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ORIGINAL ARTICLE

Dissecting the IL-6 pathway in cardiometabolic disease: A Mendelian randomization study on both *IL6* and *IL6R*

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Aims: Chronic inflammation is a risk factor for cardiovascular disease (CVD). IL-6 signalling perturbation through IL-6 or IL-6R blockade may have potential benefit on cardiovascular risk. It is unknown whether targeting either IL-6 or IL-6 receptor may result in similar effects on CVD and adverse events. We compared the anticipated effects of targeting IL-6 and IL-6 receptor on cardiometabolic risk and potential side effects.

Methods: We constructed four instruments: two main instruments with genetic variants in the *IL6* and *IL6R* loci weighted for their association with CRP, and two after firstly filtering variants for their association with IL-6 or IL-6R expression. Analyses were performed for coronary artery disease (CAD), ischemic stroke, atrial fibrillation (AF), heart failure, type 2 diabetes (T2D), rheumatoid arthritis (RA), infection endpoints, and quantitative haematological, metabolic and anthropometric parameters.

Results: A 1 mg/L lower CRP by the *IL6* instrument was associated with lower CAD (odds ratio [OR] 0.86, 95% confidence interval [CI] 0.77;0.96), AF and T2D risk. A 1 mg/L lower CRP by the *IL6R* instrument was associated with lower CAD (OR 0.90, 95% CI 0.86;0.95), any stroke and ischemic stroke, AF, RA risk and higher pneumonia risk. The eQTL-filtered results were in concordance with the main results, but with wider confidence intervals.

Conclusions: IL-6 signalling perturbation by either *IL6* or *IL6R* genetic instruments is associated with a similar risk reduction for multiple cardiometabolic diseases, suggesting that both IL-6 and IL-6R are potential therapeutic targets to lower CVD. Moreover, IL-6 rather than IL-6R inhibition might have a more favourable pneumonia risk.

KEYWORDS
cardiovascular disease, classical signalling, IL-6, trans-signalling

1 | INTRODUCTION

The burden of cardiovascular disease (CVD) on morbidity and mortality remains high, despite major advances in the (early) diagnosis and treatment. The residual risk for CVD is at least partly attributed to chronic inflammation.[1] Inflammatory markers, including **interleukin-6 (IL-6)** and C-reactive protein (CRP), have been consistently associated with adverse cardiovascular outcomes both in subjects with and

without a history of CVD.[1–4] However, dissecting the causative nature of the different markers of inflammation has proven troublesome often due to confounding and reverse causation.[5]

Finding the culprit factor in the inflammatory process is pivotal to curb residual risk. The Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) showed that subcutaneously administered canakinumab, a monoclonal antibody directed against **IL-1 beta**, reduced both CRP levels by a median of 54.1% (interquartile range [IQR]: −74.4, −16.5%) from baseline and the incidence of cardiovascular disease by 15% (odds ratio [OR] 0.85, 95% confidence interval [CI] 0.74–0.98).[6] A post hoc analysis revealed that CVD treatment benefit was most pronounced in patients who achieved interleukin-6 (IL-6) levels below 1.65 ng/L (OR for MACE: 0.68, 95% CI 0.56–0.82, compared to an OR of 0.90 for participants with IL-6 levels above the median of 1.65 ng/L [95% CI 0.76–1.07]).[7] While conditioning on post-randomization events may induce selection bias,[8] baseline IL-6 concentrations and future CVD event rates have been correlated independently in epidemiological studies.[9,10] However, no cardiovascular outcome trial addressing the potential beneficial role of direct lowering of IL-6 signalling has been conducted to date.

Mendelian randomization (MR) studies can be of help in estimating the potential effects in clinical intervention trials.[11] MR studies have robustly shown the causal effect of **IL-6R** perturbation on coronary heart disease (CAD) and related phenotypes such as ischaemic stroke, aortic aneurysm, atrial fibrillation and carotid plaque.[12–15] The effect of IL-6 perturbation has, however, not been studied with similar vigour.

