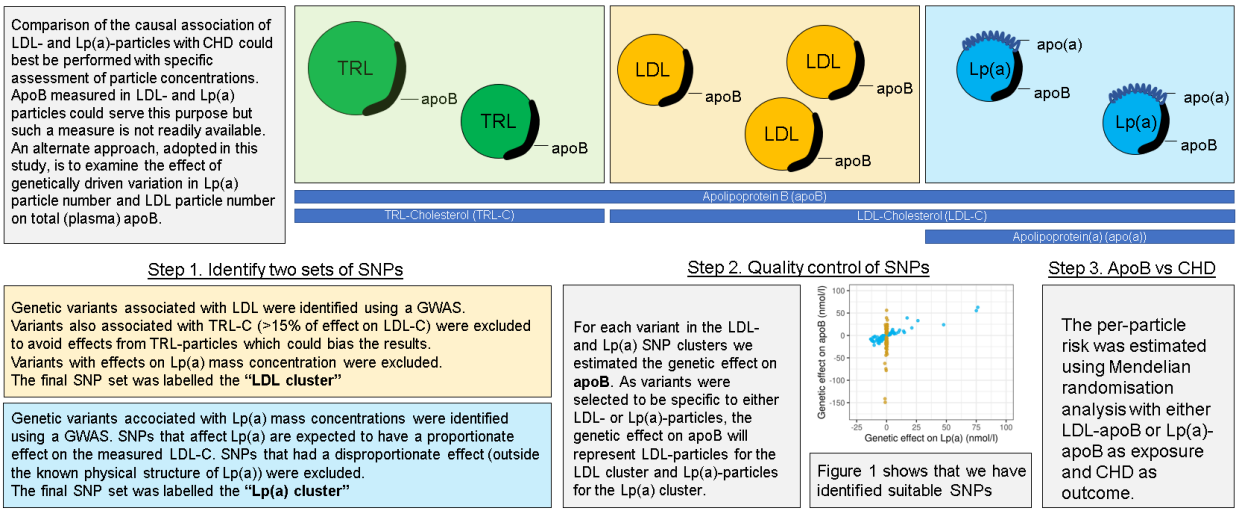
# SUPPLEMENTAL ONLINE MATERIAL

## Lipoprotein(a) is markedly more atherogenic than LDL - an apolipoprotein B-based genetic analysis in the UK Biobank

Content

**Online Expanded Rationale and Methodology.**

The novel approach to determining the CHD risk associated with variation in Lp(a) adopted in this study warrants further explanation beyond that given in the main text. Our approach is summarised in the following schematic:




Our investigation depended on:

(i) the identification in a GWAS of SNPs associated specifically with Lp(a) as measured in the UK Biobank with the Randox assay (the '***Lp(a)' SNP cluster***) (see **Online Table 2 and Figure 1**).

(ii) the identification of SNPs associated with LDL cholesterol (LDL-C) as measured in the UK Biobank cohort (the ***'LDL' SNP cluster***). Many SNPs linked to LDL-C are also associated with triglyceride rich lipoproteins (TRL) and their remnants[1] and SNPs which had a beta coefficient for TRL/remnant-cholesterol that was >15% of the beta coefficient for LDL-C (both in mmol/l) were excluded. Likewise, any SNPs associated with Lp(a) mass concentration were excluded from the 'LDL' cluster.

(iii) the quality of the apoB assay in the UK Biobank study.

Further:

(iv) it should be noted that our principal analyses were **not** based on relating the measurement of Lp(a) mass in the Randox assay to CHD risk and so any shortcomings in the assay of this lipoprotein were not a limitation in the interpretation of the results.

(v) The underlying rationale was that since both Lp(a) and LDL contain one apoB polypeptide (Lp(a) comprises a particle of LDL covalently bound to the apolipoprotein (a) protein), assessment of the genetically predicted variation in apoB using the 'Lp(a)' cluster SNPs or the 'LDL' cluster SNPs will be dependent on the accuracy and precision of the same biochemical assay for the determination of effect sizes, i.e. the assay of total plasma apoB.

