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BRIEF COMMUNICATION

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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BACKGROUND: Although trials suggest that anti-inflammatory approaches targeting interleukin (IL)-6 signaling can reduce cardiovascular risk, it remains unknown whether targeting IL-6 signaling could reduce risk additively to low-density lipoprotein cholesterol (LDL-C) lowering. Here, we assess interactions in associations of genetic downregulation of IL-6 signaling and LDL-C lowering with lifetime cardiovascular disease risk.

METHODS AND RESULTS: Genetic scores for IL-6 signaling downregulation and LDL-C lowering were used to divide 408 225 White British individuals in UK Biobank into groups of lifelong exposure to downregulated IL-6 signaling, lower LDL-C, or both. Associations with risk of cardiovascular disease (coronary artery disease, ischemic stroke, peripheral artery disease, aortic aneurysm, vascular death) were explored in factorial Mendelian randomization. Compared with individuals with genetic IL-6 and LDL-C scores above the median, individuals with LDL-C scores lower than the median but IL-6 scores above the median had an odds ratio (OR) of 0.96 (95% CI, 0.93–0.98) for cardiovascular disease. A similar OR (0.96; 95% CI, 0.93–0.98) was estimated for individuals with genetic IL-6 scores below the median but LDL-C scores above the median. Individuals with both genetic scores lower than the median were at lower odds of cardiovascular disease (OR, 0.92; 95% CI, 0.90–0.95). There was no interaction between the 2 scores (relative excess risk attributed to interaction index, 0; synergy index, 1; *P* for multiplicative interaction=0.51). Genetic IL-6 score below the median was associated with lower cardiovascular disease risk across measured LDL-C strata (<100 or ≥100 mg/dL).

CONCLUSIONS: Genetically downregulated IL-6 signaling and genetically lowered LDL-C are associated with additively lower lifetime risk of cardiovascular disease. Future trials should explore combined IL-6 inhibition and LDL-C lowering treatments for cardiovascular prevention.

Key Words: atherosclerosis ■ inflammation ■ interleukin-6 ■ low-density lipoprotein ■ Mendelian randomization

Accumulating evidence supports the fact that targeting inflammation can offer benefits in atherosclerosis independently of lipid-lowering approaches. First, the Canakinumab Anti-Inflammatory Thrombosis Outcome Study (CANTOS),[1] Colchicine Cardiovascular Outcomes Trial (COLCOT),[2] and Low-Dose Colchicine 2 (LoDoCo2)[3] trials showed that anti-inflammatory approaches directly or indirectly targeting

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upstream regulators of interleukin (IL)-6 signaling can lower cardiovascular risk without affecting low-density lipoprotein cholesterol (LDL-C) levels. Second, Mendelian randomization analyses showed genetic downregulation of IL-6 signaling to be associated with a lower risk of vascular events[4,5] and a more favorable cardiometabolic profile,[6] but not LDL-C levels.[6] Third, even at very low LDL-C, high CRP (C-reactive protein) levels—a marker of IL-6 signaling activation—predict vascular events, thus suggesting the presence of residual inflammatory risk beyond cholesterol lowering. These results have motivated efforts aiming to directly interfere with the IL-6 signaling cascade in patients with cardiovascular disease, which are already at the phase of clinical testing with promising results.[7]

Yet, it remains unknown whether there would be any interaction between lipid-lowering and anti-inflammatory strategies regarding the effects of IL-6-targeting and LDL-C-lowering approaches on cardiovascular risk. The Pravastatin Inflammation/CRP Evaluation (PRINCE)[8] and Justification for the Use of Statin in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER)[9] trials have shown that statins also reduce CRP levels beyond the expected reductions in LDL-C levels and this effect has been associated with additional reductions in vascular event rates. Conversely, tocilizumab, a monoclonal antibody targeting the IL-6 receptor (IL-6R), has been shown to elevate circulating cholesterol levels.[4] Thus, it is not clear whether aggressive targeting of both lipid accumulation and inflammation to minimize residual risk would offer additive benefits in patients with atherosclerosis. To test such a hypothesis, a 2×2 factorial trial design has been proposed that would test the efficacy of a combination therapy of IL-6 signaling inhibition and LDL-C lowering.[10]

Here, we use large-scale data to test whether there is genetic support for this hypothesis before investing in a clinical trial. Specifically, we used a 2×2 factorial Mendelian randomization study design to compare the associations of (1) downregulated IL-6 signaling attributed to variation in the gene-encoding IL-6R, (2) lower LDL-C levels as a result of variation in genes encoding lipid-lowering drug targets (PCSK9 [proprotein convertase subtilisin/kexin type 9] inhibitors, statins, ezetimibe), or (3) both with the lifetime risk of cardiovascular disease. Evidence of an interaction between genetically predicted LDL-C levels and genetically predicted IL-6 signaling activity, and specifically an attenuation of the effect of the latter in genetically lowered LDL-C levels, would indicate a relevance of the IL-6 signaling pathway only under high LDL-C levels. We hypothesized that genetically regulated IL-6 signaling and genetically predicted LDL-C levels are additively associated with the lifetime risk of cardiovascular disease and as such might represent independent targets for lowering residual cardiovascular risk.

METHODS

The data used in these analyses are available from the UK Biobank (UKB) upon approval of a submitted research proposal. The UKB has institutional review board 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 UKB Ethics and Governance Council (Application No. 2532). The genetic variants used to generate the genetic risk scores for the presented analyses are available in Data S1. A detailed description of the methods is provided in Data S1.

