SUPPLEMENTARY MATERIAL

Georgakis MK, Malik R, Burgess S, Dichgans M. Additive Effects of Genetic Interleukin-6 Signaling Downregulation and Low-Density Lipoprotein Cholesterol Lowering on Cardiovascular Disease: A 2×2 Factorial Mendelian Randomization Analysis

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Data S1. Supplementary Methods

Study population

We performed this analysis in the UK Biobank, a population-based study of 503,317 individuals aged 40-69 years recruited between 2006 and 2010. The UK Biobank has approval from the Northwest Multi-Center Research Ethics Committee. All participants provided written informed consent. We accessed the data following approval of an application by the UK Biobank Ethics and Governance Council (application #2532). The current analysis was based on unrelated White British individuals (excluded those with pihat>0.1875) with available genetic, biomarker, and outcome data. UK Biobank genotype imputation was conducted based on a merged reference panel of the Haplotype Reference Consortium (HRC) panel, the UK10K panel and the 1000 Genome Phase 3 panel.

Genetic instrument selection

To construct an instrument for LDL-C-lowering through currently used drug targets, we performed a meta-analysis of 188,577 European individuals of the Global Lipids Genetics Consortium (GLGC) GWAS with 318,366 White British individuals from the UK Biobank. We selected genetic variants associated with LDL-C at $p<5\times10^{-8}$ (clumped at $r^2<0.1$) and located within 300 kB of the genes encoding the respective drug targets for PCSK9 inhibitors, statins, and ezetimibe ($PCSK9$, $HMGCR$, $NPC1L1$). We restricted our selection to these genes to proxy LDL-C lowering through variation in targets of drug classes currently in use for lowering LDL-C. We then constructed a genetic risk score of LDL-C-lowering (LDL-C-score) using the association estimates of these variants with LDL-C from GLGC. For validation, we explored associations with Apolipoprotein-B levels, as well as with cholesterol levels across different LDL particles (small, medium, large). These analyses were done in an independent dataset of 24,495 individuals of European ancestry from 10 cohorts, among whom 123 human blood lipid and metabolite concentrations were quantified by high-throughput nuclear magnetic resonance spectroscopy metabolomics.

To construct an instrument for IL-6R-mediated downregulation of the IL-6 signaling cascade, we selected variants within the $IL6R$ gene or a region 300 kB upstream or downstream of it that were associated CRP, a downstream biomarker of IL-6 signaling reflecting its activity. We meta-analyzed a GWAS for CRP levels of 204,402 European (Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium) with data from 318,279 White British individuals in the UK Biobank. We selected variants associated with CRP levels ($p<5\times10^{-8}$) after clumping for linkage disequilibrium at $r^2<0.1$ (1000G European reference panel). We then created a genetic risk score for IL-6 signaling activity (IL-6R-score) using as weights the association estimates of the identified variants with CRP levels in CHARGE. To validate this genetic score, we explored associations with circulating levels of IL-6 in a Finnish sample of 8,293 individuals and soluble IL-6R which was quantified in the context of a blood proteomics analyses among 3,301 European ancestry individuals in the INTERVAL study, which are upstream to IL-6R-mediated signaling and both increase as a result of IL-6 signaling downregulation. We also explored associations with fibrinogen levels in the CHARGE, Consortium (N=120,246 individuals of European
ancestry), which is downstream to I-6R-mediated signaling and decreases following IL-6 signaling downregulation.

In alternative analyses aiming to address winner’s curse bias, we constructed instruments for LDL-C lowering through drug targets of currently used medications and IL-6R-mediated downregulation of the IL-6 signaling cascade by selecting genetic variants strictly on the basis of the GLGC and the CHARGE data, respectively. Specifically, we used 11 variants in PCSK9, 4 variants in HMGCR, and 3 in NPC1L1, for LDL-C-lowering and 7 variants in IL6R, in accordance with previous work.