Differentiating between IL-6 and IL-6R perturbation is important because IL-6 signalling is activated by binding of the circulating IL-6 ligand to either the soluble or membrane-bound IL-6R.[16] Signal transduction via the soluble IL-6R (trans-signalling) is generally considered to be pro-inflammatory, while transduction through membrane-bound IL-6R (classical signalling) is considered anti-inflammatory.[16] Paradoxically, *IL6R* variants associated with reduced risk of CAD are associated with increased soluble IL-6R.[12,15,17] Blockade of the IL-6R by tocilizumab and inhibition of IL-6 by siltuximab results in inhibition of both the classical and trans-signalling pathways, and the observed increase of soluble IL-6R by *IL6R* variants complicate the translation of results from *IL6R* MR studies to pharmacological effects. Moreover, multiple other ligands, including CNTF and IL-30, have also shown to bind to IL-6R.[18]

Downregulation of IL-6 signalling through *IL6* variants will likely lead to both reduced trans- and classical signalling, without incorporating effects of any other ligands binding to IL-6R. It is unknown whether perturbation of the IL-6 ligand results in similar effects compared to perturbation of IL-6R. Here, we performed a two-sample MR study to evaluate and compare the phenotypic consequences of IL-6 signalling perturbation through IL-6R and IL-6.

What is already known about this subject

- The residual risk for CVD is at least partly attributed to chronic inflammation.
- MR studies have robustly shown the effect of IL-6 receptor genetic variants on CVD.
- Some *IL6R* variants are likely to upregulate IL-6 trans-signalling, while *IL6* variants likely inhibit both the classical and trans-signalling pathway.

What this study adds

- Inhibition of the IL-6 signalling pathway, by targeting either IL-6 or IL-6R, is likely to result in beneficial effects on the risk for CAD, stroke, AF and type 2 diabetes.
- IL-6 receptor perturbation but not IL-6 perturbation was associated with increased pneumonia risk, which suggests a differential effect.

2 | METHODS

In this MR study, we used the naturally occurring variation within and around the *IL6* and *IL6R* gene to estimate the effect of inhibition of IL-6 and IL-6R on various clinical biomarkers and outcome. These models allow exploration of the directionality of a therapeutic compound on clinical outcome in trials and exploration of unanticipated on-target side effects.[19,20] MR for drug–target discovery and validation and its assumptions have been reviewed elsewhere.[11] We used publicly available data, and the original studies were all approved by the relevant ethical committees.

2.1 | Construction of genetic instruments

We constructed two distinct genetic instruments for IL-6 and IL-6R, by selecting genetic variants from within a 100 kb window around the genes encoding IL-6 (*IL6*, ENSG00000136244) and IL-6R (*IL6R*, ENSG00000160712). Instruments were extracted from a UK Biobank genome-wide association study (GWAS), based on their associations (P-value < 5×10^{-4}) with CRP (http://www.nealelab.is/uk-biobank/).[21] We selected CRP levels as our exposure variable since, unlike IL-6 levels, CRP levels are available for 361 194 participants. This results in more potential genetic variants compared to smaller IL-6 level GWAS and additionally protects against weak instrument bias and bias through measurement error.[22] To strengthen our results and to support the assumption that our main genetic instruments indeed affect the targeted gene, we additionally created two genetic instruments following an eQTL criterion, first removing variants from the initial pool of potential CRP-associated variants within 100 kb around the genes that did not associate with mRNA expression of *IL6* or *IL6R*, using data on expression levels from eQTLGen and GTEx portal release V8 (results accessed on 6 May 2020)[23,24] (P-value < 5×10^{-4}; Figure 1). From the eQTL-filtered variants we identified