We performed this analysis in 408 225 unrelated White British individuals from the UKB, a population-based study of individuals aged 40 to 69 years. To construct a score for IL6 signaling downregulation (IL-6 score),[5] we selected variants within 300 kB of the *IL6R* gene that were associated at $P<5\times10^{-8}$ ($r^2<0.1$) with CRP, a downstream biomarker of IL6 signaling,[4] in a meta-analysis of 522 681 European individuals from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium and UKB. To avoid bias due to winner's curse, we weighed the score solely on the basis of the effects of the identified genetic variants on CRP levels in the CHARGE Consortium (and not in the meta-analysis with UKB). Also, in sensitivity analyses, we restricted our selection to variants strictly selected on the basis of the CHARGE data, as previously described.[5] For validation, we explored associations with IL6, soluble IL6R, and fibrinogen levels (please see Data S1 for a description of the sample).[5] To construct a genetic score for LDL-C lowering through currently used drug targets (LDL-C score), 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 drug targets for PCSK9 inhibitors, statins, and ezetimibe (*PCSK9*, *HMGCR*, *NPC1L1*) in a meta-analysis of 504 943 European individuals from the Global Lipids Genetics Consortium (GLGC) Consortium and UKB. To avoid bias attributed to winner's curse, we weighed the score solely on the basis of the effects of the identified genetic variants on LDL-C levels in the GLGC Consortium (and not in the meta-analysis with UKB). Also, in sensitivity analyses, we restricted our selection to variants strictly selected on the basis of the GLGC data, as previously described.[11] For validation, we explored associations with apolipoprotein B levels and cholesterol levels across LDL particles (please see Data S1 for a description of the sample).

The primary combined outcome included coronary artery disease, ischemic stroke, peripheral artery disease,

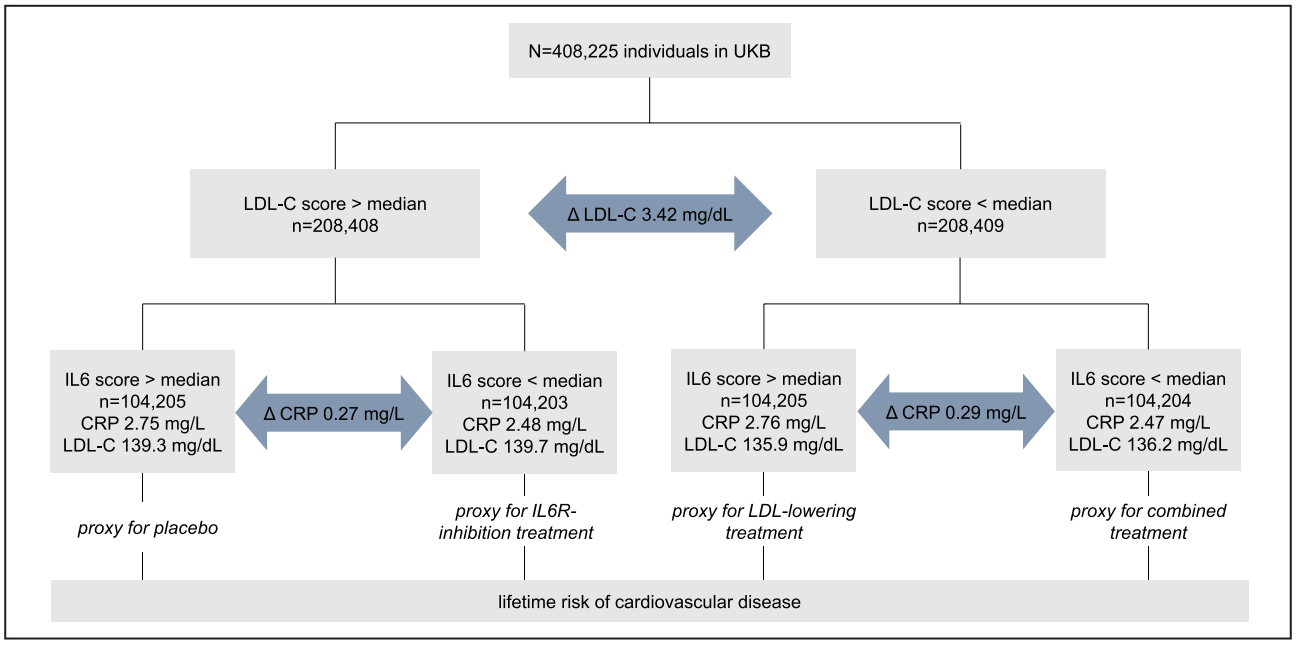


Figure 1. Study design of the 2×2 Mendelian randomization analysis in the UK Biobank (UKB).
Participants were divided into 4 groups according to their genetic risk scores for interleukin (IL)-6 signaling and low-density lipoprotein cholesterol (LDL-C). CRP indicates C-reactive protein.

aortic aneurysm, and cardiovascular death (Table S1). Secondary outcomes included the 5 individual outcomes. In the primary analysis, we combined prevalent and incident cases, whereas in sensitivity analyses, we explored associations with time-to-incident events among individuals free of cardiovascular disease at baseline. We performed 2×2 factorial Mendelian randomization analysis,[12] splitting our sample to 4 groups based on the IL6 and LDL-C scores, as depicted in Figure 1. Although this 2×2 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 attributed to arbitrary dichotomization and to maximize power, we also analyzed the 2 scores as quantitative traits, also exploring their interaction.[13] Furthermore, we explored the associations of the each score as a quantitative continuous variable with cardiovascular outcomes across deciles of the other. 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 Biobank Lung Exome Variant Evaluation Axiom array or UKB Axiom array).

