 (Figure 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 (Figure 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 manuscript are contained within the manuscript 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.

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# SUPPLEMENTAL MATERIAL

## EXTENDED METHODS

### 1 Included studies and genotyping platforms

#### *1.1 The Netherlands Epidemiology of Obesity Study*

##### *1.1.1 Study Population*

This study is part of the Netherlands Epidemiology of Obesity (NEO) study, a population-based prospective cohort study of men and women aged 45 to 65 years. All inhabitants from the greater area of Leiden, The Netherlands, with a self-reported body mass index (BMI) of 27 kg/m$^2$ or higher were eligible to participate. Moreover, inhabitants from a nearby municipality (Leiderdorp, The Netherlands) in the same age group were asked to participate regardless of their BMI, thereby forming a population with a reference BMI distribution. Between September 2008 and September 2012, a total of 6,671 participants were included in the study.

Participants visited the NEO study centre for extensive physical examination. Research nurses used medication inventory to record current medication use. Prior to the study visit, participants completed questionnaires at home with respect to demographic, lifestyle, and clinical information. After an overnight fast of at least 10 hours, fasting blood samples were taken at the NEO study centre.

The NEO study was approved by the medical ethics committee of the Leiden University Medical Center (LUMC), and all participants gave their written informed consent. The study conformed to the principles outlined in the Declaration of Helsinki. Detailed information about the study design and data collection has been described elsewhere[1].

##### *1.1.2 Genotyping and imputation*

DNA was isolated from venous blood samples. Genotyping was performed using the Illumina HumanCoreExome-24 BeadChip (Illumina Inc., San Diego, California, United States of America). In the process of quality control, participants were excluded when 1) the sample call rate was <98%, 2) there was a sex mismatch, 3) heterozygosity rate was not within ±3 SD of mean heterozygosity rate, 4) participants widely diverged based on the first two principal components (PCs) (±3.5 SD), 5) samples were duplicates, and 6) concordance with another DNA sample was >0.25 (related

individuals). Genetic variants were excluded when 1) genotype call rate was <98%, and 2) variants were not in Hardy-Weinberg equilibrium (p-value $<1\times10^{-6}$). Detailed quality control steps have been described elsewhere[2]. Subsequently, genotypes were imputed to the 1000 Genome Project reference panel[3] (v3 2011) using IMPUTE (v2.2) software[4].

### *1.2 Oxford Biobank (OBB) Study*

#### *1.2.1 Study population*

The OBB is a population-based cohort of randomly selected healthy participants aged 30 to 50 years from Oxfordshire (UK). Individuals with a history of myocardial infarction, diabetes mellitus, heart failure, untreated malignancy, other ongoing systemic diseases or ongoing pregnancy were not eligible for study inclusion. Participants were included between 1999 and May 2015. The OBB cohort comprises 7,185 individuals. A more detailed description of the study recruitment criteria and population characteristics is reported elsewhere[5].

#### *1.2.2 Genotyping*

For each OBB participant, 35 mL aliquots of whole blood were collected and frozen at -80°C for isolation of genomic DNA. Genotyping was performed using the Illumina Infinium Human Exome Beadchip 12v1 array platform for the first consecutive 5900 DNAs, and Affymetrix UK Biobank Axiom Array chip on the first consecutive 7500 participants[5]. In total 6,999 genotyped participants were included in the current study.

### *1.3 UK Biobank cohort*

#### *1.3.1 Study Population*

The UK Biobank cohort is a prospective general population cohort. Baseline assessments took place between 2006 and 2010 in 22 different assessment centers across the United Kingdom[6]. A total of 502,628 participants between the age of 40 and 70 years were recruited from the general population. Invitation letters were sent to eligible adults registered to the National Health Services (NHS) and living within a 25 miles distance from one of the study assessment centers. The UK Biobank study was approved by the North-West Multi-center Research Ethics Committee (MREC). Access for information to invite participants was approved by the Patient Information Advisory Group (PIAG) from England and Wales. All participants in the UK Biobank study provided written informed consent. The project was completed under project number 56340.

### *1.3.2 Genotyping and genetic imputations in UK Biobank*

UK Biobank genotyping was conducted by Affymetrix using a bespoke BiLEVE Axium array for approximately 50,000 participants; the remaining participants were genotyped using the Affymetrix UK Biobank Axiom array. All genetic data were quality controlled centrally by UK Biobank resources. More information on the genotyping processes can be found online (https://www.ukbiobank.ac.uk).

Based on the genotyped SNPs, UK Biobank resources performed centralized imputations on the autosomal SNPs using the UK10K haplotype[7], 1000 Genomes Phase 3 [3] and Haplotype Reference Consortium reference panels[8]. 2

## 2 Selection of genetic variants

For this study, in both NEO and OBB we calculated two independent weighted *APOA5* and *LPL* genetic scores using TG-lowering alleles. Three common haplotypes comprise more than 95% of all variation of *APOA5* within the population and are defined by five different variants in the *APOA5* locus: rs3135506, rs662799, rs651821, rs2072560 and rs2266788 [9,10]. Since the last four variants are in full linkage disequilibrium, genotypes of rs3135506 and one of the four linkage disequilibrium variants are sufficient to define the three *APOA5* haplotypes. For this study we used the TG-lowering alleles of rs3135506 (rs3135506-G) and rs662799 (rs662799-A), which have been shown to be strongly associated with TG[10,11]. For each participant, we calculated weighted *APOA5* genetic TG score by summing the number of TG-lowering alleles for each variant, weighted by their effect on TG levels in the analyses of the Global Lipids Genetics Consortium (GLGC)[12]. Likewise, the *LPL* genetic score was constructed using variants associated with TG levels that were mapped to the *LPL* gene (rs268, rs301, rs326, rs328 and rs10096633), which were weighted by their effect on TG levels in the analyses of the GLGC[12]. All variants were independently and strongly associated with TG. More details on the selection of these *LPL* variants are described by Lotta et al[13]. In UK Biobank, in addition to the *APOA5* and *LPL* genetic scores calculated as described above, we extracted, from published genome-wide association studies in which the UK Biobank did not contribute, the independent lead variants (p-value<$5x10^{-8}$) previously identified in relation LDL-C levels (188,577 individuals; 15 SNPs)[12]. Using the beta estimates of the independent lead variants, we calculated weighted genetic risk scores per participant. To limit bias by pleiotropy, we did not allow overlap in independent lead variants between LDL-C and the other lipid traits.

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**Supplementary table 1**. Included Studies and Genotyping Platforms

| Study | Total No. Participants included | Genotyping Platform |
|---|---|---|
| NEO | 4,838 | Illumina HumanCoreExome-24 BeadChip |
| OBB | 6,999 | Illumina Infinium Human Exome Beadchip 12v1<br>&<br>Affymetrix UK Biobank Axiom Array |
| UK Biobank | 309,780 | Affymetrix UK Biobank Axiom array |

Abbreviations: NEO, Netherlands Epidemiology of Obesity; OBB, Oxford BioBank;

**Supplementary table 2** List of five triglyceride-lowering *APOA5* and *LPL* variants and LDL-C lowering variants via 19 genetic genetic regions investigated in this study