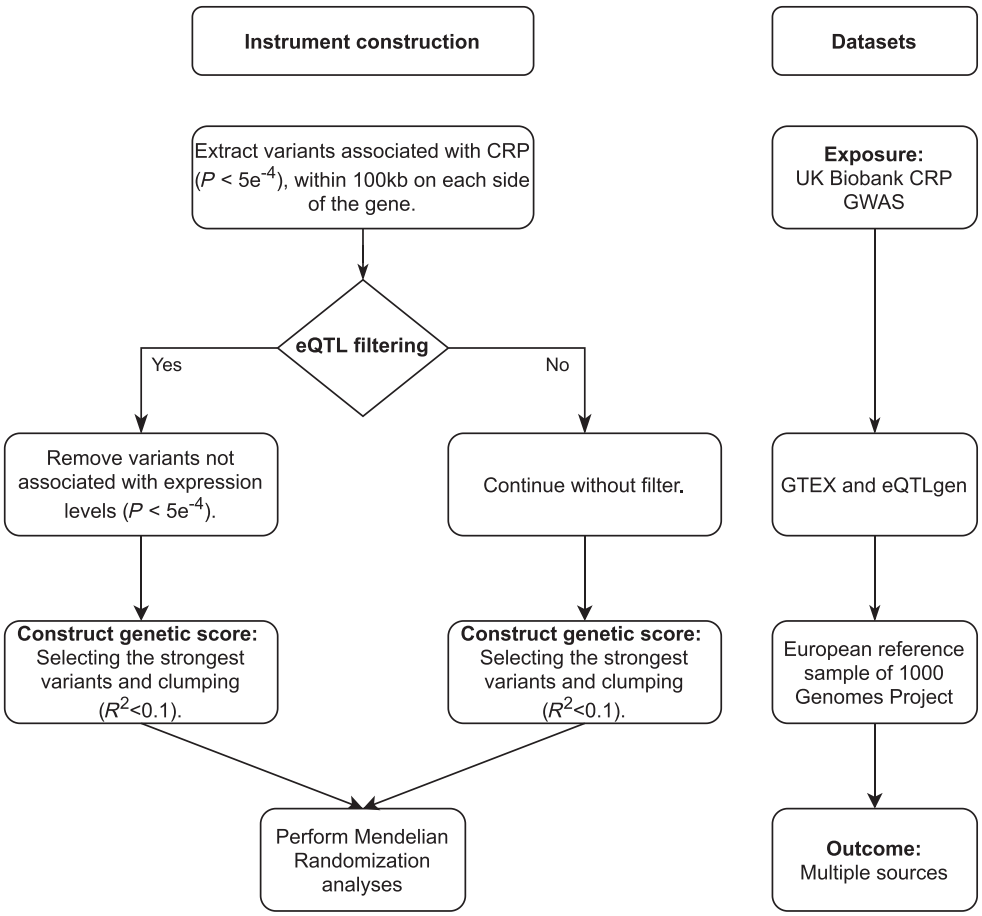

FIGURE 1 Genetic instrument construction. Diagram showing the protocol for selecting the genetic instruments used in this study. CRP, C-reactive protein; eQTL, expression quantitative trait loci; GWAS, genome-wide association study

the final set of variants based on their CRP association (*P*-value < 5×10^{-4}). While this approach results in fewer variants available for analysis and thus less power, it ensures selected variants have an impact on the targeted gene. MR analyses were subsequently performed on both the main set of instruments (the *IL6* and *IL6R* instruments without eQTL filter), and the eQTL-filtered set. For each instrument, only variants with a minor allele frequency (MAF) of >0.01 and in low linkage disequilibrium (LD) (R^2 < 0.1, based on a 1000 Genomes European reference sample set[25]) with the other variants in the genetic instrument eligible for inclusion. Clumping of the genetic instruments was performed using ieugwasr.[26] Due to differences in array coverage of the various GWAS, proxy variants (R^2 > 0.9, 1000 Genomes European reference sample set) were used to substitute unavailable variants where necessary. Variants for which no proxy was available were omitted from the analysis for that specific outcome.

2.2 | Data and contributing studies

We sought to validate our genetic instruments by testing for their associations with IL-6 levels and IL-6 receptor levels, available from a GWAS employing Olink protein assays.[27,28] We tested our genetic instruments for associations with the following clinical outcome parameters of interest for future cardiovascular outcome trials: coronary artery disease (CAD),[29] any stroke and ischaemic stroke,[30] heart failure (HF),[31] type 2 diabetes (T2D)[32] and atrial fibrillation (AF).[33] Because therapeutic agents targeting IL-6R are available for rheumatoid arthritis (RA), we also included RA in our analysis as a positive control.[34] To assess possible adverse impact of IL-6 signalling therapy, we tested the associations of our genetic instruments with immunity biomarkers including white blood cell counts and differentiation (WBC), retrieved from studies including the UK Biobank and blood cell consortium.[35,36] Since tocilizumab treatment in RA patients results in increased LDL-C levels, we extracted lipid and lipoprotein levels from a GWAS from the MAGNETIC consortium.[37] A previous study observed a suggestive effect of IL-6 signalling on the risk for T2D.[13] Therefore, we included GWAS on glucose, HbA1c and body mass index (BMI) in our analysis.[37–39] As infections will be the main safety cause of concern in cardiovascular outcome trials, we assessed the possible adverse impact of IL-6-targeted therapy by including ICD10 summary data on any infection, and pneumonia from participants of the FinnGen study.[40] A full list of the datasets used for this analysis is provided in Table S1 in the Supporting Information.