RESULTS

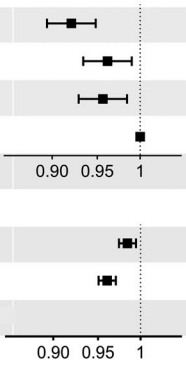
We identified 26 variants in the *IL6R* gene as genetic proxies for IL-6 signaling downregulation (Table S2) and 36 variants as proxies for LDL-C lowering (17 in the *PCSK9*, 15 in the *HMGCR*, 4 in the *NPC1L1* locus; Table S3). The genetic IL-6 score was associated with lower levels of fibrinogen and higher levels of IL-6 and soluble IL-6R (Figure S1), whereas the genetic LDL-C score was associated with lower apolipoprotein B and lower levels of cholesterol in all LDL particles (Figure S2). In the UKB, the genetic IL-6 score was strongly associated with CRP levels, but not LDL-C levels, and the genetic LDL-C score was strongly associated with LDL-C, but not CRP levels (Figure S3). There was no correlation between the 2 scores (Spearman ρ=0.0016).

The baseline characteristics of the 4 groups are presented in Table S4. There was no statistically significant difference with regard to age, sex, body mass index, blood pressure, or smoking status between the 4 groups. CRP levels were significantly lower in individuals with a mean genetic IL-6 score lower than the median (mean 2.75±4.54 versus 2.47±4.23 mg/L), whereas LDL-C levels were lower among individuals with a median genetic LDL-C score below the median (mean 139.5±34.4 versus 136.0±33.4 mg/L; Figure 1). Furthermore, individuals with median genetic LDL-C score below the median had lower apolipoprotein B levels, whereas individuals with median genetic IL-6 scores below the median had slightly higher HDL-C and apolipoprotein A1 levels, as well as lower hemoglobin A1c, as has been previously described.[6]

In the 2×2 factorial Mendelian randomization analysis, both a lower genetic IL-6 score and a lower genetic LDL-C score were associated with a lower risk of

A

Analysis	N	Change in LDL-C	Change in CRP	N events	OR [95%CI]	P
2x2 analysis		mg/dL [95%CI]	log(mg/L)			
Both scores < median	104,204	-3.01 [-3.30 to -2.70]	-0.047 [-0.051 to -0.043]	12,236	0.92 [0.90-0.95]	$1.8x10^{-7}$
IL6 score < median	104,203	+0.35 [-0.01 to +0.70]	-0.049 [-0.054 to -0.046]	12,762	0.96 [0.93-0.99]	0.008
LDL-C score < median	104,205	-3.36 [-3.66 to -3.06]	+0.001 [-0.003 to +0.004]	12,787	0.96 [0.93-0.98]	$5.5x10^{-4}$
Both scores ≥ median	104,205	reference	reference	13,644	reference	
Continuous analysis						
IL6 score (1 SD decr.)	416,817	+0.11 [-0.01 to +0.23]	-0.031 [-0.032 to -0.029]	51,429	0.98 [0.97-0.99]	0.007
LDL-C score (1 SD decr.)	416,817	-2.73 [-2.84 to -2.63]	+0.001 [-0.001 to +0.003]	51,429	0.96 [0.95-0.97]	$3.2x10^{-13}$
Interaction of two scores						0.507



B

Analysis	N	Change in LDL-C	Change in CRP	N events	OR [95%CI]	P
2x2 analysis		mg/dL	log(mg/L)			
Both scores < median	104,204	-38.67	-0.50	12,236	0.43 [0.31-0.59]	$1.8x10^{-7}$
IL6 score < median	104,203	0	-0.50	12,762	0.67 [0.50-0.90]	0.008
LDL-C score < median	104,205	-38.67	0	12,787	0.55 [0.40-0.77]	$5.5x10^{-4}$
Both scores ≥ median	104,205	reference	reference	13,644	reference	
Continuous analysis						
IL6 score	416,817	0	-0.50	51,429	0.80 [0.67-0.94]	0.007
LDL-C score	416,817	-38.67	0	51,429	0.58 [0.50-0.67]	$3.2x10^{-13}$
Interaction of two scores						0.507

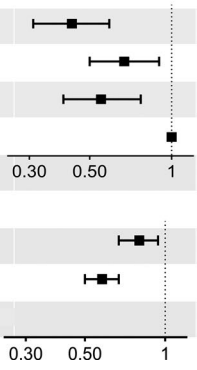


Figure 2. Independent associations between genetic scores for IL-6 signaling and LDL-C with risk of cardiovascular disease.