| Genetic score | SNP | Gene | EA[a]/NEA | EAF | Phenotype | Effect size (SD units) | SE | Reference[b] |
|---|---|---|---|---|---|---|---|---|
| Lower TG via *APOA5* | | | | | | | | |
| | rs662799 | *APOA5* | A/G | 0.92 | INT-triglycerides | -0.2474 | 0.0071 | 24097068 |
| | rs3135506 | *APOA5* | G/C | 0.94 | INT-triglycerides | -0.1837 | 0.0096 | 24097068 |
| Lower TG via *LPL* | | | | | | | | |
| | rs10096633 | *LPL* | T/C | 0.13 | INT-triglycerides | -0.1471 | 0.0050 | 24097068 |
| | rs301 | *LPL* | C/T | 0.24 | INT-triglycerides | -0.1089 | 0.0039 | 24097068 |
| | rs326 | *LPL* | G/A | 0.31 | INT-triglycerides | - 0.0869 | 0.0050 | 24097068 |
| | rs328 | *LPL* | G/C | 0.10 | INT-triglycerides | -0.1670 | 0.0058 | 24097068 |
| | rs268 | *LPL* | A/G | 0.98 | INT-triglycerides | -0.1971 | 0.0364 | 30326043[c] |
| Lower LDL-C via 19 genetic regions | | | | | | | | |
| | rs11136341 | *PLEC1* | A/G | 0.40 | INT-LDL-C | -0.045 | 0.0066 | 24097068 |
| | rs11563251 | *UGT1A1* | C/T | 0.12 | INT-LDL-C | -0.034 | 0.0062 | 24097068 |
| | rs12027135 | *LDLRAP1* | A/T | 0.46 | INT-LDL-C | -0.03 | 0.0039 | 24097068 |
| | rs12916 | *HMGCR* | T/C | 0.40 | INT-LDL-C | -0.073 | 0.0039 | 24097068 |
| | rs2030746 | *LOC84931* | C/T | 0.40 | INT-LDL-C | -0.021 | 0.0037 | 24097068 |
| | rs2072183 | *NPC1L1* | G/C | 0.29 | INT-LDL-C | -0.039 | 0.0048 | 24097068 |
| | rs2328223 | *SNX5* | A/C | 0.21 | INT-LDL-C | -0.03 | 0.0052 | 24097068 |
| | rs2642442 | *MOSC1* | C/T | 0.33 | INT-LDL-C | -0.036 | 0.0055 | 24097068 |
| | rs2710642 | *EHBP1* | G/A | 0.35 | INT-LDL-C | -0.024 | 0.0041 | 24097068 |
| | rs2902940 | *MAFB* | G/A | 0.30 | INT-LDL-C | -0.027 | 0.0040 | 24097068 |
| | rs2954029 | *TRIB1* | T/A | 0.47 | INT-LDL-C | -0.056 | 0.0037 | 24097068 |
| | rs4299376 | *ABCG5* | T/G | 0.31 | INT-LDL-C | -0.081 | 0.0045 | 24097068 |
| | rs4942486 | *BRCA2* | C/T | 0.48 | INT-LDL-C | -0.024 | 0.0036 | 24097068 |

**Supplementary table 2** List of five triglyceride-lowering *APOA5* and *LPL* variants and LDL-C lowering variants via 19 genetic genetic regions investigated in this study (continued)

| Genetic score | SNP | Gene | EA[a]/NEA | EAF | Phenotype | Effect size (SD units) | SE | Reference[b] |
|---|---|---|---|---|---|---|---|---|
| | rs514230 | *IRF2BP2* | A/T | 0.48 | INT-LDL-C | -0.036 | 0.0053 | 24097068 |
| | rs6029526 | *TOP1* | T/A | 0.47 | INT-LDL-C | -0.044 | 0.0051 | 24097068 |
| | rs7206971 | *OSBPL7* | G/A | 0.49 | INT-LDL-C | -0.029 | 0.0057 | 24097068 |
| | rs9488822 | *FRK* | A/T | 0.36 | INT-LDL-C | -0.031 | 0.0060 | 24097068 |
| | rs964184 | *APOA1* | C/G | 0.84 | INT-LDL-C | -0.086 | 0.0081 | 24097068 |
| | rs9987289 | *PPP1R3B* | A/G | 0.10 | INT-LDL-C | -0.071 | 0.0071 | 24097068 |

Abbreviations: EA, effect allele; NEA, non-effect allele; EAF, effect allele frequency; SNP, single nucleotide polymorphism; SE, standard error; *LPL, lipoprotein lipase*; LDL-C, low-density lipoprotein cholesterol; *PLEC1, Plectin; UGT1A, UDP Glucuronosyltransferase Family 1 Member A; LDLRAP, Low Density Lipoprotein Receptor Adaptor Protein 1 HMGCR, 3-Hydroxy-3-Methylglutaryl-CoA Reductase; NPC1L1, Niemann-Pick C1-Like 1; PCSK9, Proprotein convertase subtilisin/kexin type 9; SNX5, Sorting Nexin 5; MOSC1, Molybdenum Cofactor Sulfurase C-terminal Domain-Containing; EHBP1, EH Domain Binding Protein 1; MAFB, MAF BZIP Transcription Factor B; TRIB1, Tribbles Pseudokinase 1; ABCG5, ATP Binding Cassette Subfamily G Member 5; BRCA2, Breast Cancer Type 2 Susceptibility Protein; IRF2BP2, Interferon Regulatory Factor 2 Binding Protein 2; TOP1, DNA Topoisomerase I; OSBPL7, Oxysterol Binding Protein Like 7; FRK, Fyn Related Src Family Tyrosine Kinase; APOA1, Apolipoprotein A1; PPP1R3B, Protein Phosphatase 1 Regulatory Subunit 3B.*

a The effect allele is the triglyceride-lowering allele or the lipid-lowering allele.

b PubMed ID of the original manuscript from which beta coefficients and standard errors are derived.

c Estimated in EPIC-Norfolk

**Supplementary table 3** Characteristics of the UK BioBank study stratified by the LDL-C, *LPL* and *APOA5* genetic risk scores

| Characteristics | LDL-C-lowering | | TG-lowering via *LPL* | | TG-lowering via *APOA5* | | LDL-C lowering + TG-lowering via *LPL* | | LDL-C-lowering + TG-lowering via *APOA5* | | TG-lowering via *LPL* and *APOA5* | | LDL-C-lowering + TG-lowering via *LPL* and *APOA5* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Yes | No | Yes | No | Yes | No | Yes | No | Yes | No | Yes | No | Yes | No |
| N | 154,800 | 154,980 | 90,749 | 219,031 | 238,806 | 70,974 | 45,502 | 109,733 | 119,081 | 35,255 | 7,0014 | 50,239 | 35,008 | 25,014 |
| LDL-C GRS (median (IQR)) | 0.56 (0.19) | 0.26 (0.19) | 0.41 (0.30) | 0.41 (0.30) | 0.41 (0.30) | 0.41 (0.30) | 0.56 (0.19) | 0.26 (0.19) | 0.56 (0.19) | 0.26 (0.19) | 0.41 (0.30) | 0.41 (0.30) | 0.56 (0.19) | 0.26 (0.19) |
| *LPL* GRS (median (IQR)) | 0.09 (0.23) | 0.09 (0.20) | 0.40 (0.17) | 0.00 (0.09) | 0.09 (0.23) | 0.09 (0.20) | 0.40 (0.17) | 0.00 (0.09) | 0.09 (0.23) | 0.09 (0.20) | 0.40 (0.17) | 0.00 (0.09) | 0.40 (0.17) | 0.00 (0.09) |
| *APOA5* GRS (median (IQR)) | 0.86 (0.00) | 0.86 (0.00) | 0.86 (0.00) | 0.86 (0.00) | 0.86 (0.00) | 0.61 (0.06) | 0.86 (0.00) | 0.86 (0.00) | 0.86 (0.00) | 0.61 (0.06) | 0.86 (0.00) | 0.61 (0.06) | 0.86 (0.00) | 0.61 (0.06) |
| | | | | | | | | | | | | | | |
| Age, years | 56.8 (8.0) | 56.7 (8.0) | 56.8 (8.0) | 56.7 (8.0) | 56.8 (8.0) | 56.8 (8.0) | 56.8 (8.0) | 56.7 (8.0) | 56.8 (8.0) | 56.7 (8.0) | 56.8 (8.0) | 56.7 (8.0) | 56.8 (8.0) | 56.7 (8.0) |
| Sex, men | 46 | 46 | 46 | 46 | 46 | 46 | 46 | 46 | 46 | 45 | 46 | 45 | 46 | 45 |
| Body mass index, kg/m2 | 27.4 (4.8) | 27.4 (4.8) | 27.3 (4.7) | 27.4 (4.8) | 27.4 (4.8) | 27.4 (4.8) | 27.4 (4.7) | 27.4 (4.8) | 27.4 (4.8) | 27.4 (4.8) | 27.3 (4.7) | 27.4 (4.8) | 27.4 (4.8) | 27.4 (4.8) |
| Fasting serum concentrations (mmol/L): | | | | | | | | | | | | | | |
| TG (median (IQR)) | 1.48 (1.10) | 1.50 (1.12) | 1.42 (1.04) | 1.52 (1.13) | 1.44 (1.05) | 1.67 (1.29) | 1.40 (1.03) | 1.54 (1.15) | 1.43 (1.04) | 1.68 (1.30) | 1.38 (0.98) | 1.70 (1.31) | 1.36 (0.98) | 1.71 (1.33) |
| Total cholesterol | 5.59 (1.09) | 5.84 (1.17) | 5.71 (1.13) | 5.71 (1.15) | 5.69 (1.13) | 5.78 (1.18) | 5.59 (1.08) | 5.84 (1.18) | 5.56 (1.08) | 5.91 (1.21) | 5.69 (1.11) | 5.78 (1.18) | 5.56 (1.06) | 5.91 (1.22) |
| LDL cholesterol | 3.46 (0.83) | 3.68 (0.89) | 3.56 (0.86) | 3.57 (0.87) | 3.46 (0.83) | 3.61 (0.89) | 3.45 (0.82) | 3.56 (0.86) | 3.44 (0.82) | 3.72 (0.92) | 3.55 (0.85) | 3.62 (0.89) | 3.43 (0.81) | 3.73 (0.92) |