***Evaluation of genetically predicted variation in apoB in the 'Lp(a)' and 'LDL' SNP clusters.***

For each of the 107 SNPs in the 'Lp(a)' cluster and the 143 SNPs in the 'LDL' cluster the beta coefficient (effect size) on apoB was estimated. In this way we could determine an effect size for ***Lp(a)-apoB*** and for ***LDL-apoB.***

By way of validation of the 'Lp(a)' and 'LDL' SNP clusters, we were able to demonstrate that:-

- SNP variants in the 'LDL' cluster had no effect on Lp(a) mass concentration (see main text **Figure 1**).
- SNPs in the 'Lp(a)' cluster had an effect size on apoB in Lp(a) that appeared equal numerically to the effect size on Lp(a) mass concentration when both were expressed in nmol/L (main text **Figure 1**). This is consistent with 1 mole of Lp(a) particles containing 1 mole of apoB.
- SNPs in the 'Lp(a)' cluster had effect sizes on LDL-C and apoB that were consistent with the known structure of Lp(a). That is, an LDL particle (with its contained cholesterol and apoB) is incorporated into Lp(a) (**Online Figure 2**).
- All the identified SNPs in the 'Lp(a)' cluster were located in the region of the *LPA* gene on chromosome 6.

Note this experimental design did not require the biochemical assay of apoB in Lp(a) particles. Rather, the SNP-based estimation of genetically predicted variation in Lp(a)-apoB and LDL-apoB permitted evaluation of the **causal** association of these variables with CHD outcomes. The relationship of genetically predicted variation in Lp(a)-apoB and LDL-apoB was assessed both for individual SNPs (main text **Figure 2**) and through use of a polygenic score for each cluster (main text **Figure 3**). Further, when considering the polygenic scores, the difference in mean measured apoB between ventiles using the 'LP(a)' cluster SNPs is attributable to the apoB component in Lp(a). Likewise, for the 'LDL' cluster SNPs, the difference between ventiles is due to the apoB in LDL.

***Use of Lp(a) mass assay measurements***

Apart from in the GWAS to identify SNPs associated with Lp(a) mass concentration, the other instances in which we employed the measured Lp(a) results were (i) as a sense check of the Lp(a)-apoB beta coefficients (main text **Figure 1B**) and (ii) the genetically predicted differences in Lp(a)-apoB relative to differences in Lp(a) mass concentration across ventiles of the polygenic score based on the 'Lp(a)' SNP cluster (**Online Figure 3**).

**Online Table 1. Definition of CHD outcomes**

| **CHD outcome** | | **Individuals, n = 487,202** |
|---|---|---|
| Non-fatal myocardial infarction (MI) | ICD 9 codes 410, 4110, 412, 42979<br>ICD 10 codes I21, I22, I23, I241, I252 | Prevalent events n = 6,577<br>Incident events n = 17,356 |
| Fatal MI | ICD 10 codes I21, I23, I241, I251, 1252, I253, I255-I259 | Incident events n = 3,850 |
| Coronary revascularisation | **Operational procedures**<br>Codes K501, K40-K44 | Prevalent events n = 2,845<br>Incident events n = 3,571 |
| Unique CHD outcomes | First event of above | Prevalent events n = 8,391<br>Incident events n = 20,792<br>Total events = 29,183 |

Incident events based on approximately 12 years of follow (as of January 2021).