A, Nonscaled associations. **B**, Associations scaled to 38.67 mg/dL (1 mmol/L) decrement in LDL-C levels and 0.50 log(mg/L) decrement in log-transformed C-reactive protein levels. The upper panels of (**A** and **B**) represent associations from the 2×2 analysis dividing participants into 4 groups according to the median genetic IL-6 and LDL-C scores. The lower panels of (**A** and **B**) represent associations from an analysis where the 2 genetic scores were included on a continuous scale as well as the interaction between the 2 scores. The results are derived from logistic regression models adjusted for age, sex, the first 10 principal components of population structure, and the genotyping array. IL6 indicates interleukin-6; LDL-C, low-density lipoprotein cholesterol; and OR, odds ratio.

cardiovascular disease, whereas scoring less than the median in both scores showed an approximately log-additive lower risk (Figure 2). Specifically, when scaled to 50% decrement in CRP levels (0.5 log-decrement in log-transformed CRP levels), a lower genetic IL-6 score was associated with 33% lower odds for cardiovascular disease (odds ratio [OR], 0.67; 95% CI, 0.50–0.90), whereas a lower genetic LDL-C score scaled to a 38.67 mg/dL decrement in LDL-C levels was associated with 45% lower odds for cardiovascular disease (OR, 0.55; 95% CI, 0.40–0.77); a combined exposure showed an OR of 0.43 (95% CI, 0.31–0.59; Figure 2). This corresponded to a relative excess risk attributed to interaction index of 0 and a synergy index of 1 indicating an absolute lack of additive interaction. In the continuous analysis, both scores were also independently associated with a lower risk of cardiovascular disease, with no evidence of multiplicative interaction between the 2 scores (Figure 2), and the results were consistent when splitting the sample in deciles of the genetic LDL-C and IL-6 scores (Figure S4). Furthermore, to avoid bias attributed to arbitrary dichotomization of the genetic scores and to explore the impact of potentially hidden nonlinear effects on the examined interaction, we then tested in spline models the association between the 2 genetic scores with the risk of cardiovascular disease. There was no evidence for nonlinear effects (Figure S5). Introducing an interaction term between the 2 spline factors to the model (4×4 equally split splines of each genetic score) to explore if potential nonlinearities in the associations of the 2 variables with cardiovascular outcome cloud any interaction, we again found no significant interaction across any of the 16 interaction terms.

In sensitivity analyses restricted to incident cardiovascular events, as well as when excluding individuals

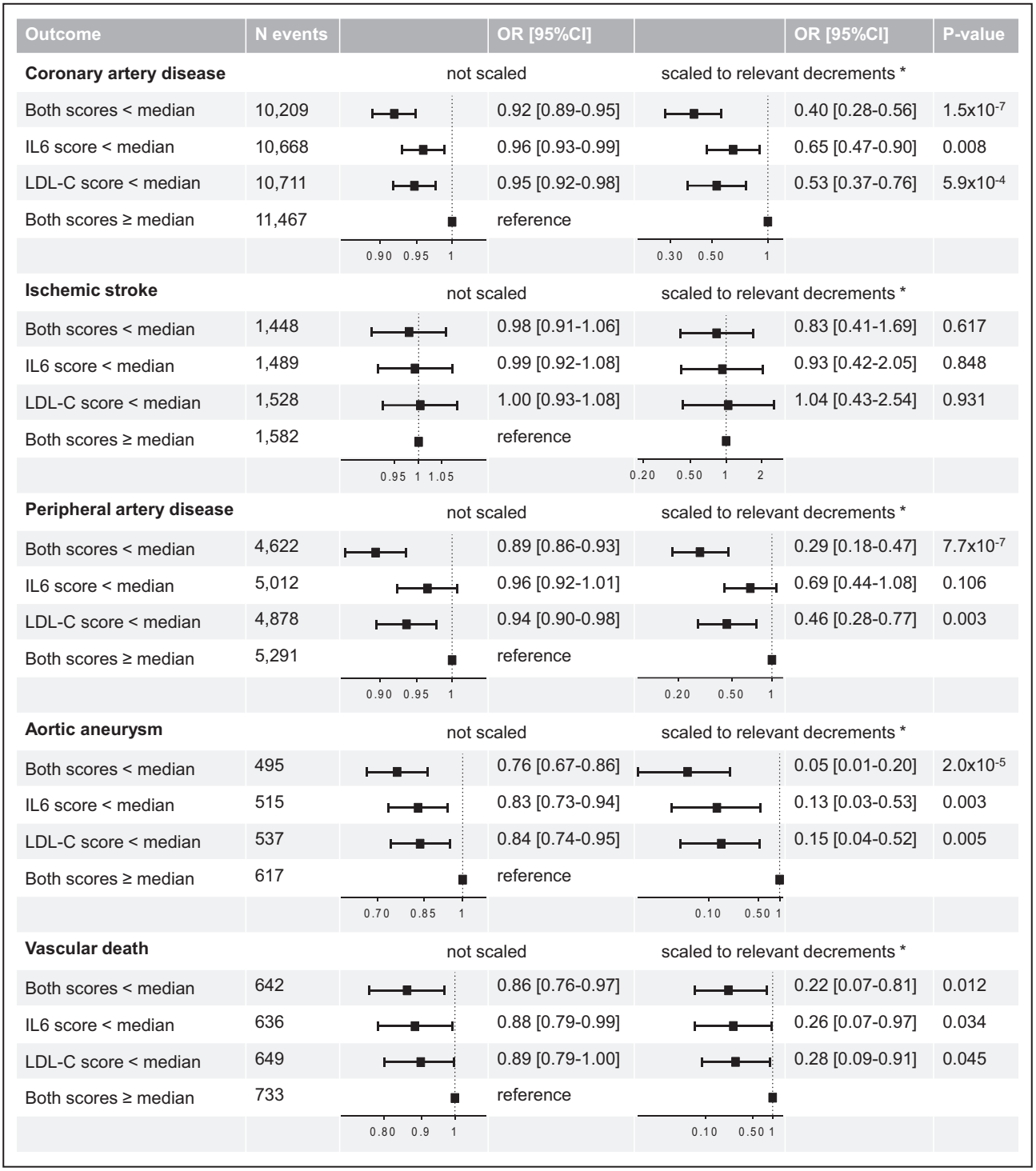


Figure 3. Independent associations between genetic scores for IL-6 signaling and LDL-cholesterol with risk of individual cardiovascular outcomes.