**Study outcomes**

The data were linked with inpatient hospital episode records, primary general practitioner data, and death registry for longitudinal follow-up. The outcomes of the current study included a combined cardiovascular outcome of coronary artery disease, ischemic stroke, peripheral artery disease, aortic aneurysm, and cardiovascular death. The detailed codes used to define these outcomes are provided in Table S1. Secondary outcomes included the five separate components of the combined outcome. Incident and prevalent cases were combined in the primary analysis under the assumption that all events are incident to genetic exposures. Still, in sensitivity analyses, we explored associations with time-to-incident events among individuals free of cardiovascular disease at baseline.

**Statistical analysis**

For the main analysis, we performed 2x2 factorial Mendelian randomization analysis. In this analysis, we split our sample to 4 groups: individuals with genetic LDL-C and IL-6R-scores above median (proxy for placebo), individuals with IL-6R-score below median and LDL-C score above median (proxy for IL-6R inhibition treatment), individuals with LDL-C score below median and IL-6R-score above median (proxy for LDL-C-lowering treatment), and individuals with both scores below median (proxy for combined treatment) (Figure 1). We then explored in logistic regression models associations with the primary and secondary endpoints with the first group (proxy for placebo) as reference. To derive clinically relevant association estimates, we scaled the derived odds ratios to 38.67 mg/dL (1 mmol/L) decrement in LDL-C and 0.50 log(mg/L) decrement in log-transformed CRP levels (to approximate a 50% reduction which was reported in the CANTOS trial).

While this 2x2 method based on dichotomization might arbitrarily group participants across different levels of IL6 and LDL-C- genetic scores, it provides sufficient power to meaningfully test interactions and is also offering estimates that are easier to interpret in the clinical setting. To avoid biased estimates due to arbitrary dichotomization and to maximize power, we also analyzed the two scores as quantitative traits, also exploring their interaction. Furthermore, we explored the associations of the each score as a quantitative continuous variable with cardiovascular outcomes across deciles of the other. Finally, in an alternative approach, to test for potential non-linear effects we introduced spline factors of the two genetic scores in the same model (split in 4 equal splines). To tests if potential non-linearities in the associations

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of the two genetic scores with cardiovascular disease cloud potential interactions we also introduced to the model an interaction term between the two spline factors (4x4=16 interactions).

We explored associations with the primary and secondary outcomes in logistic regression models. Our models were adjusted for age, sex, the first 10 principal components of population structure, and the array used for genotyping (UK BILEVE Axiom array or UK Biobank Axiom array).

In sensitivity analyses, we also performed Cox regression analyses on incident events after excluding individuals with known cardiovascular disease at baseline, as well as individuals on lipid-lowering treatment at baseline. Finally, we analyzed the effects of the genetic IL-6R-score on risk of cardiovascular events across strata of measured LDL-C levels (<100 mg/dL and ≥100 mg/dL) among individuals on or not on lipid-lowering treatment at baseline.

The effects of the genetic LDL-C score on Apolipoprotein B and LDL particle levels, as well as the effects of the genetic IL-6 score on IL-6, soluble IL-6R, and fibrinogen levels were explored with two-sample inverse-variance weighted Mendelian randomization analyses.

All analyses were performed in R (v3.5.0; The R Foundation for Statistical Computing). Statistical significance threshold was set at a two-tailed P <0.05.

Table S1. Definition of outcomes in the current analysis.