**Online Table 2. SNPs with largest effect size in 'LDL' and 'Lp(a)' clusters**

| SNP | Gene | Chr | Position | Beta apoB | Beta LDL-C | Beta Lp(a) |
|---|---|---|---|---|---|---|
| **LDL cluster** | | | | | | |
| rs12721109 | APOC2_APOC4 | 19 | 45447221 | -0.1173 | -0.3467 | -0.96 |
| rs11083761 | NKPD1_MARK4 | 19 | 45655636 | -0.08209 | -0.2383 | -1.169 |
| rs76560105 | CBLC | 19 | 45299199 | -0.07773 | -0.2247 | -1.34 |
| rs139659653 | NPC1L1 | 7 | 44578747 | -0.03434 | -0.1509 | -1.44 |
| rs62119261 | IGSF23_CEACAM22P | 19 | 45122043 | -0.04299 | -0.1263 | -0.3006 |
| rs11881756 | CEACAM16_BCL3 | 19 | 45220896 | -0.04093 | -0.1207 | -0.5626 |
| rs148790687 | DNM2 | 19 | 10883157 | -0.02434 | -0.103 | -0.5396 |
| rs56315738 | SMARCA4_LDLR | 19 | 11175823 | -0.02315 | -0.08902 | 0.4909 |
| rs405509 | APOE_TOMM40 | 19 | 45408836 | 0.03089 | 0.08451 | 0.03189 |
| rs505151 | PCSK9 | 1 | 55529187 | 0.02159 | 0.08037 | -0.02321 |
| rs74257940 | LDLR | 19 | 11214475 | 0.01892 | 0.07496 | 0.6599 |
| rs62116988 | KCNN4_LYPD5 | 19 | 44296689 | -0.02026 | -0.06204 | -0.05688 |
| rs41302083 | PSRC1 | 1 | 109824680 | -0.02119 | -0.05995 | 0.03732 |
| rs1800961 | HNF4A | 20 | 43042364 | -0.01017 | -0.05591 | -0.5274 |
| rs115357389 | GDF7_HS1BP3 | 2 | 20863092 | -0.01542 | -0.05489 | -0.2616 |
| rs1229984 | ADH1B | 4 | 100239319 | -0.01091 | -0.05444 | 0.4704 |
| rs78755596 | ABO_OBP2B | 9 | 136124590 | 0.01085 | 0.05316 | -0.7721 |
| rs111282584 | SPC24 | 19 | 11262229 | 0.01319 | 0.05283 | -1.035 |
| rs77231091 | MAP2K6_KCNJ16 | 17 | 67547201 | 0.01282 | 0.05191 | 0.2098 |
| rs117490455 | SMARCA4 | 19 | 11170677 | 0.01325 | 0.0508 | 0.2682 |
| rs11244084 | SURF6_ABO | 9 | 136191010 | 0.01077 | 0.0495 | 0.1267 |
| rs62116303 | ZNF229_ZNF180 | 19 | 44957310 | -0.01632 | -0.04908 | -0.2577 |
| rs76797241 | ZNF223_ZNF222 | 19 | 44554289 | -0.01721 | -0.04739 | 0.3756 |
| rs12609269 | CEACAM20 | 19 | 45033285 | -0.01549 | -0.04652 | -0.5303 |
| rs10205003 | APOB_LOC645949 | 2 | 21482439 | -0.01601 | -0.04626 | -0.4984 |
| **Lp(a) cluster** | | | | | | |
| rs8177505 | SLC22A2 | 6 | 160258624 | 0.03455 | 0.1257 | 76.35 |
| rs3918291 | SLC22A3 | 6 | 160828142 | 0.03043 | 0.1197 | 75.09 |
| rs117446263 | SLC22A3 | 6 | 160847571 | 0.01313 | 0.04454 | 47.74 |
| rs146534110 | SLC22A1 | 6 | 160578069 | 0.01813 | 0.07418 | 26.2 |
| rs10945656 | SLC22A2_SLC22A1 | 6 | 160635886 | 0.00927 | 0.03313 | 21.11 |
| rs2282143 | SLC22A1 | 6 | 160557643 | 0.02147 | 0.0813 | 17.21 |
| rs73020718 | FLJ27255_SOD2 | 6 | 160032984 | 0.003599 | 0.01211 | 14.9 |
| rs6415084 | LPA | 6 | 160980330 | 0.005354 | 0.01945 | 14.26 |
| rs41267809 | LPA | 6 | 160953642 | -0.004511 | -0.01597 | -13.3 |
| rs143431368 | LPA | 6 | 160969693 | -0.004652 | -0.02006 | -13.08 |
| rs555754 | SLC22A3 | 6 | 160769423 | -0.006421 | -0.02238 | -12.31 |
| rs35509017 | PLG_MAP3K4 | 6 | 161217544 | -0.006298 | -0.02219 | -11.54 |
| rs41259144 | LPA | 6 | 161022107 | -0.00247 | -0.01394 | -11.43 |
| rs41272114 | LPA | 6 | 161006077 | -0.007306 | -0.02414 | -11.41 |
| rs75975688 | AGPAT4_PARK2 | 6 | 161701726 | 0.006523 | 0.02313 | 10.89 |
| rs118001500 | SLC22A2_SLC22A3 | 6 | 160683206 | -0.007847 | -0.0249 | -10.43 |
| rs74907759 | SLC22A3 | 6 | 160857193 | -0.01198 | -0.04092 | -10.42 |

| | | | | | | |
|---|---|---|---|---|---|---|
| rs58364041 | SLC22A3_LPAL2 | 6 | 160874677 | -0.002012 | -0.009743 | -10.38 |
| rs117332585 | SOD2_FNDC1 | 6 | 159857839 | 0.004119 | 0.01268 | 9.99 |
| rs4252129 | PLG | 6 | 161152905 | -0.001074 | -0.008043 | -9.784 |
| rs3798158 | SLC22A2 | 6 | 160646222 | -0.004815 | -0.02033 | -8.178 |
| rs35589108 | TCP1 | 6 | 160207845 | -0.006199 | -0.01968 | -8.148 |
| rs1937475 | PLG_MAP3K4 | 6 | 161315724 | 0.002591 | 0.007853 | 8.017 |
| rs783147 | PLG | 6 | 161137990 | -0.001907 | -0.007753 | -7.713 |
| rs74334585 | LPA | 6 | 161011907 | -0.009035 | -0.03293 | -7.12 |

Abbreviations: Chr, chromosome; beta, beta coefficient.