**Supplementary table 3** Characteristics of the UK BioBank study stratified by the LDL-C, *LPL* and *APOA5* genetic risk scores (continued)

| Characteristics | LDL-C-lowering | | TG-lowering via *LPL* | | TG-lowering via *APOA5* | | LDL-C lowering + TG-lowering via *LPL* | | LDL-C-lowering + TG-lowering via *APOA5* | | TG-lowering via *LPL* and *APOA5* | | LDL-C-lowering + TG-lowering via *LPL* and *APOA5* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Yes | No | Yes | No | Yes | No | Yes | No | Yes | No | Yes | No | Yes | No |
| HDL cholesterol | 1.46 (0.39) | 1.45 (0.38) | 1.48 (0.39) | 1.44 (0.38) | 1.46 (0.39) | 1.42 (0.38) | 1.49 (0.39) | 1.46 (0.38) | 1.47 (0.39) | 1.41 (0.37) | 1.49 (0.39) | 1.41 (0.37) | 1.50 (0.39) | 1.40 (0.37) |

Values are mean (SD), unless otherwise specified. GRS unit is in SD. [a]Due to nonavailability of rs301, the *LPL* GRS for the UK BioBank was calculated based on five variants (rs268, rs326, rs328 and rs10096633) versus the six variants (rs268, rs301, rs326, rs328 and rs10096633) used in the NEO and OBB cohorts.
Abbreviations: BMI, body mass index; GRS, genetic risk score; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; TG, triglycerides

**Supplementary table 4** Characteristics of the combined cohort of the Netherlands Epidemiology of Obesity (NEO) study and the Oxford Biobank (OBB) study stratified by the *LPL* and *APOA5* genetic risk scores

| Characteristics | 1: Reference | 2: TG-lowering via *LPL* | 3: TG-lowering via *APOA5* | 4: TG-lowering via both |
|---|---|---|---|---|
| Total (n) | 1,908 | 1,657 | 4,547 | 3,725 |
| Age (years) | 49.0 (6.0) | 48.8 (6.0) | 46.5 (5.8) | 46.7 (5.9) |
| Men (%) | 41.8 | 40.3 | 44.3 | 43.5 |
| BMI (kg/m$^2$) | 26.1 (4.5) | 25.9 (4.5) | 25.9 (4.4) | 25.8 (4.5) |
| ***Fasting serum concentrations (mmol/L):*** | | | | |
| Total cholesterol | 5.61 (1.01) | 5.55 (1.03) | 5.37 (0.98) | 5.36 (0.97) |
| LDL-cholesterol | 3.57 (0.91) | 3.49 (0.94) | 3.41 (0.87) | 3.38 (0.88) |
| HDL-cholesterol | 1.44 (0.41) | 1.52 (0.43) | 1.44 (0.40) | 1.50 (0.42) |
| Triglycerides (median (IQR) | 1.05 (0.77; 1.58) | 1.02 (0.72; 1.47) | 0.96 (0.70; 1.37) | 0.89 (0.66; 1.26) |
| *LPL* GRS (median (IQR) | 0.39 (0.00) | 0.72 (0.31) | 0.39 (0.00) | 0.72 (0.31) |
| *APOA5* GRS (median (IQR) | 0.64 (0.14) | 0.64 (0.14) | 0.86 (0.00) | 0.86 (0.00) |

Values are mean (SD), unless otherwise specified. Genetic risk score unit is in SD.
Data represent averaged results from the individual analyses in NEO and OBB cohort
In NEO, results are based on analyses weighted towards the reference BMI distribution of the general Dutch population.
Abbreviations: BMI, body mass index; GRS, genetic risk score; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; TG, triglycerides

**SUPPLEMENTARY table 5** The associations between genetically-influenced lower triglyceride levels via *APOA5* and *LPL*, separately and in combination and 145 NMR-based metabolomic measures in the combined cohort of Netherlands Epidemiology of Obesity (NEO) study (n = 4,838) and the OBB study (n= 6,999)

| | Lower TG via *LPL* | | | Lower LDL-C | | | Lower TG via *LPL* & *APOA5* | | |
|---|---|---|---|---|---|---|---|---|---|
| **Metabolic measure** | **BETA*** | **SE** | ***P* VALUE** | **BETA** | **SE** | ***P* VALUE** | **BETA*** | **SE** | ***P* VALUE** |
| *Very-low-density lipoproteins (VLDL)* | | | | | | | | | |
| XXL.VLDL.C | -0.161 | 0.065 | 1.41E-02 | -0.106 | 0.060 | 7.58E-02 | -0.316 | 0.062 | 3.25E-07 |
| XXL.VLDL.CE | -0.141 | 0.064 | 2.70E-02 | -0.109 | 0.059 | 6.41E-02 | -0.285 | 0.060 | 2.28E-06 |
| XXL.VLDL.FC | -0.156 | 0.066 | 1.77E-02 | -0.082 | 0.060 | 1.70E-01 | -0.313 | 0.062 | 4.29E-07 |
| XXL.VLDL.L | -0.163 | 0.066 | 1.40E-02 | -0.096 | 0.060 | 1.06E-01 | -0.326 | 0.062 | 1.55E-07 |
| XXL.VLDL.P | -0.163 | 0.066 | 1.39E-02 | -0.096 | 0.059 | 1.05E-01 | -0.327 | 0.062 | 1.44E-07 |
| XXL.VLDL.PL | -0.170 | 0.067 | 1.15E-02 | -0.099 | 0.059 | 9.62E-02 | -0.327 | 0.062 | 1.16E-07 |
| XXL.VLDL.TG | -0.162 | 0.066 | 1.44E-02 | -0.094 | 0.059 | 1.41E-01 | -0.326 | 0.062 | 1.44E-07 |
| XL.VLDL.C | -0.131 | 0.064 | 4.01E-02 | -0.117 | 0.060 | 5.00E-02 | -0.284 | 0.058 | 1.26E-06 |
| XL.VLDL.CE | -0.136 | 0.063 | 3.20E-02 | -0.114 | 0.059 | 5.64E-02 | -0.279 | 0.058 | 1.52E-06 |
| XL.VLDL.FC | -0.120 | 0.065 | 6.30E-02 | -0.115 | 0.060 | 5.75E-02 | -0.289 | 0.060 | 1.40E-06 |
| XL.VLDL.L | -0.120 | 0.066 | 6.93E-02 | -0.086 | 0.062 | 1.67E-01 | -0.289 | 0.062 | 2.95E-06 |
| XL.VLDL.P | -0.118 | 0.066 | 7.39E-02 | -0.082 | 0.062 | 1.85E-01 | -0.287 | 0.062 | 3.49E-06 |
| XL.VLDL.PL | -0.122 | 0.066 | 6.47E-02 | -0.106 | 0.062 | 8.73E-02 | -0.303 | 0.062 | 9.45E-07 |
| XL.VLDL.TG | -0.115 | 0.066 | 8.11E-02 | -0.074 | 0.062 | 2.36E-01 | -0.284 | 0.062 | 4.67E-06 |
| L.VLDL.C | -0.132 | 0.057 | 2.04E-02 | -0.109 | 0.055 | 4.57E-02 | -0.321 | 0.056 | 1.20E-08 |
| L.VLDL.CE | -0.135 | 0.056 | 1.61E-02 | -0.112 | 0.054 | 3.91E-02 | -0.331 | 0.056 | 4.31E-09 |
| L.VLDL.FC | -0.125 | 0.058 | 3.22E-02 | -0.102 | 0.056 | 6.89E-02 | -0.288 | 0.056 | 2.43E-07 |
| L.VLDL.L | -0.133 | 0.059 | 2.36E-02 | -0.102 | 0.056 | 7.10E-02 | -0.304 | 0.057 | 8.01E-08 |
| L.VLDL.P | -0.133 | 0.059 | 2.45E-02 | -0.100 | 0.056 | 7.63E-02 | -0.303 | 0.057 | 9.43E-08 |
| L.VLDL.PL | -0.134 | 0.059 | 2.41E-02 | -0.105 | 0.056 | 6.19E-02 | -0.308 | 0.057 | 6.18E-08 |
| L.VLDL.TG | -0.130 | 0.059 | 2.79E-02 | -0.095 | 0.057 | 9.44E-02 | -0.295 | 0.057 | 2.08E-07 |