| Outcome                  | N Cases | ICD-9                        | ICD-10                       | OPCS                      | Self-report* |
|--------------------------|---------|------------------------------|------------------------------|---------------------------|--------------|
| Coronary artery disease  | 43,055  | 410, 411, 412, 414.0, 414.8, 414.9 | I21, I22, I23, I24, I25.1, I25.2, I25.5, I25.6, I25.8, I25.9 | K40, K41, K42, K43, K44, K45, K46, K49, K50.1, K50.2, K50.4, K75 | 20002        |
| Ischemic stroke          | 5,747   | 434, 436                     | I63, I64                     |                           | 20002        |
| Peripheral artery disease| 19,803  | 4400, 4402, 4438, 4439       | I70.0, I70.00, I70.01, I70.02, I70.20, I70.21, I70.8, I70.80, I70.9, I70.90, I73.8, I73.9 | L21.6, L51.3, L51.6, L51.8, L52.1, L52.2, L54.1, L54.4, L54.8, L59.1, L59.2, L59.3, L59.4, L59.5, L59.6, L59.7, L59.8, L60.1, L60.2, L63.1, L63.5, L63.9, L66.7 | 20002        |
| Aortic aneurysm          | 2,164   | 441                          | I71.1-I71.9                  | L18, L19, L27, L28        | 20002        |
| Cardiovascular death     | 2,660   |                              | I chapter                    |                           |              |

* Variable coding in the UK Biobank.

ICD: International Classification of disease. OPCS: Office of Population Censuses and Surveys Classification of Surgical Operations and Procedures Classification of Interventions and Procedures.
Table S2. Genetic variants included in the genetic risk score for interleukin-6 (IL6) signaling downregulation.

| SNP     | chrom | bp_hg19 | effect allele | other allele | beta CHARGE | SE CHARGE | p-value CHARGE | beta META | SE META | p-value META |
|---------|-------|---------|---------------|--------------|-------------|------------|----------------|-----------|---------|--------------|
| rs3766925 | 1     | 154564712 | a             | t            | -0.0093     | 0.0049     | 0.057701       | -0.0148   | 0.0025  | 2.69E-09     |
| rs78035035 | 1     | 154273429 | a             | c            | 0.052       | 0.0248     | 0.036014       | 0.0457    | 0.0081  | 1.43E-08     |
| rs112203594 | 1   | 154553430 | a             | c            | 0.0591      | 0.024      | 0.013797       | 0.0396    | 0.0071  | 2.09E-08     |
| rs3738028 | 1     | 154698817 | a             | c            | 0.0121      | 0.0047     | 0.010039       | 0.0137    | 0.0023  | 1.19E-09     |
| rs116141616 | 1   | 154416069 | a             | g            | 0.0614      | 0.0197     | 0.001829       | 0.0387    | 0.0069  | 1.76E-08     |
| rs12406117 | 1     | 154740879 | a             | g            | 0.0136      | 0.0043     | 0.001563       | 0.0124    | 0.0021  | 3.83E-09     |
| rs144029367 | 1   | 154455249 | t             | c            | -0.0752     | 0.0185     | 4.81E-05       | 0.0498    | 0.008   | 5.43E-10     |
| rs61806853 | 1     | 154154587 | t             | c            | 0.0456      | 0.0112     | 4.67E-05       | 0.0437    | 0.005   | 1.23E-18     |
| rs76289529 | 1     | 154516404 | t             | c            | -0.0715     | 0.0171     | 2.90E-05       | -0.0519   | 0.006   | 4.78E-18     |
| rs6698385 | 1     | 154652572 | a             | g            | -0.0216     | 0.0051     | 2.28E-05       | -0.035    | 0.0026  | 3.74E-41     |
| rs145262901 | 1 | 154394484 | a             | g            | -0.0844     | 0.0183     | 3.99E-06       | -0.061    | 0.0102  | 2.38E-09     |
| rs145909430 | 1   | 154391504 | t             | c            | 0.1396      | 0.0272     | 2.86E-07       | 0.1001    | 0.0082  | 2.86E-34     |
| rs41269913 | 1     | 154461480 | t             | c            | -0.0779     | 0.0147     | 1.16E-07       | -0.0424   | 0.0058  | 2.28E-13     |
| rs77994623 | 1     | 154505106 | t             | c            | 0.0332      | 0.0061     | 5.25E-08       | 0.046     | 0.0029  | 1.50E-58     |
| rs183641528 | 1   | 154499328 | a             | g            | -0.1034     | 0.0178     | 6.29E-09       | -0.0851   | 0.008   | 1.62E-26     |
| rs113580743 | 1 | 154420333 | a             | g            | 0.0708      | 0.0112     | 2.59E-10       | 0.055     | 0.0055  | 1.09E-23     |
| rs34693607 | 1     | 154661369 | c             | g            | 0.0368      | 0.0057     | 1.07E-10       | 0.0328    | 0.0026  | 3.83E-36     |
| rs56100876 | 1     | 154496473 | a             | g            | -0.18       | 0.0262     | 6.41E-12       | -0.117    | 0.0086  | 3.27E-42     |
| rs12735458 | 1     | 154361406 | a             | g            | 0.126       | 0.0183     | 5.77E-12       | 0.0842    | 0.0092  | 4.53E-20     |
| rs73026617 | 1     | 154369981 | t             | c            | 0.0474      | 0.0068     | 3.16E-12       | 0.0467    | 0.0034  | 1.69E-42     |
| rs7525477 | 1     | 154394297 | a             | g            | 0.0382      | 0.0051     | 6.88E-14       | 0.0296    | 0.0023  | 1.35E-38     |
| rs11264224 | 1     | 154568086 | a             | c            | 0.0465      | 0.0057     | 3.41E-16       | 0.0418    | 0.0028  | 1.60E-49     |
| rs16836054 | 1     | 154462195 | a             | g            | 0.0453      | 0.0054     | 4.91E-17       | 0.0516    | 0.0028  | 1.51E-75     |
| rs12059682 | 1     | 154579585 | t             | c            | -0.0441     | 0.0049     | 2.26E-19       | -0.0474   | 0.0025  | 2.11E-77     |
| rs12083537 | 1     | 154381103 | a             | g            | 0.0643      | 0.0053     | 7.14E-34       | 0.0679    | 0.0026  | 3.03E-156    |
| rs2228145 | 1     | 154426970 | a             | c            | 0.0899      | 0.0042     | 1.21E-101      | 0.0947    | 0.0021  | 3.00E-307    |