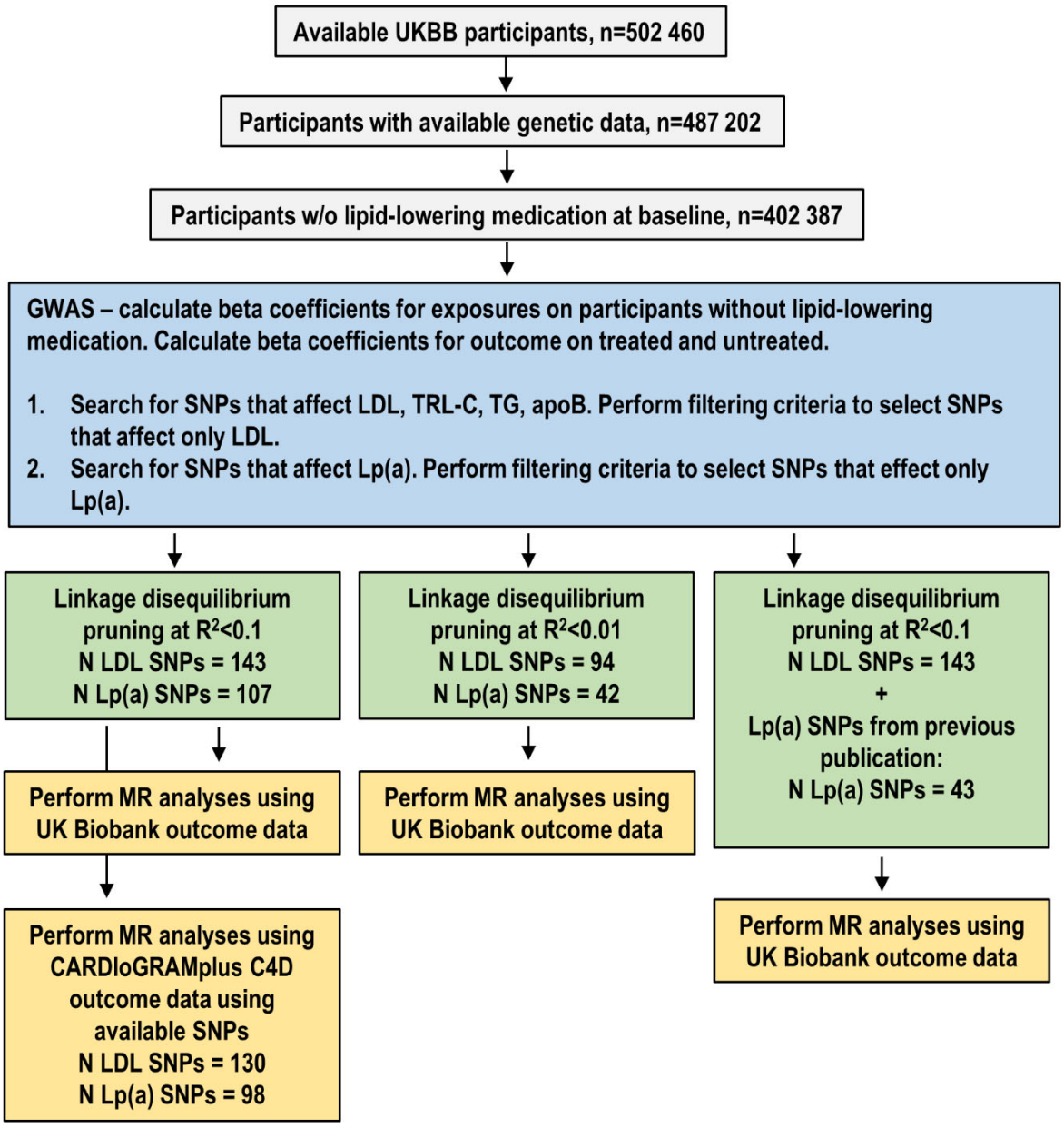
The beta-coefficients can be interpreted as the expected change in the exposure (apoB, LDL-C or Lp(a)) by one extra effect-allele. Negative beta-coefficients indicate a lipoprotein-lowering effect of the SNP (minor/less frequent allele vs major allele) and positive values indicate a lipoprotein-raising effect.