**SUPPLEMENTARY table 5** The associations between genetically-influenced lower triglyceride levels via *APOA5* and *LPL*, separately and in combination and 145 NMR-based metabolomic measures in the combined cohort of Netherlands Epidemiology of Obesity (NEO) study (n = 4,838) and the OBB study (n= 6,999) (continued)

| | Lower TG via *LPL* | | | Lower LDL-C | | | Lower TG via *LPL* & *APOA5* | | |
|---|---|---|---|---|---|---|---|---|---|
| **Metabolic measure** | **BETA*** | **SE** | ***P* VALUE** | **BETA** | **SE** | ***P* VALUE** | **BETA*** | **SE** | ***P* VALUE** |
| M.VLDL.C | -0.189 | 0.057 | 9.32E-04 | -0.157 | 0.054 | 3.73E-03 | -0.380 | 0.054 | 3.55E-12 |
| M.VLDL.CE | -0.181 | 0.057 | 1.56E-03 | -0.163 | 0.054 | 2.50E-03 | -0.378 | 0.055 | 5.34E-12 |
| M.VLDL.FC | -0.189 | 0.057 | 9.11E-04 | -0.146 | 0.054 | 7.54E-03 | -0.364 | 0.054 | 2.22E-11 |
| M.VLDL.L | -0.194 | 0.057 | 7.10E-04 | -0.143 | 0.054 | 8.29E-03 | -0.374 | 0.055 | 1.11E-11 |
| M.VLDL.P | -0.194 | 0.057 | 7.06E-04 | -0.142 | 0.054 | 8.97E-03 | -0.372 | 0.055 | 1.36E-11 |
| M.VLDL.PL | -0.193 | 0.057 | 7.56E-04 | -0.149 | 0.054 | 6.27E-03 | -0.379 | 0.055 | 6.85E-12 |
| M.VLDL.TG | -0.190 | 0.057 | 8.42E-04 | -0.131 | 0.054 | 1.61E-02 | -0.361 | 0.055 | 5.75E-11 |
| S.VLDL.C | -0.195 | 0.061 | 1.55E-03 | -0.184 | 0.058 | 1.38E-03 | -0.424 | 0.061 | 4.41E-12 |
| S.VLDL.CE | -0.182 | 0.062 | 3.12E-03 | -0.188 | 0.058 | 1.33E-03 | -0.405 | 0.062 | 6.95E-11 |
| S.VLDL.FC | -0.203 | 0.060 | 7.91E-04 | -0.173 | 0.057 | 2.24E-03 | -0.412 | 0.058 | 1.90E-12 |
| S.VLDL.L | -0.212 | 0.060 | 4.17E-04 | -0.165 | 0.056 | 3.36E-03 | -0.415 | 0.058 | 1.25E-12 |
| S.VLDL.P | -0.212 | 0.060 | 3.88E-04 | -0.161 | 0.056 | 4.22E-03 | -0.410 | 0.058 | 1.72E-12 |
| S.VLDL.PL | -0.206 | 0.061 | 7.88E-04 | -0.169 | 0.057 | 3.15E-03 | -0.409 | 0.059 | 4.27E-12 |
| S.VLDL.TG | -0.201 | 0.058 | 5.11E-04 | -0.133 | 0.055 | 1.60E-02 | -0.370 | 0.056 | 4.64E-11 |
| XS.VLDL.C | -0.111 | 0.059 | 6.11E-02 | -0.161 | 0.057 | 4.46E-03 | -0.315 | 0.061 | 2.26E-07 |
| XS.VLDL.CE | -0.108 | 0.060 | 6.89E-02 | -0.149 | 0.057 | 8.87E-03 | -0.301 | 0.061 | 7.84E-07 |
| XS.VLDL.FC | -0.112 | 0.059 | 5.80E-02 | -0.175 | 0.056 | 1.66E-03 | -0.326 | 0.060 | 6.24E-08 |
| XS.VLDL.L | -0.145 | 0.060 | 1.56E-02 | -0.178 | 0.057 | 1.68E-03 | -0.372 | 0.060 | 6.92E-10 |
| XS.VLDL.P | -0.154 | 0.060 | 1.06E-02 | -0.179 | 0.057 | 1.69E-03 | -0.384 | 0.060 | 1.77E-10 |
| XS.VLDL.PL | -0.104 | 0.058 | 7.00E-02 | -0.180 | 0.054 | 9.20E-04 | -0.321 | 0.058 | 3.25E-08 |
| XS.VLDL.TG | -0.199 | 0.060 | 9.14E-04 | -0.139 | 0.057 | 1.52E-02 | -0.373 | 0.058 | 1.21E-10 |

**SUPPLEMENTARY table 5** The associations between genetically-influenced lower triglyceride levels via *APOA5* and *LPL*, separately and in combination and 145 NMR-based metabolomic measures in the combined cohort of Netherlands Epidemiology of Obesity (NEO) study (n = 4,838) and the OBB study (n= 6,999) (continued)

| | Lower TG via *LPL* | | | Lower LDL-C | | | Lower TG via *LPL* & *APOA5* | | |
|---|---|---|---|---|---|---|---|---|---|
| **Metabolic measure** | **BETA*** | **SE** | ***P* VALUE** | **BETA** | **SE** | ***P* VALUE** | **BETA*** | **SE** | ***P* VALUE** |
| *Intermediate-density lipoproteins (IDL)* | | | | | | | | | |
| IDL.C | -0.044 | 0.056 | 4.33E-01 | -0.163 | 0.053 | 2.07E-03 | -0.251 | 0.058 | 1.38E-05 |
| IDL.CE | -0.056 | 0.057 | 3.28E-01 | -0.168 | 0.054 | 1.85E-03 | -0.274 | 0.059 | 3.17E-06 |
| IDL.FC | -0.016 | 0.054 | 7.61E-01 | -0.150 | 0.051 | 3.26E-03 | -0.189 | 0.055 | 5.55E-04 |
| IDL.L | -0.051 | 0.056 | 3.60E-01 | -0.163 | 0.053 | 1.98E-03 | -0.259 | 0.057 | 5.52E-06 |
| IDL.P | -0.057 | 0.056 | 3.10E-01 | -0.164 | 0.053 | 1.88E-03 | -0.269 | 0.057 | 2.56E-06 |
| IDL.PL | -0.043 | 0.055 | 4.28E-01 | -0.163 | 0.052 | 1.70E-03 | -0.238 | 0.056 | 2.02E-05 |
| IDL.TG | -0.120 | 0.058 | 3.97E-02 | -0.125 | 0.057 | 2.80E-02 | -0.285 | 0.057 | 6.20E-07 |
| *Low-density lipoproteins (LDL)* | | | | | | | | | |
| L.LDL.C | -0.043 | 0.056 | 4.44E-01 | -0.170 | 0.052 | 1.21E-03 | -0.255 | 0.057 | 9.07E-06 |
| L.LDL.CE | -0.052 | 0.057 | 3.60E-01 | -0.174 | 0.053 | 9.93E-04 | -0.273 | 0.058 | 2.61E-06 |
| L.LDL.FC | -0.017 | 0.054 | 7.56E-01 | -0.156 | 0.051 | 2.20E-03 | -0.199 | 0.055 | 2.94E-04 |
| L.LDL.L | -0.045 | 0.056 | 4.18E-01 | -0.170 | 0.052 | 1.15E-03 | -0.261 | 0.057 | 4.94E-06 |
| L.LDL.P | -0.049 | 0.056 | 3.86E-01 | -0.172 | 0.053 | 1.10E-03 | -0.267 | 0.057 | 3.03E-06 |
| L.LDL.PL | -0.047 | 0.056 | 4.01E-01 | -0.175 | 0.052 | 8.08E-04 | -0.267 | 0.057 | 2.80E-06 |
| L.LDL.TG | -0.057 | 0.056 | 3.05E-01 | -0.122 | 0.054 | 2.44E-02 | -0.218 | 0.055 | 7.75E-05 |
| M.LDL.C | -0.042 | 0.057 | 4.63E-01 | -0.167 | 0.053 | 1.65E-03 | -0.247 | 0.057 | 1.83E-05 |
| M.LDL.CE | -0.046 | 0.057 | 4.20E-01 | -0.168 | 0.053 | 1.56E-03 | -0.250 | 0.058 | 1.47E-05 |
| M.LDL.FC | -0.026 | 0.057 | 6.46E-01 | -0.162 | 0.053 | 2.21E-03 | -0.235 | 0.057 | 4.52E-05 |
| M.LDL.L | -0.047 | 0.057 | 4.12E-01 | -0.171 | 0.053 | 1.34E-03 | -0.261 | 0.057 | 5.58E-06 |
| M.LDL.P | -0.049 | 0.057 | 3.91E-01 | -0.171 | 0.053 | 1.30E-03 | -0.264 | 0.057 | 4.21E-06 |