chrom: chromosome; bp_hg19: genomic position according to the GRCh37/hg19 reference genome. SE: standard error; META: meta-analysis
Table S3. Genetic variants included in the genetic risk score for low-density lipoprotein cholesterol (LDL-C) lowering.

| SNP       | gene   | chrom | bp_hg19   | effect allele | other allele | beta  | SE    | p-value  | beta  | SE    | p-value  | beta  | SE    | p-value  |
|-----------|--------|-------|-----------|---------------|--------------|--------|-------|----------|--------|-------|----------|--------|-------|----------|
| rs10888896 | PCSK9  | 1     | 55509213  | c             | g            | 0.0426 | 0.0049 | 2.14E-14 | 0.0414 | 0.0024 | 3.44E-68 |        |       |          |
| rs11206510 | PCSK9  | 1     | 55496039  | t             | c            | 0.0831 | 0.005  | 2.38E-53 | 0.0667 | 0.0026 | 1.03E-149 |       |       |          |
| rs11583974 | PCSK9  | 1     | 55551718  | a             | g            | 0.0646 | 0.0117 | 3.95E-09 | 0.0518 | 0.0052 | 4.19E-23 |        |       |          |
| rs11591147 | PCSK9  | 1     | 55505647  | g             | t            | 0.497  | 0.018  | 8.58E-143| 0.4408 | 0.0078 | 2.54E-701 |       |       |          |
| rs1475701  | PCSK9  | 1     | 55638546  | c             | t            | 0.0904 | 0.0092 | 1.46E-20 | 0.0901 | 0.0054 | 1.27E-61 |        |       |          |
| rs17111483 | PCSK9  | 1     | 55485098  | t             | c            | 0.0345 | 0.0084 | 2.30E-06 | 0.0308 | 0.0036 | 1.28E-17 |        |       |          |
| rs207145   | PCSK9  | 1     | 55808143  | t             | c            | 0.0495 | 0.0057 | 6.19E-18 | 0.0284 | 0.0031 | 3.25E-20 |        |       |          |
| rs2479394  | PCSK9  | 1     | 55486064  | g             | a            | 0.0386 | 0.0041 | 1.58E-19 | 0.0347 | 0.0022 | 1.19E-55 |        |       |          |
| rs2479409  | PCSK9  | 1     | 55504650  | g             | a            | 0.0642 | 0.0041 | 2.52E-50 | 0.0488 | 0.0021 | 1.29E-118 |       |       |          |
| rs2500340  | PCSK9  | 1     | 55464743  | c             | t            | 0.0159 | 0.0066 | 0.01869  | 0.0181 | 0.0026 | 3.55E-12 |        |       |          |
| rs2647280  | PCSK9  | 1     | 55725200  | g             | a            | 0.0171 | 0.0059 | 0.006112| 0.0136 | 0.0022 | 6.69E-10 |        |       |          |
| rs2647281  | PCSK9  | 1     | 55724704  | g             | a            | 0.0589 | 0.0095 | 2.27E-09 | 0.0307 | 0.0046 | 2.83E-11 |        |       |          |
| rs4927207  | PCSK9  | 1     | 55713628  | g             | a            | 0.0692 | 0.0049 | 2.36E-39 | 0.0574 | 0.0028 | 6.07E-96 |        |       |          |
| rs4927218  | PCSK9  | 1     | 55749649  | a             | g            | 0.0468 | 0.0116 | 0.0003197| 0.0457 | 0.0047 | 2.67E-22 |        |       |          |
| rs585131   | PCSK9  | 1     | 55524116  | t             | c            | 0.0637 | 0.005  | 2.70E-35 | 0.0402 | 0.0026 | 4.08E-54 |        |       |          |
| rs6662286  | PCSK9  | 1     | 55730327  | c             | t            | 0.0989 | 0.0073 | 6.30E-36 | 0.0588 | 0.0037 | 5.23E-56 |        |       |          |
| rs7552841  | PCSK9  | 1     | 55518752  | t             | c            | 0.0368 | 0.0044 | 5.40E-15 | 0.0314 | 0.0022 | 1.82E-47 |        |       |          |
| rs10447161 | HMGCR  | 5     | 74449472  | g             | c            | 0.0303 | 0.0073 | 8.42E-06 | 0.0288 | 0.0032 | 6.73E-19 |        |       |          |