**Online Table 3. Evaluation of potential bias in Mendelian randomisation (MR) analysis.**

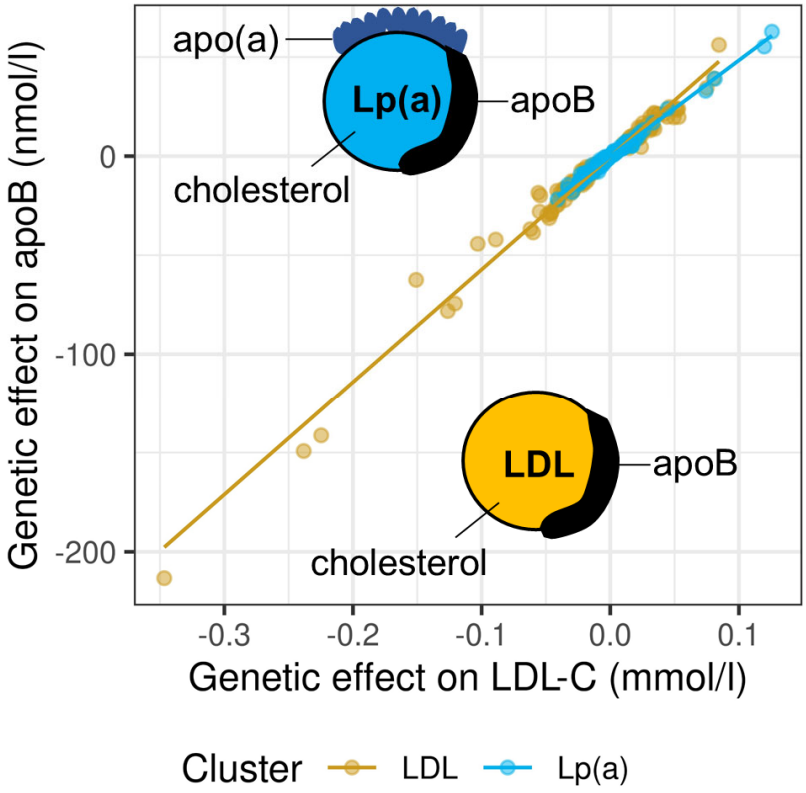
| Method | Cluster | OR per 50 nmol/l | CI low | CI high | P-value |
|---|---|---|---|---|---|
| Simple median | LDL | 1,051 | 1,035 | 1,066 | 9,6E-11 |
| Weighted median | LDL | 1,038 | 1,028 | 1,049 | 2,9E-13 |
| Penalized weighted median | LDL | 1,039 | 1,028 | 1,049 | 2,8E-13 |
| IVW | LDL | 1,038 | 1,029 | 1,048 | 3,2E-17 |
| Penalized IVW | LDL | 1,044 | 1,035 | 1,052 | 4,1E-25 |
| Robust IVW | LDL | 1,040 | 1,032 | 1,048 | 3,3E-22 |
| Penalized robust IVW | LDL | 1,043 | 1,037 | 1,050 | 9,2E-38 |
| MR-Egger | LDL | 1,030 | 1,019 | 1,042 | 2,5E-07 |
| (intercept) | LDL | 1,00013 | 1,00002 | 1,00024 | 0,026 |
| | | | | | |
| Simple median | Lp(a) | 1,27 | 1,19 | 1,35 | 1,5E-13 |
| Weighted median | Lp(a) | 1,28 | 1,22 | 1,34 | 8,9E-26 |
| Penalized weighted median | Lp(a) | 1,26 | 1,21 | 1,33 | 5,9E-23 |
| IVW | Lp(a) | 1,28 | 1,24 | 1,33 | 4,1E-47 |
| Penalized IVW | Lp(a) | 1,27 | 1,24 | 1,31 | 1,2E-64 |
| Robust IVW | Lp(a) | 1,28 | 1,22 | 1,34 | 1,8E-26 |
| Penalized robust IVW | Lp(a) | 1,27 | 1,23 | 1,31 | 6,5E-51 |
| MR-Egger | Lp(a) | 1,29 | 1,23 | 1,34 | <1E-100 |
| (intercept) | Lp(a) | 1,00 | 1,00 | 1,00 | 0,89 |

The close agreement of causal estimates across the statistical approaches provides a high degree of confidence in the application of the inverse-variance weighted (IVW) method in Mendelian randomisation models used in the present analysis. Briefly, in addition to the IVW method there are several methods or weighting-schemes that are designed to offer robustness against outlying genetic instruments. The median methods (simple, weighted and penalized weighted) all are robust methods when a minority of genetic instruments are outliers or invalid instrumental variables. The robust IVW implements a robust regression method instead of the standard linear regression method. Penalized robust IVW applies a penalty to down-weight outliers. Lastly, the MR-Egger tests for pleiotropic effects by introducing an intercept term. For further information, see the documentation to the MendelianRandomization R package (https://cran.r-project.org/web/packages/MendelianRandomization/MendelianRandomization.pdf). For each analysis, results are expressed as an odds ratio (OR) and 95% confidence interval (CI).