2.3 | Statistical analyses

The effects of on-target IL-6 or IL-6R perturbation were estimated using Mendelian randomization, specifically using the inverse-variance weighed (IVW) and the more robust MR-Egger estimators.[41,42] MR-

Egger provides valid estimates even in extreme settings were 100% of the variants show a horizontal pleiotropy effect; however, this comes at the cost of comparatively low power. Hence, we subsequently applied the Rücker model selection framework to decide between both estimates.[42] First, we calculated both the IVW and Egger models. If the difference in Q – Q' between the IVW and the Egger model is significant, we considered the Egger model a better fit. Using Cochran's Q and Rucker's Q' statistic in assessment of pleiotropy improves prediction by penalizing outlying variants with a random effects model. Thus, based on the chosen model's Q, we then chose a fixed or random-effects model. We present the results of the model with the best fit in the main text, and the fixed- or random-effect results of all methods in Tables S3–S6 in the Supporting Information. In further sensitivity analyses, we also used the weighted median method. The potential for horizontal pleiotropy bias was further limited by selecting variants based on CRP from small cis-regions known to encode IL-6 or IL-6R, and through the development of two additional genetic scores by firstly filtering based on the association with IL-6 and IL-6R mRNA and afterwards on CRP. All effects are standardized to a 1 mg/L reduction in CRP levels, and presented as mean difference (MD) or odds ratio (OR) with 95% confidence interval (CI). We provide estimates using a 95% confidence interval, and indicate which outcomes achieve a multiple testing threshold (defined as 0.05/28 outcomes/2 genes $= 8.92 \times 10^{-4}$) with a '#'. Furthermore, we employed the Kolmogorov–Smirnov test on the instrument-specific 28 *P*-values to assess if our results are due to multiple testing.[43] Finally, we provide precision of our results by calculating the squared standard error (Table S3–S6 in the Supporting Information), where a lower value indicates a higher precision, compared to the other analyses for that outcome. All analyses were performed using R version 3.6.1.[44] The plots were made using ggplot2.[45]

2.4 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.[46]

3 | RESULTS

3.1 | Characteristics of genetic instruments for IL6-signalling

Depending on the variant coverage of the outcome dataset available for analysis, up to eight variants were used in our *IL6* instrument, and up to three variants using the eQTL filtering approach (Figures S1 and S2 in the Supporting Information). The combined *F*-statistic for the main *IL6* instrument was 164, and for the instrument with eQTL filtering 66. For *IL6R*, up to 19 variants were available for our instrument, while up to six variants were available for the eQTL-filtered instrument. The combined *F*-statistic for the main *IL6R* instrument was 1221, and for the instrument with eQTL filtering 770 (Figures S3–S4 in the Supporting Information). All variants included in the genetic instruments are listed in Table S2 in the Supporting Information and the number of variants available per analysis is listed in Table S3–S6 in the Supporting Information.

3.2 | Effect of genetic instruments on IL-6 levels

Genetically predicted lower CRP by the main instrument for *IL6* was indeed associated with reduced IL-6 levels (−0.17 SD units, 95% CI −0.30;−0.04; Figure 2) and genetically predicted lower CRP by the main *IL6R* instrument was associated with increased IL-6 receptor levels (5.16 SD units, 95% CI: 3.86;6.45) and increased IL-6 levels (0.43 SD units, 95% CI: 0.26;0.60; Figure 2). The associations of the main *IL6R* instrument with IL-6 and IL-6R levels and the eQTL-filtered *IL6R* instrument with IL-6R levels reached the multiple testing threshold. The results for the eQTL-filtered instruments were consistent with the conventionally selected instruments (Tables S3–S6 in the Supporting Information).