**SUPPLEMENTARY table 5** The associations between genetically-influenced lower triglyceride levels via *APOA5* and *LPL*, separately and in combination and 145 NMR-based metabolomic measures in the combined cohort of Netherlands Epidemiology of Obesity (NEO) study (n = 4,838) and the OBB study (n= 6,999) (continued)

| | Lower TG via *LPL* | | | Lower LDL-C | | | Lower TG via *LPL* & *APOA5* | | |
|---|---|---|---|---|---|---|---|---|---|
| **Metabolic measure** | **BETA*** | **SE** | ***P* VALUE** | **BETA** | **SE** | ***P* VALUE** | **BETA*** | **SE** | ***P* VALUE** |
| M.LDL.PL | -0.065 | 0.059 | 2.65E-01 | -0.185 | 0.054 | 6.37E-04 | -0.308 | 0.058 | 1.15E-07 |
| M.LDL.TG | -0.031 | 0.055 | 5.70E-01 | -0.119 | 0.054 | 2.71E-02 | -0.176 | 0.055 | 1.37E-03 |
| S.LDL.C | -0.032 | 0.057 | 5.76E-01 | -0.162 | 0.053 | 2.22E-03 | -0.231 | 0.057 | 5.63E-05 |
| S.LDL.CE | -0.037 | 0.057 | 5.20E-01 | -0.163 | 0.053 | 1.95E-03 | -0.232 | 0.057 | 4.73E-05 |
| S.LDL.FC | -0.021 | 0.057 | 7.18E-01 | -0.156 | 0.054 | 3.66E-03 | -0.226 | 0.058 | 1.10E-04 |
| S.LDL.L | -0.039 | 0.058 | 4.96E-01 | -0.168 | 0.053 | 1.63E-03 | -0.253 | 0.058 | 1.17E-05 |
| S.LDL.P | -0.043 | 0.058 | 4.59E-01 | -0.170 | 0.053 | 1.48E-03 | -0.260 | 0.058 | 6.94E-06 |
| S.LDL.PL | -0.039 | 0.058 | 5.01E-01 | -0.173 | 0.054 | 1.36E-03 | -0.270 | 0.058 | 3.32E-06 |
| S.LDL.TG | -0.101 | 0.057 | 7.75E-02 | -0.149 | 0.055 | 6.91E-03 | -0.287 | 0.055 | 1.99E-07 |
| *High-density lipoproteins (HDL)* | | | | | | | | | |
| XL.HDL.C | 0.127 | 0.054 | 1.88E-02 | -0.027 | 0.052 | 6.06E-01 | 0.126 | 0.054 | 1.98E-02 |
| XL.HDL.CE | 0.123 | 0.054 | 2.40E-02 | -0.034 | 0.052 | 5.15E-01 | 0.115 | 0.055 | 1.58E-02 |
| XL.HDL.FC | 0.138 | 0.053 | 9.45E-03 | -0.007 | 0.051 | 8.93E-01 | 0.153 | 0.054 | 4.57E-03 |
| XL.HDL.L | 0.132 | 0.052 | 1.09E-02 | -0.008 | 0.050 | 8.80E-01 | 0.157 | 0.053 | 2.91E-03 |
| XL.HDL.P | 0.131 | 0.052 | 1.14E-02 | -0.007 | 0.050 | 8.81E-01 | 0.157 | 0.053 | 2.93E-03 |
| XL.HDL.PL | 0.138 | 0.052 | 7.74E-03 | -0.014 | 0.050 | 7.84E-01 | 0.196 | 0.052 | 1.88E-04 |
| XL.HDL.TG | 0.015 | 0.059 | 8.01E-01 | -0.061 | 0.054 | 2.55E-01 | -0.081 | 0.056 | 1.49E-01 |
| L.HDL.C | 0.176 | 0.054 | 1.13E-03 | 0.069 | 0.051 | 1.82E-01 | 0.235 | 0.054 | 1.63E-05 |
| L.HDL.CE | 0.176 | 0.054 | 1.08E-03 | 0.070 | 0.052 | 1.74E-01 | 0.236 | 0.054 | 1.54E-05 |
| L.HDL.FC | 0.175 | 0.054 | 1.20E-03 | 0.066 | 0.051 | 2.02E-01 | 0.232 | 0.054 | 2.00E-05 |
| L.HDL.L | 0.169 | 0.053 | 1.39E-03 | 0.057 | 0.051 | 2.61E-01 | 0.216 | 0.054 | 5.54E-05 |

**SUPPLEMENTARY table 5** The associations between genetically-influenced lower triglyceride levels via *APOA5* and *LPL*, separately and in combination and 145 NMR-based metabolomic measures in the combined cohort of Netherlands Epidemiology of Obesity (NEO) study (n = 4,838) and the OBB study (n= 6,999) (continued)

| | Lower TG via *LPL* | | | Lower LDL-C | | | Lower TG via *LPL* & *APOA5* | | |
|---|---|---|---|---|---|---|---|---|---|
| **Metabolic measure** | **BETA*** | **SE** | ***P* VALUE** | **BETA** | **SE** | ***P* VALUE** | **BETA*** | **SE** | ***P* VALUE** |
| L.HDL.P | 0.168 | 0.053 | 1.47E-03 | 0.055 | 0.051 | 2.76E-01 | 0.213 | 0.054 | 6.83E-05 |
| L.HDL.PL | 0.163 | 0.053 | 1.88E-03 | 0.048 | 0.051 | 3.41E-01 | 0.203 | 0.053 | 1.47E-04 |
| L.HDL.TG | 0.104 | 0.055 | 5.69E-02 | 0.005 | 0.051 | 9.18E-01 | 0.093 | 0.055 | 9.16E-02 |
| M.HDL.C | 0.140 | 0.053 | 7.58E-03 | 0.011 | 0.050 | 8.27E-01 | 0.145 | 0.051 | 4.32E-03 |
| M.HDL.CE | 0.144 | 0.053 | 6.68E-03 | 0.016 | 0.050 | 7.54E-01 | 0.151 | 0.051 | 3.23E-03 |
| M.HDL.FC | 0.126 | 0.052 | 1.44E-02 | -0.007 | 0.049 | 8.81E-01 | 0.123 | 0.050 | 1.44E-02 |
| M.HDL.L | 0.117 | 0.052 | 2.50E-02 | -0.010 | 0.050 | 8.38E-01 | 0.101 | 0.051 | 4.78E-02 |
| M.HDL.P | 0.112 | 0.052 | 3.25E-02 | -0.014 | 0.050 | 7.77E-01 | 0.090 | 0.051 | 7.76E-02 |
| M.HDL.PL | 0.110 | 0.052 | 3.34E-02 | -0.017 | 0.050 | 7.30E-01 | 0.092 | 0.051 | 7.19E-02 |
| M.HDL.TG | -0.165 | 0.061 | 6.71E-03 | -0.128 | 0.057 | 2.43E-02 | -0.309 | 0.058 | 1.05E-07 |
| S.HDL.C | 0.044 | 0.059 | 4.54E-01 | -0.067 | 0.053 | 2.06E-01 | -0.049 | 0.056 | 3.81E-01 |
| S.HDL.CE | 0.033 | 0.058 | 5.68E-01 | -0.079 | 0.053 | 1.34E-01 | -0.070 | 0.055 | 2.09E-01 |
| S.HDL.FC | 0.063 | 0.060 | 2.93E-01 | 0.011 | 0.056 | 8.42E-01 | 0.044 | 0.057 | 4.44E-01 |
| S.HDL.L | 0.027 | 0.063 | 6.64E-01 | -0.040 | 0.057 | 4.89E-01 | -0.048 | 0.061 | 4.32E-01 |
| S.HDL.P | 0.017 | 0.063 | 7.86E-01 | -0.044 | 0.058 | 4.50E-01 | -0.062 | 0.061 | 3.13E-01 |
| S.HDL.PL | 0.032 | 0.061 | 5.98E-01 | 0.018 | 0.056 | 7.55E-01 | 0.017 | 0.059 | 7.75E-01 |
| S.HDL.TG | -0.189 | 0.059 | 1.48E-03 | -0.100 | 0.057 | 8.00E-02 | -0.298 | 0.057 | 2.15E-07 |
| *Lipoprotein particle size* | | | | | | | | | |
| VLDL particle size | -0.165 | 0.054 | 2.12E-03 | -0.082 | 0.052 | 1.15E-01 | -0.266 | 0.052 | 3.83E-07 |
| LDL particle size | -0.009 | 0.061 | 8.78E-01 | 0.043 | 0.053 | 4.12E-01 | 0.050 | 0.058 | 3.84E-01 |
| HDL particle size | 0.164 | 0.061 | 1.95E-03 | 0.027 | 0.051 | 5.90E-01 | 0.221 | 0.053 | 3.26E-05 |