**Online Figure 1. Flowchart of GWAS design and SNP selection**



**Online Figure 2. Relationship of genetically predicted variation in apoB with genetically predicted variation in LDL cholesterol (LDL-C) for SNPs in the 'Lp(a)' and 'LDL' clusters.**

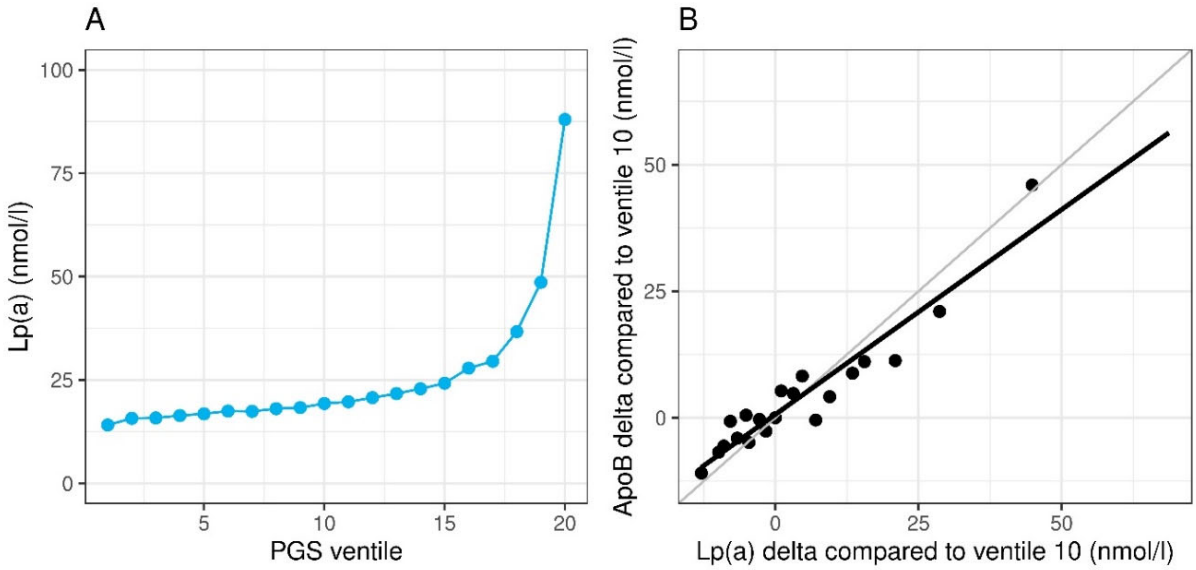



Cholesterol in Lp(a) particles is included in the measurement of LDL-C, and apoB in Lp(a) particles is included in the measurement of total plasma apoB (however, in most subjects the contribution of Lp(a) to total LDL-C and apoB is small due to the low Lp(a) mass concentration). Since an Lp(a) particle incorporates an LDL particle, then in theory the ratio of cholesterol to apoB in Lp(a) should equal the ratio of cholesterol to apoB in LDL particles.

This is what we observed. The figure above shows that the relationship of effect sizes for LDL-C and apoB for the 107 SNPs in the 'Lp(a)' cluster (due to variation in Lp(a) particle number) was the same as the relationship of effect sizes for LDL-C and apoB for the 143 SNPs in the 'LDL' cluster (due to variation in LDL particle number).