The results represent associations from the 2×2 analysis dividing participants into 4 groups according to the median genetic IL-6 and LDL-C scores. The results are derived from logistic regression models adjusted for age, sex, the first 10 principal components of population structure, and the genotyping array. *ORs are scaled to 38.67 mg/dL (1 mmol/L) decrement in LDL-C levels and 0.5 log(mg/L) decrement in log-transformed C-reactive protein levels. IL6 indicates interleukin-6; LDL-C, low-density lipoprotein cholesterol; and OR, odds ratio.

on lipid-lowering treatments at baseline, the results were stable (Table S5). Furthermore, the results were similar in sensitivity analyses of genetic scores from variants strictly selected on the basis of the CHARGE and GLGC Consortium data (Table S6). Directionally consistent and significant results were similarly

obtained for the individual end points including coronary artery disease, peripheral artery disease, aortic aneurysm, and vascular death, but not ischemic stroke (Figure 3).

To explore whether the effects of the IL-6 score were also independent of measured LDL-C levels, we examined associations with incident cardiovascular events among individuals with baseline LDL-C levels <100 and ≥100 mg/dL stratified by the intake of lipid-lowering medications at baseline. Interestingly, there was no evidence of differential effects by LDL-C levels or use of lipid-lowering treatment at baseline (Figure S6).

DISCUSSION

Among 408 225 community-based individuals, genetically downregulated IL-6 signaling was associated with a lower risk of cardiovascular events additively to genetically lowered and measured LDL-C levels. Although several trials support the fact that targeting IL-6 signaling could reduce vascular risk, it remains unknown whether a combined treatment of LDL-C-lowering and IL-6-signaling inhibition would offer additive reductions in risk. Our results provide genetic support that targeting residual inflammatory risk and residual cholesterol risk could indeed offer additive benefits in patients with atherosclerosis.

Although several trials now support the fact that targeting the inflammasome–IL-1β–IL-6 axis could lower vascular risk among patients with myocardial infarction, post hoc analyses from the CANTOS trial support the concept that there remains substantial residual inflammatory risk related to IL-6 after interventions targeting upstream regulators of IL-6,[14] thus indicating that targeting IL-6 signaling directly could be a more effective approach than targeting upstream molecules in the pathway. Our results support this notion and further expand these findings by showing that targeting IL-6 signaling by blocking IL-6R could reduce cardiovascular risk additively to current lipid-lowering approaches. Beyond genetically determined lipid levels, in an analysis of actually measured LDL-C levels, we were able to show that even among individuals with relatively low LDL-C levels (<100 mg/dL) either on or off lipid-lowering treatments, genetically downregulated IL-6 signaling is still associated with a lower risk of vascular events. Cumulatively, these results provide support that a combined strategy of lowering LDL-C and inflammation could offer additive benefits in lowering cardiovascular risk and as such should be tested in the future in a 2×2 factorial clinical trial.

We found that genetically downregulated IL-6 signaling is consistently associated with lower risk beyond lipid lowering for a number of vascular end points including coronary artery disease, peripheral artery disease, aortic aneurysm, and vascular death, thus supporting the utility of the approach for lowering vascular events in general. Still, there were differences in the effect sizes across different end points. For example, we found a particularly strong association between the genetic IL-6 score and aortic aneurysm in accord with previous reports.[5] IL-6 signaling might contribute to the formation of aortic aneurysms through mechanisms aside from atherosclerosis, thus explaining the large effect. For instance, IL-6 signaling is a key pathway in the pathogenesis of giant cell arteritis,[15] which are strongly associated with the formation of aortic aneurysms. In contrast, and in opposition to our previous findings,[5] we found no significant association of genetic IL-6 signaling downregulation with ischemic stroke. Although the direction of the association was consistent with other end points, the lack of a significant effect might relate to limited power or to the heterogeneous nature of ischemic stroke. Atherosclerosis accounts for only about 30% of the cases, and because the UKB does not have data on stroke subtypes, we could not perform analyses by stroke etiology.

Our study has limitations. First, we did not explore the effects of medications but, rather, the effects of the lifetime changes as a result of genetic variation in the targets of IL-6R inhibitors and lipid-lowering treatments, which might differ from those of a short-acting treatment on vascular events. Second, the results from the current analysis reflect the effects of IL-6 signaling on incident vascular events and might thus not be applicable for secondary prevention. Third, our results reflect the effects of genetic downregulation of IL-6 signaling attributed to variations in the *IL6R* gene and may thus differ from other approaches targeting other molecules upstream to IL-6. Still, post hoc analyses from the CANTOS and Cardiovascular Inflammation Reduction Trial (CIRT) support the fact that there is residual inflammatory risk that is explained by posttreatment IL-6 levels,[14] thus providing indirect evidence that targeting IL-6 directly might be the optimal strategy. Fourth, as we selected variants on genes encoding the primary targets of available lipid-lowering drugs, our results cannot inform on potential off-target pleiotropic effects of statins, other lipid-lowering medications, or IL-6R-targeting monoclonal antibodies.