**SUPPLEMENTARY table 5** The associations between genetically-influenced lower triglyceride levels via *APOA5* and *LPL*, separately and in combination and 145 NMR-based metabolomic measures in the combined cohort of Netherlands Epidemiology of Obesity (NEO) study (n = 4,838) and the OBB study (n= 6,999) (continued)

| | Lower TG via *LPL* | | | Lower LDL-C | | | Lower TG via *LPL* & *APOA5* | | |
|---|---|---|---|---|---|---|---|---|---|
| **Metabolic measure** | **BETA*** | **SE** | ***P* VALUE** | **BETA** | **SE** | ***P* VALUE** | **BETA*** | **SE** | ***P* VALUE** |
| *Apolipoproteins* | | | | | | | | | |
| ApoA1 | 0.139 | 0.054 | 5.54E-03 | -0.041 | 0.049 | 3.97E-01 | 0.068 | 0.051 | 1.85E-01 |
| ApoB | -0.133 | 0.068 | 2.37E-02 | -0.185 | 0.055 | 8.07E-01 | -0.387 | 0.058 | 3.64E-11 |
| *Cholesterols* | | | | | | | | | |
| Esterified cholesterol | -0.003 | 0.055 | 9.58E-01 | -0.162 | 0.052 | 1.77E-03 | -0.219 | 0.056 | 9.02E-05 |
| Free cholesterol | -0.017 | 0.054 | 7.61E-01 | -0.146 | 0.052 | 5.00E-03 | -0.195 | 0.056 | 4.91E-04 |
| Total cholesterol in HDL2 | 0.170 | 0.050 | 6.13E-04 | 0.014 | 0.049 | 7.71E-01 | 0.198 | 0.050 | 6.70E-05 |
| Total cholesterol in HDL3 | 0.113 | 0.051 | 2.67E-02 | -0.058 | 0.050 | 2.48E-01 | 0.052 | 0.051 | 3.09E-01 |
| Total cholesterol in HDL | 0.174 | 0.050 | 4.89E-04 | 0.010 | 0.049 | 8.30E-01 | 0.195 | 0.050 | 8.66E-05 |
| Total cholesterol in LDL | -0.038 | 0.056 | 4.99E-01 | -0.166 | 0.053 | 1.57E-03 | -0.250 | 0.057 | 1.34E-05 |
| Remnant cholesterol | -0.135 | 0.059 | 2.18E-02 | -0.178 | 0.055 | 1.26E-03 | -0.380 | 0.059 | 9.15E-11 |
| Serum total cholesterol | -0.007 | 0.055 | 8.94E-01 | -0.158 | 0.052 | 2.23E-03 | -0.216 | 0.056 | 1.07E-04 |
| Total cholesterol in VLDL | -0.184 | 0.059 | 1.80E-03 | -0.163 | 0.055 | 3.11E-03 | -0.407 | 0.058 | 2.43E-12 |
| *Fatty acids* | | | | | | | | | |
| Conjugated linoleic acid | 0.012 | 0.069 | 8.61E-01 | -0.090 | 0.064 | 1.61E-01 | -0.070 | 0.066 | 2.86E-01 |
| Docosahexaenoic acid | -0.030 | 0.062 | 6.30E-01 | -0.044 | 0.060 | 4.65E-01 | -0.111 | 0.062 | 7.40E-02 |
| Fatty acid chain length | -0.045 | 0.066 | 4.99E-01 | 0.005 | 0.062 | 9.34E-01 | 0.049 | 0.064 | 4.41E-01 |
| Omega-3 | -0.035 | 0.058 | 5.53E-01 | -0.089 | 0.057 | 1.15E-01 | -0.210 | 0.060 | 4.51E-04 |
| Omega-6 | -0.077 | 0.058 | 1.84E-01 | -0.174 | 0.055 | 1.60E-03 | -0.275 | 0.059 | 2.97E-06 |
| Linoleic acid | -0.103 | 0.055 | 6.27E-02 | -0.215 | 0.054 | 7.79E-05 | -0.327 | 0.057 | 8.71E-09 |

**SUPPLEMENTARY table 5** The associations between genetically-influenced lower triglyceride levels via *APOA5* and *LPL*, separately and in combination and 145 NMR-based metabolomic measures in the combined cohort of Netherlands Epidemiology of Obesity (NEO) study (n = 4,838) and the OBB study (n= 6,999) (continued)

| | Lower TG via *LPL* | | | Lower LDL-C | | | Lower TG via *LPL* & *APOA5* | | |
|---|---|---|---|---|---|---|---|---|---|
| **Metabolic measure** | **BETA*** | **SE** | ***P* VALUE** | **BETA** | **SE** | ***P* VALUE** | **BETA*** | **SE** | ***P* VALUE** |
| MUFA | -0.077 | 0.059 | 1.90E-01 | -0.096 | 0.055 | 7.88E-02 | -0.244 | 0.056 | 1.27E-05 |
| PUFA | -0.074 | 0.058 | 1.99E-01 | -0.166 | 0.055 | 2.60E-03 | -0.279 | 0.059 | 2.56E-06 |
| SFA | -0.055 | 0.059 | 3.50E-01 | -0.076 | 0.055 | 1.69E-01 | -0.253 | 0.059 | 1.56E-05 |
| Total fatty acids | -0.083 | 0.058 | 1.52E-01 | -0.126 | 0.054 | 2.03E-02 | -0.294 | 0.057 | 2.46E-07 |
| Degree of unsaturation | 0.023 | 0.056 | 6.79E-01 | 0.018 | 0.055 | 7.44E-01 | 0.154 | 0.055 | 4.94E-03 |
| *Glycerides* | | | | | | | | | |
| Diacylglycerol | 0.026 | 0.074 | 7.29E-01 | -0.013 | 0.069 | 8.48E-01 | -0.147 | 0.070 | 3.49E-02 |
| Triglycerides in HDL | -0.104 | 0.056 | 6.42E-02 | -0.093 | 0.053 | 8.02E-02 | -0.211 | 0.053 | 7.27E-05 |
| Triglycerides in LDL | -0.060 | 0.056 | 2.81E-01 | -0.129 | 0.054 | 1.75E-02 | -0.228 | 0.055 | 3.64E-05 |
| Total triglycerides | -0.182 | 0.057 | 1.39E-03 | -0.131 | 0.055 | 1.64E-02 | -0.354 | 0.055 | 9.77E-11 |
| Total phosphoglycerides | 0.014 | 0.060 | 8.12E-01 | -0.083 | 0.053 | 1.19E-01 | -0.120 | 0.058 | 3.93E-02 |
| Triglycerides in VLDL | -0.192 | 0.057 | 7.97E-04 | -0.128 | 0.054 | 1.89E-02 | -0.362 | 0.055 | 4.79E-11 |
| *Phospholipids* | | | | | | | | | |
| Phosphatidylcholine | 0.033 | 0.058 | 5.73E-01 | -0.123 | 0.054 | 2.32E-02 | -0.102 | 0.058 | 8.23E-02 |
| Sphingomyelins | 0.056 | 0.055 | 3.12E-01 | -0.060 | 0.052 | 2.48E-01 | -0.081 | 0.056 | 1.47E-01 |
| Total cholines | 0.042 | 0.063 | 5.05E-01 | -0.082 | 0.058 | 1.56E-01 | -0.134 | 0.064 | 3.68E-02 |
| *Amino acids* | | | | | | | | | |
| Alanine | 0.027 | 0.058 | 6.41E-01 | 0.083 | 0.058 | 1.52E-01 | -0.045 | 0.057 | 4.25E-01 |
| Glutamine | -0.108 | 0.057 | 6.10E-02 | -0.047 | 0.055 | 3.94E-01 | -0.078 | 0.058 | 1.81E-01 |
| Histidine | 0.048 | 0.058 | 4.10E-01 | -0.005 | 0.056 | 9.25E-01 | 0.009 | 0.060 | 8.74E-01 |
| Isoleucine | -0.112 | 0.054 | 3.73E-02 | -0.090 | 0.050 | 7.23E-02 | -0.199 | 0.051 | 1.13E-04 |