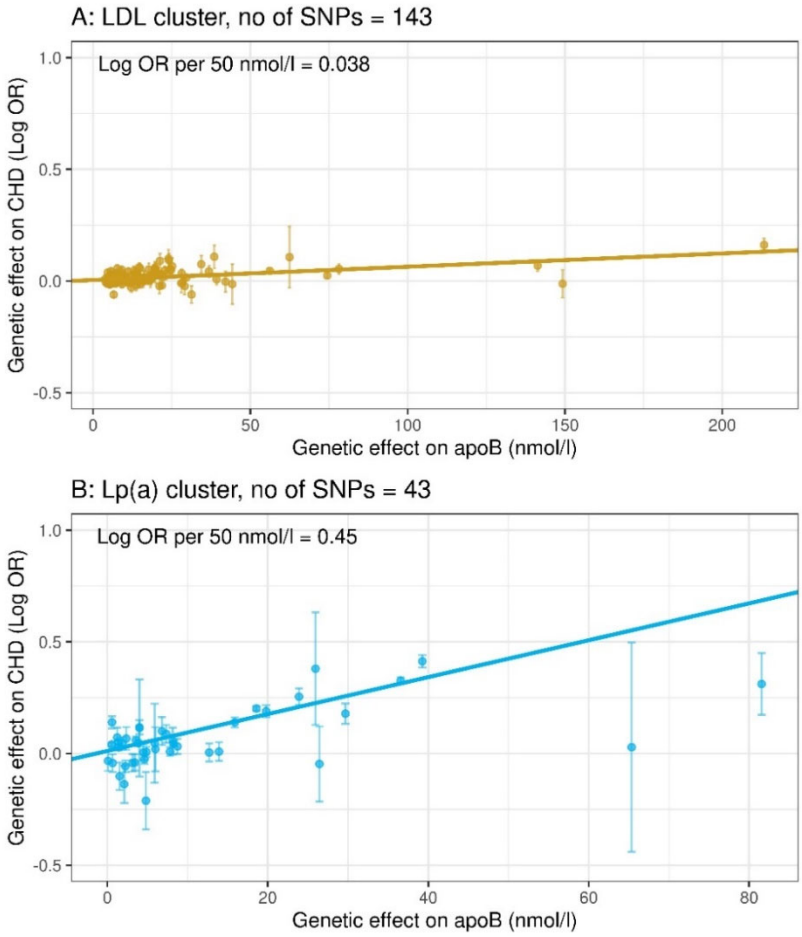
This finding indicates that the SNPs in the 'Lp(a)' cluster were providing a genetically driven variation in Lp(a) particle number that was in line with the known structural properties of Lp(a). Further, on the basis of this data it was considered unlikely that the SNPs in the 'Lp(a)' cluster were influencing any other lipoprotein species.

**Online Figure 3. Association of measured Lp(a) mass concentration with apoB by 'Lp(a)' cluster polygenic score in ventiles based on subjects not on lipid lowering therapy.**




Untreated subjects in the UK Biobank cohort (n= 415 535) were ranked by 'Lp(a)'-PGS and then divided into ventiles (20$^{ths,}$ i.e. with 20776 or 20777 subjects in each ventile). **Panel A** presents the mean measured Lp(a) mass concentration in nmol/L (Randox assay) for each ventile. In **Panel B**, the mean plasma Lp(a) and mean plasma apoB and the subsequent difference (delta) from ventile 10 (used as reference) are plotted against each other. The differences in apoB between ventiles when subjects are ranked in this way is due to the higher level of Lp(a) as shown in **Figure 1** in the main text. In theory, since one apoB is present per particle, then the two measures should show agreement, that is 1 nmol/L of Lp(a) should equal 1 nmol/L of apoB. The data presented in **Panel B** supports this contention.

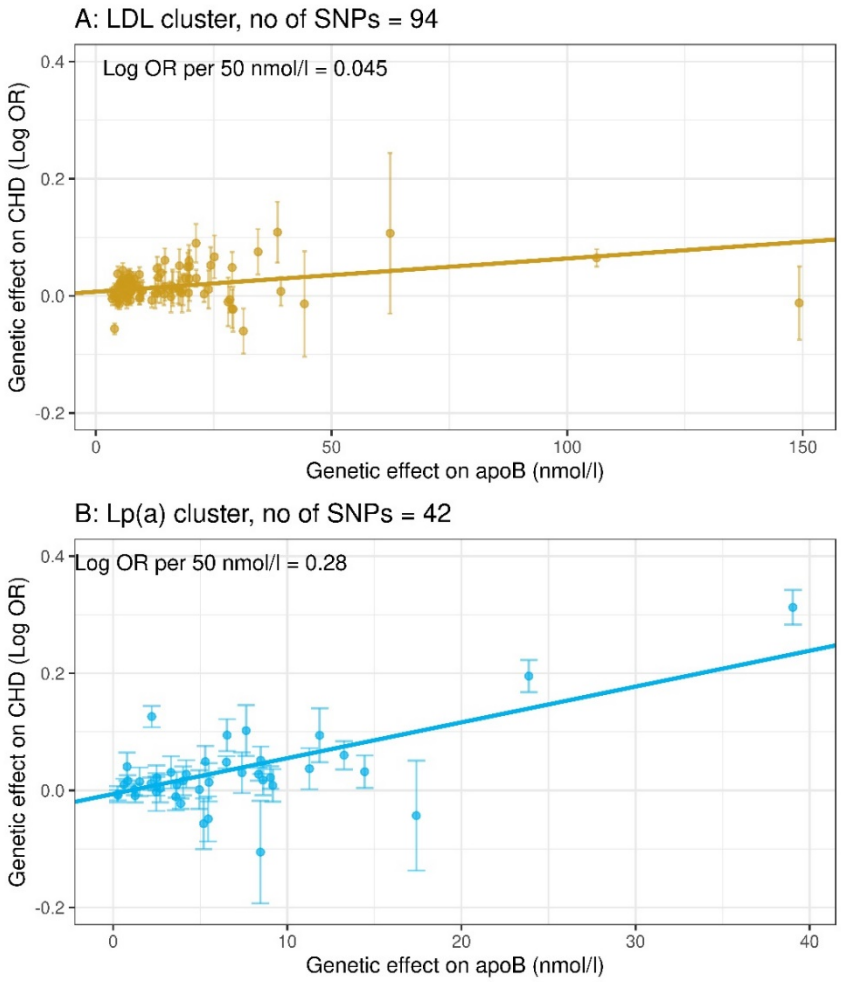
**Online Figure 4. Association of genetically-predicted apoB variation with CHD using Lp(a) SNPs from Burgess et al[2] ('LDL' cluster SNPs as in main analysis).**