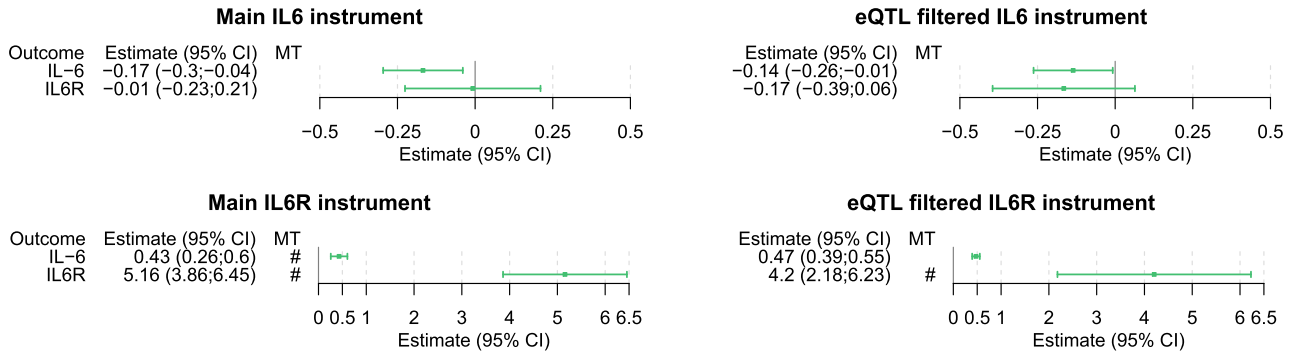

FIGURE 2 Effects of the genetic instruments on IL-6 and IL-6R levels. Forest plots representing the change in IL-6 and IL-6R levels for all genetic instruments, scaled to a 1 mg/L reduction in CRP levels. The bracket marks an association of the genetic instrument with IL-6 or IL-6R that meets the multiple testing threshold (8.92×10^{-4}). CRP, C-reactive protein; IL-6, Interleukin-6

3.3 | Effect of inhibition of interleukin 6 signalling on clinical outcomes

Genetically predicted CRP reduction by the *IL6* instrument is associated with lower odds for CAD (OR 0.86, 95% CI 0.77;0.96), AF (OR 0.86, 95% CI 0.77;0.95) and T2D (OR 0.83, 95% CI 0.75;0.92; Figure 3). The eQTL-filtered analysis showed an association between CRP reduction by the *IL6* instrument and HF (OR 0.77, 95% CI 0.63;0.94), and similar effect estimates with remaining outcomes compared to the *IL6* instrument, albeit with wider confidence intervals. Only the association of the *IL6* instrument with diabetes reached the multiple testing threshold.

The Mendelian randomization analysis of *IL6R* suggested that 1 mg/L lower CRP levels due to the *IL6R* instrument are associated with a lower risk for CAD (OR 0.90, 95% CI 0.86;0.95), any stroke (OR 0.92, 95% CI 0.85;0.98) and any ischaemic stroke (OR 0.90, 95% CI 0.84;0.97), AF (OR 0.91, 95% CI 0.84;0.97) and RA (OR 0.77, 95% CI 0.64;0.92; Figure 3). The *IL6R* instrument was also associated with an increased risk for pneumonia (OR 1.17, 95% CI 1.09;1.27). The eQTL-filtered *IL6R* analysis confirmed effects on CAD (OR 0.89, 95% CI 0.84;0.95), any stroke (OR 0.92, 95% CI 0.85;0.99), any ischaemic stroke (OR 0.91, 95% CI 0.84;0.99), AF (OR 0.86, 95% CI 0.79;0.95), RA (OR 0.71, 95% CI 0.63;0.79) and pneumonia (OR 1.20, 95% CI 1.10;1.32). The associations of the *IL6R* main instrument with CAD and with pneumonia reached the multiple testing threshold, and the associations of the eQTL-filtered *IL6R* instrument with CAD, RA and pneumonia also reached the multiple testing threshold. The estimates of the weighted median method were very similar to the effects of the IVW method (Tables S3–S6 and Figures S5–S12 in the Supporting Information).

3.4 | Effect of inhibition of interleukin 6 signalling on safety biomarkers

The *IL6* model was associated with reduced platelet counts (−0.100, 95% CI −0.16; −0.04), fibrinogen levels (−0.115, 95% CI −0.204; −0.026), HDL-C (−0.865, 95% CI −1.335; −0.395), ApoA1 (−0.763, 95% CI −1.259; −0.266) and BMI (−0.068, 95% CI −0.107; −0.029), and with increased basophil counts (0.198, 95% CI 0.091;0.304). The eQTL-filtered analysis showed associations between the *IL6* instrument and increased white blood cell (0.817, 95% CI 0.439;1.195), neutrophil (0.464, 95% CI 0.07;0.859), basophil (0.468, 95% CI 0.044;0.891) and eosinophil counts (1.38, 95% CI 0.97;1.79), with reduced platelet counts (−0.100, 95% CI −0.188; −0.013) and with lower BMI (−0.052, 95% CI −0.103; −0.001). The associations of the main *IL6* instrument with basophils, HDL-C and BMI and the associations of the eQTL-filtered *IL6* instrument with white blood cell count