| rs10474435 | HMGCR  | 5     | 74657280  | c             | t            | 0.0536 | 0.0149 | 0.002362| 0.0508 | 0.0087 | 5.29E-09 |        |       |          |
| rs114796667| HMGCR  | 5     | 74928541  | t             | c            | 0.0369 | 0.0136 | 0.009135| 0.0658 | 0.0062 | 3.70E-26 |        |       |          |
| rs12916    | HMGCR  | 5     | 74656539  | c             | t            | 0.0733 | 0.0038 | 7.79E-78 | 0.0767 | 0.002 | 1.69E-315 |        |       |          |
| rs17244939 | HMGCR  | 5     | 74631096  | a             | c            | 0.0537 | 0.0202 | 0.01483  | 0.0424 | 0.0075 | 1.24E-08 |        |       |          |
| rs2241402  | HMGCR  | 5     | 74646255  | a             | t            | 0.0328 | 0.0113 | 0.001236| 0.0314 | 0.0047 | 1.53E-11 |        |       |          |
| rs3857388  | HMGCR  | 5     | 74620377  | c             | t            | 0.0421 | 0.0059 | 2.20E-11 | 0.034  | 0.0031 | 2.22E-27 |        |       |          |
| rs4703665  | HMGCR  | 5     | 74602898  | c             | t            | 0.0241 | 0.006  | 1.34E-05 | 0.0293 | 0.0029 | 8.39E-24 |        |       |          |
| rs68160747 | HMGCR  | 5     | 74569432  | a             | c            | 0.0287 | 0.0105 | 0.009556| 0.0303 | 0.0048 | 1.87E-10 |        |       |          |
| rs74695562 | HMGCR  | 5     | 74675951  | t             | g            | 0.0315 | 0.0127 | 0.02198 | 0.0359 | 0.0052 | 3.78E-12 |        |       |          |
| SNP       | Gene  | Chrom | BP (hg19) | Minor Allele | Minor Allele Frequency | Minor Allele MAF | Major Allele Frequency | Major Allele MAF | P Value (meta-analysis) |
|-----------|-------|-------|-----------|--------------|------------------------|-----------------|------------------------|-----------------|------------------------|
| rs76733602 | HMGCR | 5     | 74562373  | g            | 0.0442                | 0.014           | 0.004198               | 0.0378          | 0.005                  | 5.07E-14 |
| rs7726378  | HMGCR | 5     | 74337139  | t            | 0.0184                | 0.0058          | 0.001492              | 0.0251          | 0.0024                 | 4.79E-25 |
| rs77443979 | HMGCR | 5     | 74779202  | c            | 0.0691                | 0.022           | 0.01642               | 0.0659          | 0.0102                 | 9.52E-11 |
| rs80324692 | HMGCR | 5     | 74717761  | c            | 0.0313                | 0.0093          | 0.001246              | 0.0327          | 0.0039                 | 6.97E-17 |
| rs9654427  | HMGCR | 5     | 74466833  | g            | 0.0494                | 0.0074          | 1.58E-10              | 0.0562          | 0.0042                 | 6.42E-41 |
| rs10257749 | NPC1L1| 7     | 44388619  | t            | 0.0164                | 0.0048          | 0.0005077             | 0.0179          | 0.0024                 | 1.57E-13 |
| rs2073547  | NPC1L1| 7     | 44582331  | g            | 0.0485                | 0.0049          | 1.92E-21              | 0.0474          | 0.0026                 | 2.34E-76 |
| rs217355   | NPC1L1| 7     | 44626377  | t            | 0.0294                | 0.0037          | 4.13E-14              | 0.0256          | 0.002                  | 9.74E-38 |
| rs2300414  | NPC1L1| 7     | 44682938  | a            | 0.0353                | 0.008           | 5.45E-06              | 0.024           | 0.004                  | 2.87E-09 |