| | N SNPs | LOG OR per 50 nmol/l | LOG OR CI low | LOG OR CI high | OR per 50 nmol/l | OR CI low | OR CI high | P-value |
|---|---|---|---|---|---|---|---|---|
| LDL cluster | 143 | 0.0378 | 0.029 | 0.0465 | 1.04 | 1.03 | 1.05 | 3.23e-17 |
| Lp(a) SNPs (Burgess et al[2]) | 43 | 0.447 | 0.393 | 0.502 | 1.56 | 1.48 | 1.65 | 2.81e-58 |

**A**: Scatter plot of effect sizes (beta-coefficients) of LDL-apoB against the genetic effects on CHD outcomes. B: Scatter plot of effect sizes of Lp(a)-apoB against the genetic effects on CHD outcomes. The slope for the 'Lp(a)' cluster was found to be greater than for the slope for the 'LDL' cluster. Table shows MR-model results produced by modelling the genetic instruments plotted in A (the 'LDL' cluster) and in B (the 'Lp(a)' cluster). MR model results for the Lp(a) SNP set was further adjusted for internal correlation, which only marginally affected the estimate (data not shown). Note that in the present study SNPs in the 'Lp(a)' cluster were identified in an 'agnostic' procedure in which SNP selection was not based on the strength of the association, only on its presence. In the previous study, SNPs for Lp(a) were 'selected' based on an algorithm that added variants only if they improved prediction of Lp(a) levels[2]. The main difference between the two SNP sets lies in the inclusion in the main analysis of SNP with weaker effect sizes in the 10-40 nmol/L range of genetically-predicted apoB (compare **Panel B** above with main text **Figure 2B**).

**Online Figure 5. Selection of SNPs in 'Lp(a)' and 'LDL' clusters using a linkage disequilibrium threshold of $r^2$<0.01.**




| | N SNPs | LOG OR per 50 nmol/l | LOG OR CI low | LOG OR CI high | OR per 50 nmol/l | OR CI low | OR CI high | P-value |
|---|---|---|---|---|---|---|---|---|
| LDL cluster | 94 | 0.0447 | 0.031 | 0.0583 | 1.05 | 1.03 | 1.06 | 1.43e-10 |
| Lp(a) cluster | 42 | 0.285 | 0.212 | 0.358 | 1.33 | 1.24 | 1.43 | 2.11e-14 |

**A:** Scatter plot of SNP effect sizes (beta-coefficients) for LDL-apoB against the genetic effects on CHD outcomes. **B:** Scatter plot of SNP effect sizes for Lp(a)-apoB against the genetic effects on CHD outcomes. The slope of the 'Lp(a)' cluster was found to be greater than for the slope of the 'LDL' cluster. Table shows the findings for the MR-model produced by modelling the genetic instruments plotted in **A** (the 'LDL' cluster) and in **B** (the 'Lp(a)' cluster).

## REFERENCES


1. Björnson E, Adiels M, Taskinen MR, Burgess S, Rawshani A, Borén J and Packard CJ. Triglyceride-rich lipoprotein remnants, low-density lipoproteins, and risk of coronary heart disease: a UK Biobank study, *Eur Heart J*. 2023. In Press.
2. Burgess S, Ference BA, Staley JR, et al. Association of LPA Variants With Risk of Coronary Disease and the Implications for Lipoprotein(a)-Lowering Therapies: A Mendelian Randomization Analysis. *JAMA Cardiol*. 2018;3:619-627.