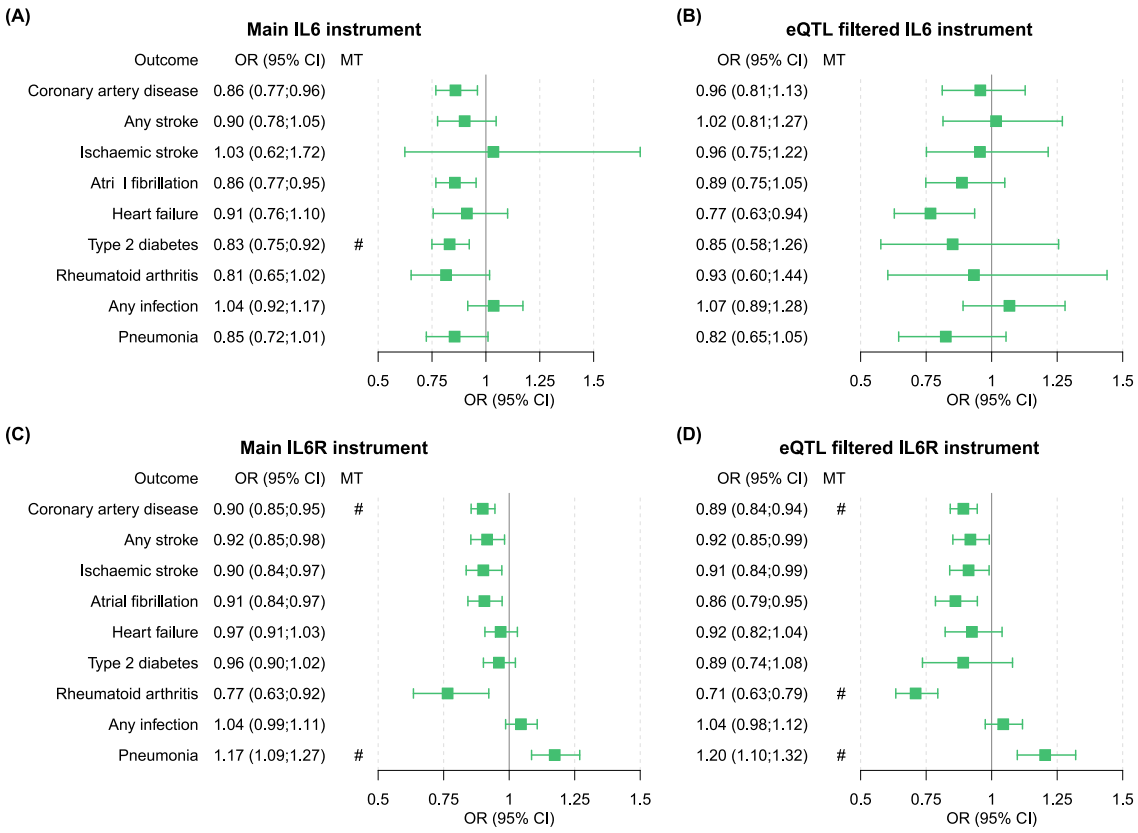

FIGURE 3 Drug target MR effects of IL-6 and IL-6R on clinical outcome, per 1 mg/L reduction in CRP. Forest plots representing the risk of clinical outcome parameters for all genetic instruments, scaled to a 1 mg/L reduction in CRP levels. The hash mark indicates an association of the genetic instrument with the clinical outcome that meets the multiple testing threshold (8.92×10^{-4})

and eosinophils reached the multiple testing threshold. The *IL6R* instrument was associated with increased monocyte (0.07, 95% CI 0.05;0.091), platelet counts (0.032, 95% CI 0.006;0.058) and with BMI (0.076, 95% CI 0.007;0.145), and was not associated with an effect on other leukocytes. The *IL6R* eQTL-filtered analysis confirmed associations with increased monocytes (0.075, 95% CI 0.059;0.09) and showed associations with increased eosinophils (0.032, 95% CI 0.004;0.06), and with reduced lymphocyte counts (−0.072, 95% CI −0.121; −0.023) (Figure 4, and point estimates provided in Tables 3–6 in the Supporting Information). The associations of the main and eQTL-filtered *IL6R* instrument with monocytes reached the multiple testing threshold. The estimates of the weighted median method for the safety biomarkers were also similar to the effects of the IVW method (Tables S3–S6 and Figures S5–S12 in the Supporting Information).