**SUPPLEMENTARY table 5** The associations between genetically-influenced lower triglyceride levels via *APOA5* and *LPL*, separately and in combination and 145 NMR-based metabolomic measures in the combined cohort of Netherlands Epidemiology of Obesity (NEO) study (n = 4,838) and the OBB study (n= 6,999) (continued)

| | Lower TG via *LPL* | | | Lower LDL-C | | | Lower TG via *LPL* & *APOA5* | | |
|---|---|---|---|---|---|---|---|---|---|
| **Metabolic measure** | **BETA*** | **SE** | ***P* VALUE** | **BETA** | **SE** | ***P* VALUE** | **BETA*** | **SE** | ***P* VALUE** |
| Leucine | -0.058 | 0.050 | 2.46E-01 | -0.088 | 0.047 | 6.45E-02 | -0.155 | 0.050 | 2.13E-03 |
| Phenylalanine | -0.009 | 0.055 | 8.67E-01 | -0.037 | 0.053 | 4.90E-01 | -0.018 | 0.057 | 7.56E-01 |
| Tyrosine | 0.001 | 0.052 | 9.85E-01 | 0.056 | 0.052 | 2.80E-01 | 0.016 | 0.056 | 7.76E-01 |
| Valine | 0.030 | 0.050 | 5.57E-01 | -0.007 | 0.047 | 8.77E-01 | 0.001 | 0.052 | 9.85E-01 |
| *Kidney function* | | | | | | | | | |
| Albumin | 0.099 | 0.062 | 1.11E-01 | -0.038 | 0.060 | 5.32E-01 | -0.022 | 0.058 | 6.98E-01 |
| Creatinine | -0.006 | 0.048 | 9.07E-01 | -0.017 | 0.044 | 7.00E-01 | -0.039 | 0.048 | 4.11E-01 |
| *Glycolysis* | | | | | | | | | |
| Citrate | -0.003 | 0.064 | 9.65E-01 | -0.020 | 0.061 | 7.40E-01 | -0.020 | 0.061 | 7.45E-01 |
| Glucose | 0.052 | 0.053 | 3.22E-01 | -0.008 | 0.050 | 8.67E-01 | -0.057 | 0.054 | 2.99E-01 |
| Lactate | 0.048 | 0.056 | 3.94E-01 | 0.084 | 0.056 | 1.38E-01 | -0.037 | 0.057 | 5.25E-01 |
| *Inflammation* | | | | | | | | | |
| Glycoprotein acetyls | -0.154 | 0.063 | 1.40E-02 | -0.102 | 0.059 | 8.43E-02 | -0.310 | 0.059 | 1.89E-07 |
| *Ketone bodies* | | | | | | | | | |
| Acetate | 0.004 | 0.061 | 9.42E-01 | -0.112 | 0.057 | 4.98E-02 | -0.120 | 0.060 | 4.66E-02 |
| Beta-hydroxybutyrate | -0.009 | 0.068 | 8.94E-01 | -0.020 | 0.064 | 7.57E-01 | -0.126 | 0.066 | 5.51E-02 |

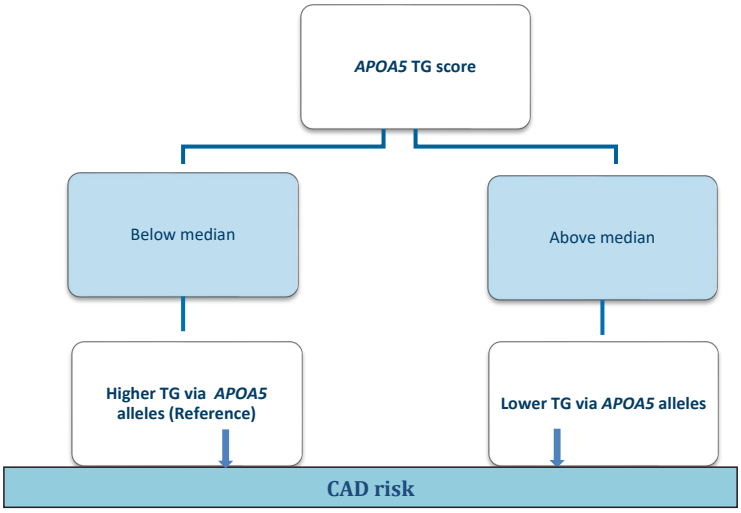



**Figure S1** Single instrument genetic analyses of TG-lowering via *APOA5* alleles.
**Note.** The same design is used for the other two single instrument genetic analyses: TG-lowering via *LPL* alleles; LDL-C-lowering.

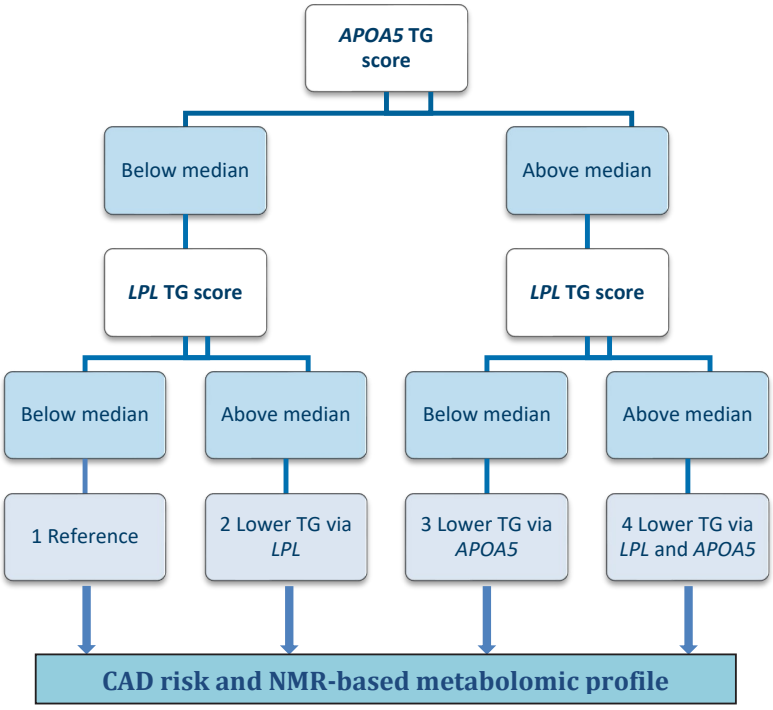



**Figure S2** Design of 2 × 2 factorial analyses TG-lowering *APOA5* alleles and TG-lowering *LPL* alleles.
**Note.** The same design is used for the other 2x2 factorial analyses: TG-lowering *APOA5* alleles and LDL-C-lowering ; TG-lowering LPL alleles and LDL-C-lowering.