chrom: chromosome; bp_hg19: genomic position according to the GRCh37/hg19 reference genome. SE: standard error; META: meta-analysis
Table S4. Baseline characteristics by groups determined by the genetic interleukin-6 (IL6) and low-density lipoprotein cholesterol (LDL-C) scores.

| Variable                      | Both scores ≥ median | IL-6-score < median | LDL-C score < median | Both scores < median | p-value  |
|-------------------------------|----------------------|---------------------|----------------------|----------------------|----------|
| N                             | 104,205              | 104,203             | 104,205              | 104,204              |          |
| Sex, % females                | 54.1                 | 54.2                | 54.0                 | 53.9                 | 0.3248   |
| Age, mean (SD)                | 56.9 (8.0)           | 56.9 (8.0)          | 56.9 (8.0)           | 56.9 (8.0)           | 0.8451   |
| BMI, mean (SD)                | 27.4 (4.8)           | 27.4 (4.8)          | 27.5 (4.8)           | 27.5 (4.8)           | 0.1341   |
| SBP, mean (SD)                | 141.6 (20.6)         | 141.6 (20.7)        | 141.5 (20.6)         | 141.6 (20.6)         | 0.6410   |
| DBP, mean (SD)                | 84.4 (11.3)          | 84.4 (11.3)         | 84.4 (11.3)          | 84.4 (11.3)          | 0.9132   |
| Smoking                       |                      |                     |                      |                      | 0.0713   |
| current, %                    | 54.6                 | 54.4                | 54.1                 | 54.6                 |          |
| former, %                     | 35.2                 | 35.6                | 35.4                 | 35.1                 |          |
| never, %                      | 10.2                 | 10.0                | 10.5                 | 10.3                 |          |
| HbA1c, mean (SD)              | 5.46 (2.77)          | 5.44 (2.76)         | 5.46 (2.75)          | 5.44 (2.75)          | 4.9x10^-06 |
| CRP, mean (SD)                | 2.74 (4.49)          | 2.48 (4.24)         | 2.76 (4.56)          | 2.47 (4.21)          | <2x10^-16 |
| LDL-C, mean (SD)              | 139.3 (34.4)         | 139.7 (34.2)        | 135.9 (33.3)         | 136.2 (33.3)         | <2x10^-16 |
| HDL-C, mean (SD)              | 55.9 (14.8)          | 56.2 (14.8)         | 55.9 (14.7)          | 56.1 (14.8)          | 0.0091   |
| TG, mean (SD)                 | 156.1 (91.3)         | 156.3 (91.2)        | 155.5 (90.8)         | 155.5 (90.3)         | 0.2071   |
| ApoB, mean (SD)               | 1.04 (0.24)          | 1.05 (0.24)         | 1.02 (0.24)          | 1.02 (0.24)          | <2x10^-16 |
| ApoA1, mean (SD)              | 1.52 (0.27)          | 1.54 (0.27)         | 1.52 (0.27)          | 1.54 (0.27)          | 1.2x10^-06 |
| Current lipid-lowering treatment, % | 19.4                 | 18.9                | 17.3                 | 17.1                 | <2x10^-16 |