The Kolmogorov–Smirnov test was 1.0×10^{-3} and 9.8×10^{-3} for the main and eQTL-filtered *IL6* instrument, respectively, and 4.2×10^{-4} and 3.0×10^{-3} for the main and eQTL-filtered *IL6R* instrument, respectively, indicating that our results are unlikely to be driven by false-positive results (Figure S13 in the Supporting Information).

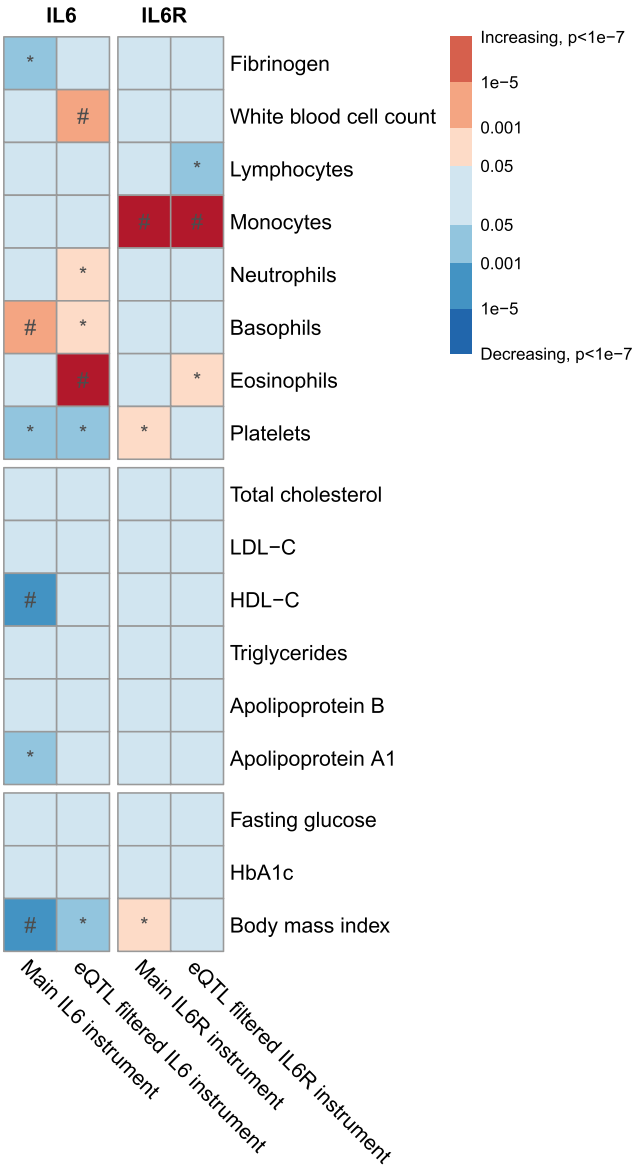

FIGURE 4 Drug target MR effects of IL-6 and IL-6R on biomarkers. The strength of the association for each genetic instrument with the outcome is depicted by the *P*-value times the direction of the effect, per 1 mg/L CRP reduction. The asterisks mark an association of the genetic instrument with the trait of $P < .05$. The hash mark indicates an association of the genetic instrument with the trait meeting the multiple testing threshold (8.92×10^{-4}). eQTL, expression quantitative trait loci

4 | DISCUSSION

In this study, we used Mendelian randomization to predict the clinical effects of reducing IL-6 signalling by pharmacologic inhibition of either IL-6 ligand or IL-6 receptor. We show that inhibition of IL-6 ligand, mimicked through *IL6*, is nominally associated with risk reductions of CAD, AF and T2D, and observed a similar effects profile of IL-6R inhibition, mimicked by *IL6R*, which was nominally associated with reduced risk for CAD, (ischaemic) stroke, AF and RA, at the potential cost of increased pneumonia risk. The association of the *IL6* instrument with T2D and *IL6R* instrument with CAD and pneumonia reached the multiple testing threshold. Targeting IL-6 signalling through pharmacologic inhibition of IL-6 or IL-6R will likely elicit similar favourable effects on clinical CVD outcomes. Unlike previous MR studies of these targets, we created additional genetic scores using variants associated with both CRP levels and mRNA expression of *IL6* or *IL6R*. The results from these analyses generally resulted in wider confidence intervals, but were largely directional concordant, and any discordant results were within the 95% CI of each other. The directional concordance of the two eQTL-filtered instruments with the main instruments provide further support for the causal effects of IL-6-signalling perturbation through either IL-6 or IL-6R on cardiometabolic disease.