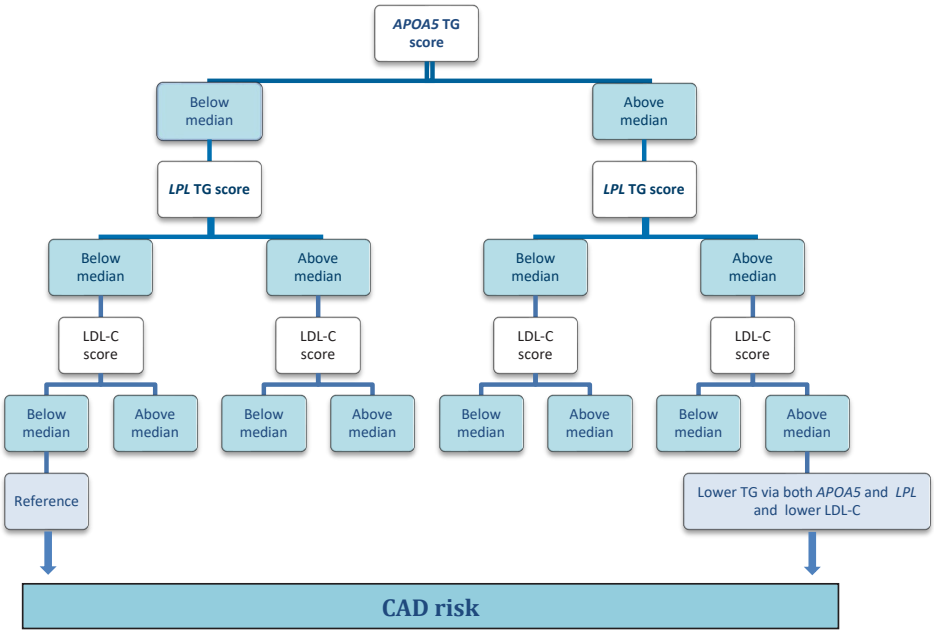



**Figure S3** Design of 2 × 2 × 2 factorial analyses of TG-lowering *APOA5* alleles, TG-lowering *LPL* alleles and LDL-C lowering alleles.

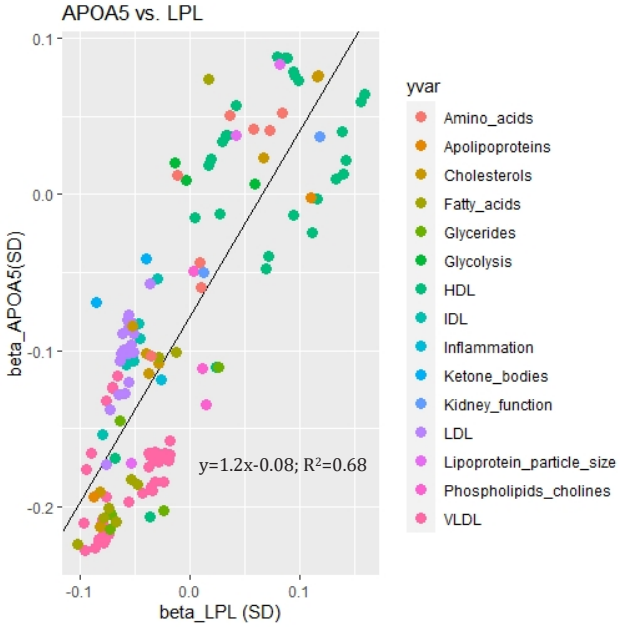



**Figure S4** The effect sizes (i.e. beta coefficients) for the associations between genetically-influenced lower TG via *APOA5* and *LPL* and 145 circulating metabolic measures in the Netherlands Epidemiology of Obesity (NEO) and in Oxford Biobank (OBB) cohort.

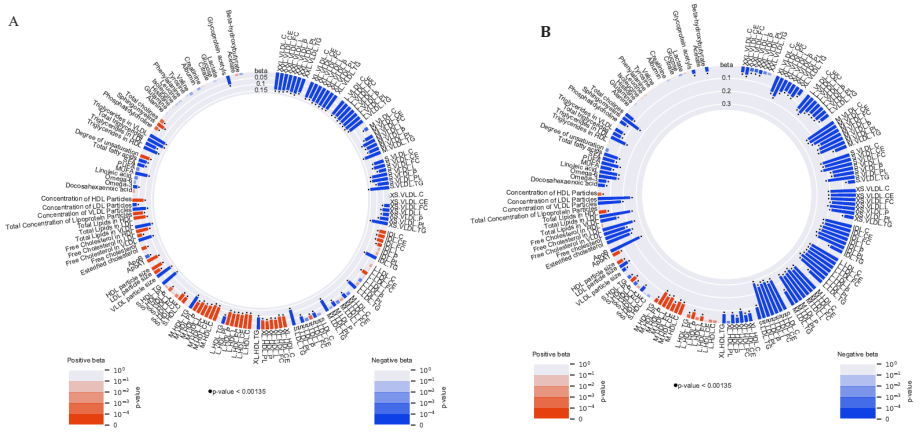



**Figure S5.** Associations of the genotype group with 145 NMR-based metabolomic measures in factorial analyses in the UK Biobank cohort (UBB) (n = 309,780): **A)** LDL-C-lowering only; **B)** lower TG via *LPL* only; **C)** lower TG via *APOA5* only; **D)** LDL-C-lowering and lower TG via *LPL*; **E)** LDL-C-lowering and lower TG via *APOA5*; **F)** lower TG via both *LPL* and *APOA5*; **G)** all scores combined.

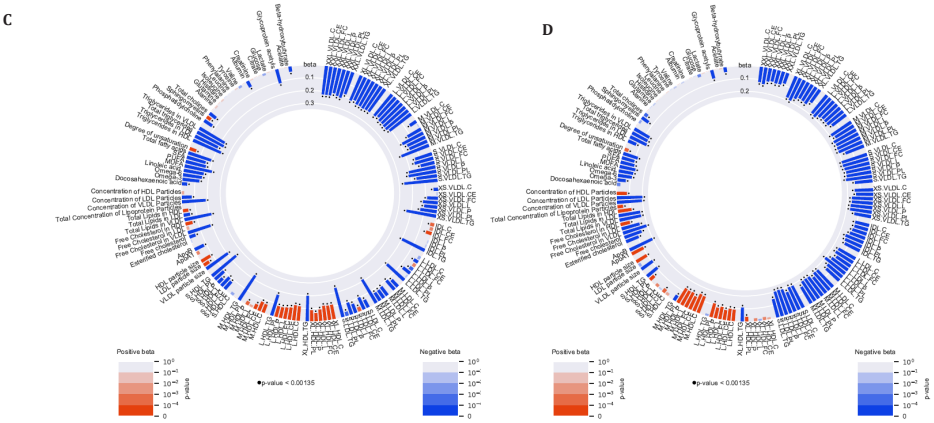
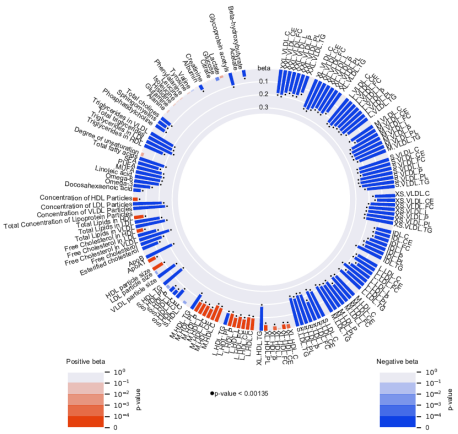



**Figure S5 continued.**

E




F

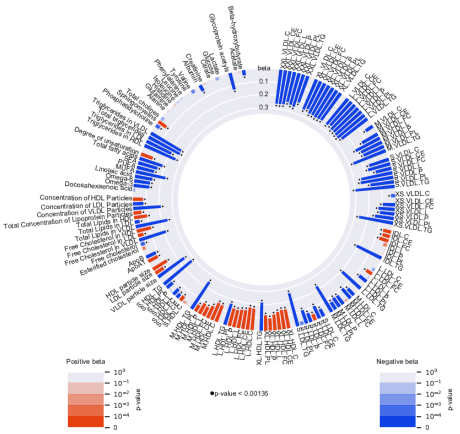



**Figure S5 continued.**

G

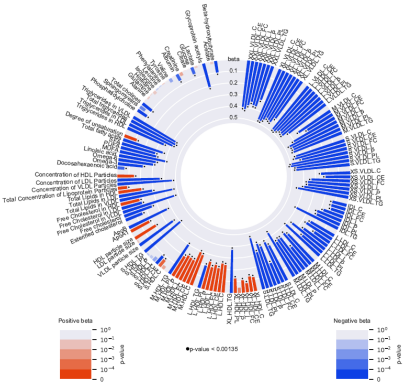



**Figure S5 continued.**