In conclusion, lifelong genetic exposure to IL-6 signaling downregulation is associated with lower cardiovascular risk additively to the genetic lowering of LDL-C levels through variation in genes encoding standard LDL-C-lowering treatments. These results suggest that inhibition of IL-6 signaling on top of LDL lowering could lead to further reductions in vascular risk and should be tested in clinical trials of patients with atherosclerosis.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Data S1
Tables S1–S6
Figures S1–S6
References 4, 5, 11–13, 16–27

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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

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.[16] 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 pi-hat>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.[17]

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[18] with 318,366 White British individuals from the UK Biobank.[19] We selected genetic variants associated with LDL-C at $p<5x10^{-8}$ (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.[20]

To construct an instrument for IL-6R-mediated downregulation of the IL-6 signaling cascade,[5] 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.[4, 21] We meta-analyzed a GWAS for CRP levels of 204,402 European (Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium)[19] with data from 318,279 White British individuals in the UK Biobank.[22] We selected variants associated with CRP levels (p<5x10-8) after clumping for linkage disequilibrium at $r2<0.1$ (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[23] 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,[24] which are upstream to IL-6R-mediated signaling and both increase as a result of IL-6 signaling downregulation.[4] We also explored associations with fibrinogen levels in the CHARGE, Consortium (N=120,246 individuals of European

ancestry),[25] which is downstream to I-6R-mediated signaling and decreases following IL-6 signaling downregulation.[4]

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.[5, 11]

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.[26] 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.[26, 12] 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[27]).

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.[13] 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

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, I70.2, 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 GLGC	SE GLGC	p-value GLGC	beta META	SE META	p-value META
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

rs76733602	*HMGCR*	5	74562373	g	a	0.0442	0.014	0.004198	0.0378	0.005	5.07E-14
rs7726378	*HMGCR*	5	74337139	t	a	0.0184	0.0058	0.001492	0.0251	0.0024	4.79E-25
rs77443979	*HMGCR*	5	74779202	c	g	0.0691	0.022	0.01642	0.0659	0.0102	9.52E-11
rs80324692	*HMGCR*	5	74717761	c	t	0.0313	0.0093	0.001246	0.0327	0.0039	6.97E-17
rs9654427	*HMGCR*	5	74466833	g	a	0.0494	0.0074	1.58E-10	0.0562	0.0042	6.42E-41
rs10257749	*NPC1L1*	7	44388619	t	c	0.0164	0.0048	0.0005077	0.0179	0.0024	1.57E-13
rs2073547	*NPC1L1*	7	44582331	g	a	0.0485	0.0049	1.92E-21	0.0474	0.0026	2.34E-76
rs217355	*NPC1L1*	7	44626377	t	c	0.0294	0.0037	4.13E-14	0.0256	0.002	9.74E-38
rs2300414	*NPC1L1*	7	44682938	a	g	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)	**4.9×10^{-06}**
CRP, mean (SD)	2.74 (4.49)	2.48 (4.24)	2.76 (4.56)	2.47 (4.21)	**$< 2 \times 10^{-16}$**
LDL-C, mean (SD)	139.3 (34.4)	139.7 (34.2)	135.9 (33.3)	136.2 (33.3)	**$< 2 \times 10^{-16}$**
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)	**$< 2 \times 10^{-16}$**
ApoA1, mean (SD)	1.52 (0.27)	1.54 (0.27)	1.52 (0.27)	1.54 (0.27)	**1.2×10^{-06}**
Current lipid-lowering treatment, %	19.4	18.9	17.3	17.1	**$< 2 \times 10^{-16}$**

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.

Full dataset	**HR**	**95%CI**		**p-value**
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**
P for interaction				0.2324
Individuals not on lipid-lowering treatments	**HR**	**95%CI**		**p-value**
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				0.8845

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.

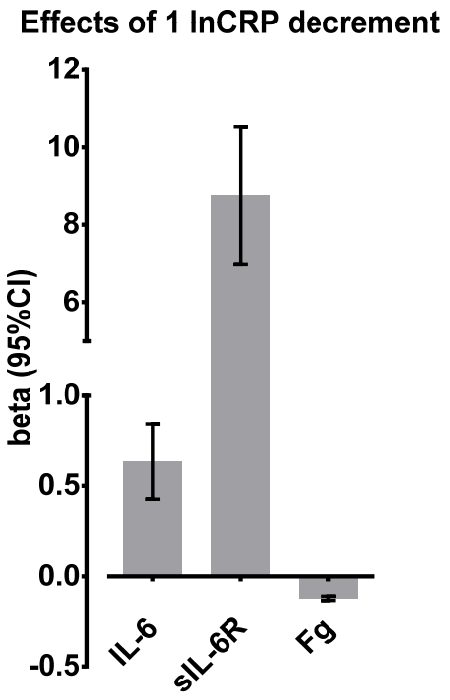
Table S6. Independent associations between genetic IL-6 and LDL-C scores with risk of cardiovascular disease based on genetic variants selected from the GLGC and CHARGE Consortia.

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.

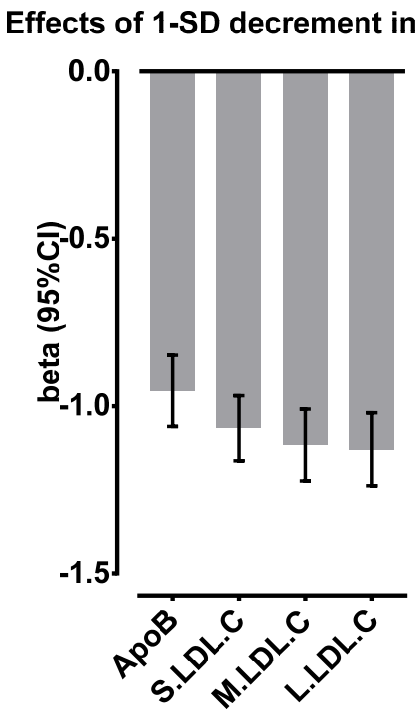
OR: odds ratio; CI: confidence intervals; IL6: interleukin-6; LDL-C: low-density lipoprotein cholesterol.