SD: standard deviation; BMI: body mass index; SBP: systolic blood pressure, DBP: diastolic blood pressure; HbA1c: glycated hemoglobin A1c; CRP: C-reactive protein; LDL-C: low-density lipoprotein cholesterol; HDL-C: high low-density lipoprotein cholesterol; TG: triglycerides; ApoB: apolipoprotein B; ApoA1: apolipoprotein A1.
**Table S5.** Time-to-event analysis for incident cardiovascular events among individuals without a history of cardiovascular disease at baseline.

|                       | HR   | 95% CI   | p-value    |
|-----------------------|------|----------|------------|
| **Full dataset**      |      |          |            |
| Both scores < median  | 0.927| 0.904    | 0.951      | 6.7E-09    |
| IL6 score < median    | 0.960| 0.936    | 0.984      | 0.0015     |
| LDL-C score < median  | 0.962| 0.938    | 0.987      | 0.0028     |
| Both scores ≥ median  | 1 (reference) |          |            |
| IL6 score per 1-SD-decrement | 0.984| 0.975    | 0.993      | 0.0006     |
| LDL-C score per 1-SD decrement | 0.969| 0.961    | 0.978      | 3.1E-11    |
| **Individuals not on lipid-lowering treatments** |      |          |            |
| Both scores < median  | 0.931| 0.902    | 0.961      | 1.1E-05    |
| IL6 score < median    | 0.985| 0.954    | 1.017      | 0.3445     |
| LDL-C score < median  | 0.982| 0.951    | 1.013      | 0.2480     |
| Both scores ≥ median  | 1 (reference) |          |            |
| IL6 score per 1-SD-decrement | 0.983| 0.972    | 0.994      | 0.0032     |
| LDL-C score per 1-SD decrement | 0.968| 0.957    | 0.978      | 8.33E-09   |
| **P for interaction** |      |          | 0.2324     |

The results are derived from Cox regression models adjusted for age, sex, the first 10 principal components of population structure, and the array used for genotyping.