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.

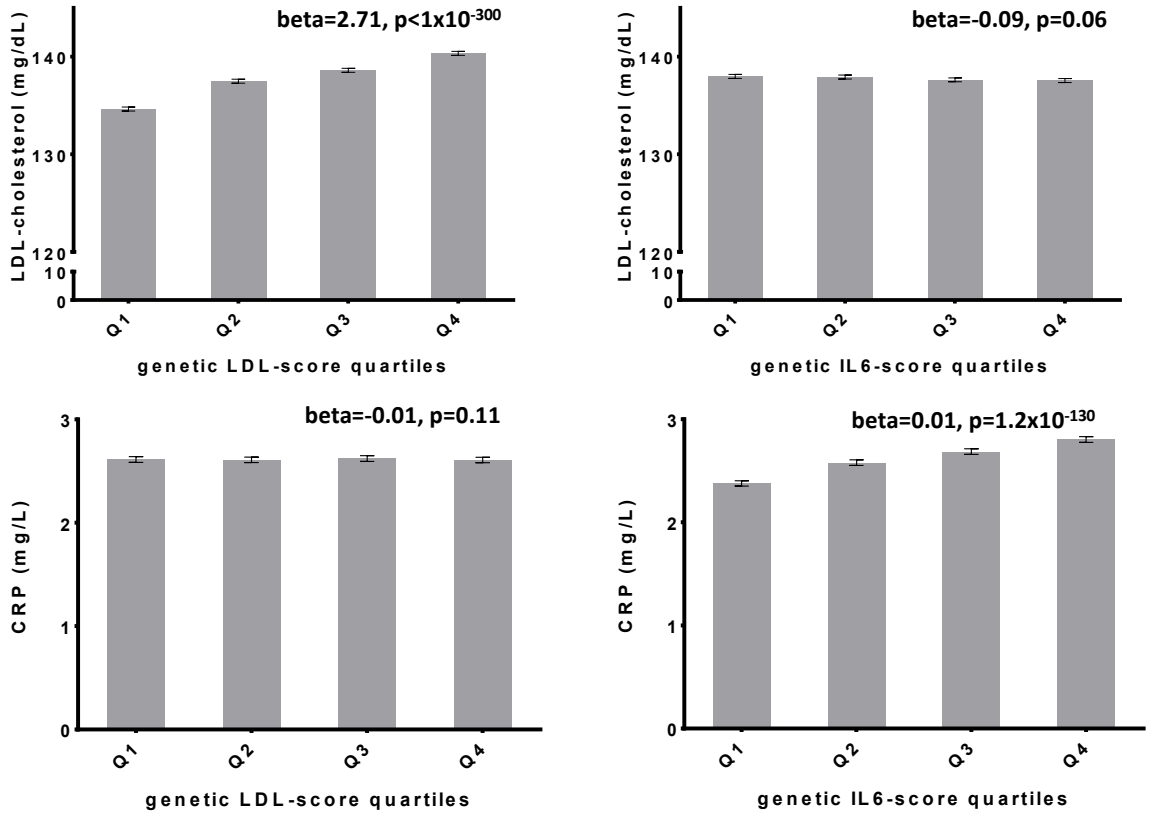
Figure S2. Effects of genetically downregulated LDL-C through known drug targets on Apolipoprotein-B and cholesterol levels across different LDL particles.



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.

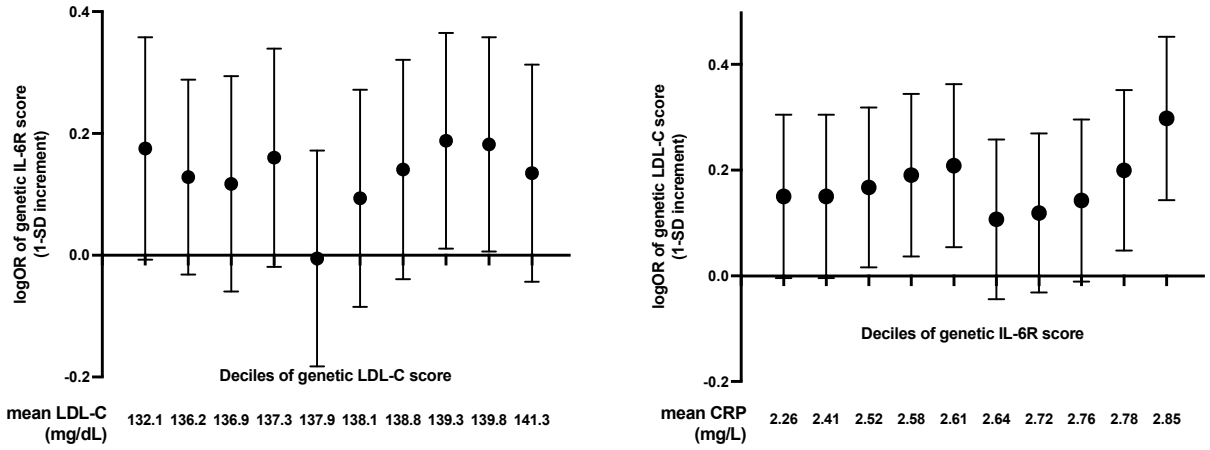
ApoB: apolipoprotein B; S.LDL.C: small low-density lipoprotein cholesterol; M.LDL.C: medium low-density lipoprotein cholesterol; L.LDL.C: large low-density lipoprotein cholesterol.