HR: hazard ratio; CI: confidence intervals; IL6: interleukin-6; LDL-C: low-density lipoprotein cholesterol.
**Table S6.** Independent associations between genetic IL-6 and LDL-C scores with risk of cardiovascular disease based on genetic variants selected from the GLGC and CHARGE Consortia.

| Exposure                              | OR    | 95%CI   | p-value    |
|---------------------------------------|-------|---------|------------|
| Both scores < median                  | 0.921 | 0.895   | 0.948      | 6.7E-09   |
| IL6 score < median                    | 0.962 | 0.934   | 0.990      | 0.0082    |
| LDL-C score < median                  | 0.956 | 0.929   | 0.985      | 0.0026    |
| Both scores ≥ median                  | 1 (reference) |         |            |
| IL6 score per 1-SD-decrement          | 0.983 | 0.974   | 0.994      | 0.0027    |
| LDL-C score per 1-SD decrement        | 0.961 | 0.951   | 0.971      | 4.1E-14   |
| *P* for interaction                   |       |         | 0.3391     |

The results are derived from logistic regression models adjusted for age, sex, the first 10 principal components of population structure, and the array used for genotyping.

**OR:** odds ratio; **CI:** confidence intervals; **IL6:** interleukin-6; **LDL-C:** low-density lipoprotein cholesterol.
Figure S1. Effects of genetically downregulated IL6 signaling on upstream and downstream molecules in the IL6 cascade.

The error lines correspond to 95% confidence intervals of beta coefficients per 1 ln-CRP decrement, as derived from fixed effects inverse-variance weighted 2-sample Mendelian randomization analyses. IL6: interleukin-6; sIL6R: soluble interleukin-6 receptor; Fg: fibrinogen.
**Figure S2.** Effects of genetically downregulated LDL-C through known drug targets on Apolipoprotein-B and cholesterol levels across different LDL particles.

**Effects of 1-SD decrement in LDL-C**

The error lines correspond to 95% confidence intervals of beta coefficients per 1 standard deviation (SD) decrement in LDL-C, as derived from fixed effects inverse-variance weighted 2-sample Mendelian randomization analyses.

ApoB: apolipoprotein B; S.LDL.C: small low-density lipoprotein cholesterol; M.LDL.C: medium low-density lipoprotein cholesterol; L.LDL.C: large low-density lipoprotein cholesterol.
Figure S3. Associations of the genetic LDL-C and IL6 scores with measured LDL-C levels and CRP levels in the UK Biobank.

The error lines correspond to 95% confidence intervals. The betas and p-values are derived from linear models for LDL-C and CRP adjusted for age, sex, and both the genetic LDL-C and IL-6 scores included (the betas correspond to SD-increments in the genetic scores).

IL6: interleukin-6; LDL-C: low-density lipoprotein cholesterol; CRP: C-reactive protein.
Figure S4. Associations between (A) genetic IL-6R score (be1-SD increment) and genetic LDL-C score (1-SD increment) with cardiovascular disease across deciles of the genetic LDL-C and IL-6R scores, respectively.

The error lines correspond to 95% confidence intervals. The logORs (log-Odds Ratio) are derived from logistic regression models adjusted for age, sex, the first 10 principal components of population structure, and the array used for genotyping. IL-6: interleukin-6; LDL-C: low-density lipoprotein cholesterol; CRP: C-reactive protein.
**Figure S5.** Non-linear associations between the (A) genetic LDL-C score and (B) genetic IL-6R score with cardiovascular disease, as derived from restricted cubic spline models.

\[ P \]-values for non-linearity derived from log-likelihood ratio test comparisons between the spline models and linear models were (A) 0.09 and (B) 0.13.

The error lines correspond to 95% confidence intervals.

The spline representations (restricted cubic splines) are derived from logistic regression models adjusted for age, sex, the first 10 principal components of population structure, and the array used for genotyping.

IL-6: interleukin-6; LDL-C: low-density lipoprotein cholesterol.
**Figure S6.** Associations between genetic IL6-score (below vs. above the median) and risk of cardiovascular disease across different groups of measured LDL-C levels depending on receipt of lipid-lowering drugs.

The results are derived from logistic regression models adjusted for age, sex, the first 10 principal components of population structure, and the array used for genotyping. The depicted estimates correspond to associations of scores below than median vs. scores above than median in genetic IL-6 score across the presented groups. OR: odds ratio; CI: confidence interval; IL6: interleukin-6; LDL-C: low-density lipoprotein cholesterol.