Figure S3. Associations of the genetic LDL-C and IL6 scores with measured LDL-C levels and CRP levels in the UK Biobank.



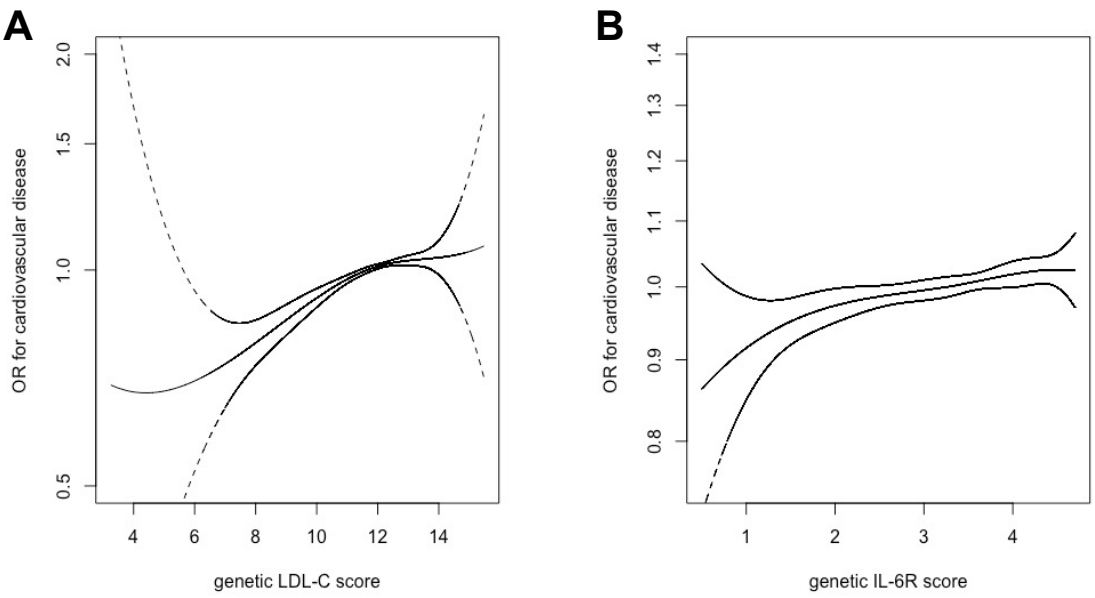
The error lines correspond to 95% confidence intervals.
The betas and p-values are derived from linear models for LDL-C and CRP adjusted for age, sex, and both the genetic LDL-C and IL-6 scores included (the betas correspond to SD-increments in the genetic scores).
IL6: interleukin-6; LDL-C: low-density 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.



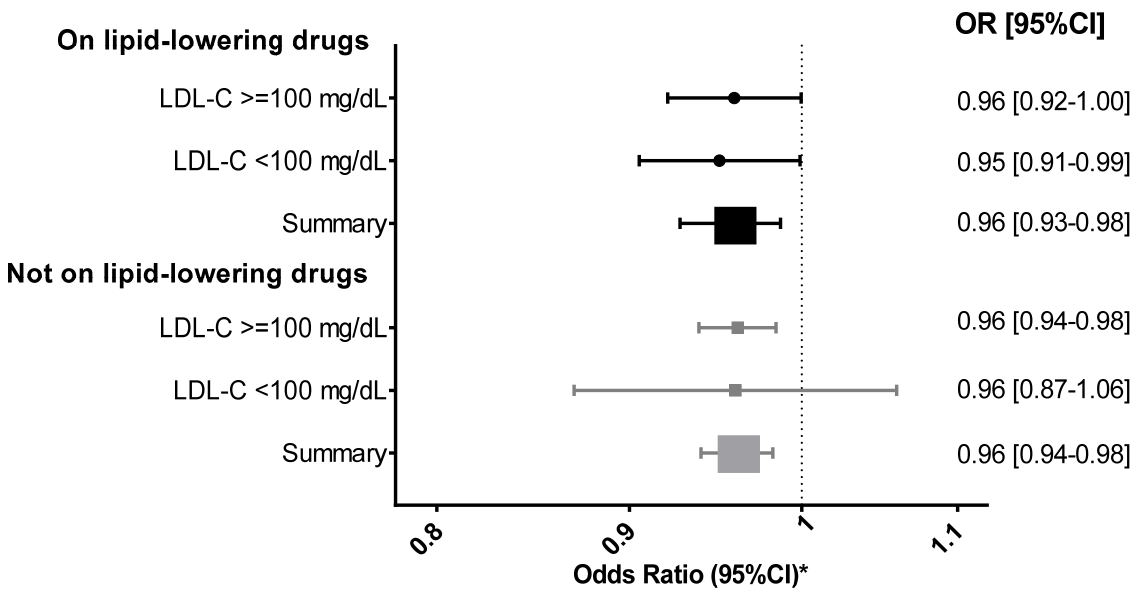
The error lines correspond to 95% confidence intervals.
The logORs (log-Odds Ratio) are derived from logistic regression models adjusted for age, sex, the first 10 principal components of population structure, and the array used for genotyping.
IL-6: interleukin-6; LDL-C: low-density lipoprotein cholesterol; 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.