Our observations that IL-6 signalling inhibition through *IL6* variants reduces the risk for CAD and atrial fibrillation is in accordance with the literature on *IL6R* variants and provides evidence for the protective benefits of perturbing IL-6 signalling through inhibition of either IL-6 or IL-6R in CAD.[12–15] This is of importance as *IL6* genetic variants likely inhibit both the classical and trans-signalling pathway, unlike *IL6R* variants, from which some are described to be likely to upregulate IL-6 trans-signalling in certain tissues.[17,47,48] For example, the Asp358Ala variant in IL6R was shown to increase soluble IL-6R and is associated with increased risk for asthma, atopic dermatitis and faster disease progression in ALS patients, and it was suggested that this is due to upregulation of the trans-signalling pathway.[47,49,50] Based on our observations that both *IL6* and *IL6R* instruments are associated with reduced CAD risk, we hypothesize that it is probably the classical-signalling pathway that is involved in cardiovascular disease. Inhibition of the IL-6 ligand may be

pharmacologically preferential over targeting IL-6R, as IL-6R monoclonal antibodies have been shown to inhibit the IL-6R ligands ciliary neurotrophic factor and IL-30.[51] It is unlikely that a similar effect will be observed in patients treated with a monoclonal antibody directed against the IL-6 ligand. Moreover, targeting the IL-6 ligand instead of the IL-6 receptor may also be preferential based on the potentially required therapeutic range of monoclonal antibodies, as levels of the IL-6 ligand are normally in the range of 1 pg/mL but can rise dramatically in periods of acute inflammation, compared to a relatively stable range of 50–75 ng/mL for IL-6R.[52] It is possible that the effective therapeutic range of IL-6 monoclonal antibodies for CAD prevention will be lower than that for IL-6R antibodies, potentially resulting in less influence of IL-6 antibodies on acute phase reactions (such as infections), compared to IL-6R antibodies.

We show that inhibition of IL-6 signalling by our *IL6* instrument is associated with a protective effect on the risk for type 2 diabetes, even after correction for multiple testing. A large epidemiological study previously showed that IL-6 levels and CRP levels are associated with the risk for T2D,[53] and genetic studies also observed a directionally consistent association between variation in the *IL6R* locus and T2D, with *IL6R* variant rs7529229 showing a trend towards a lower risk (OR 0.97, 95% CI 0.94;1.00).[13,54] Although IL-6 has been shown to reduce hepatic insulin sensitivity, the exact role of IL-6 in glucose metabolism has not been elucidated, as some studies have also shown a beneficial role of IL-6 on peripheral insulin sensitivity.[55] It is of note that we did not observe an effect on either glucose or HbA1c. The observed beneficial effect of tocilizumab on insulin sensitivity and HbA1c levels (in both T2D and non-T2D subjects) supports the notion that inhibition of the IL-6 signalling pathway may have beneficial effects on glucose metabolism,[56,57] a finding that was subsequently confirmed in clinical trials by showing improved fasting blood glucose and HbA1c levels in RA patients with T2D randomized to the IL-6R monoclonal antibody sarilumab.[58] In contrast, a number of MR studies have shown that LDL-C lowering by statins and proprotein convertase subtilisin–kexin type 9 inhibition confers a low, but consistent increased risk for T2D.[19,20,59,60]

In anticipation on any adverse effects of interest for future clinical trials, we observed that IL-6 signalling inhibition by *IL6R* variants was associated with increased risk for pneumonia. In contrast, *IL6* variants showed a trend towards a lower risk for pneumonia. These results might indicate that inhibition of IL-6 might have a more favourable infection risk profile than inhibition of IL-6R. The Asp358Ala variant in *IL6R* was associated with increased soluble IL-6R in lung tissue and was shown to act pro-inflammatorily in lung cells.[47] The variant was associated with atopic asthma, but not with COPD.[47] A recent MR study showed that *IL6R* variants were associated with increased pneumonia.[61] This is in line with findings for tocilizumab, which is associated with increased risk for opportunistic and serious bacterial infections in a dose-dependent manner, with pneumonia, urinary tract infections and cellulitis most frequently mentioned.[62] Future research is warranted to investigate the discrepancy between IL-6 inhibition through *IL6* or *IL6R* variants observed in this study.

This is the first MR study attempting to directly compare IL-6 signalling inhibition through either *IL6* or *IL6R* genetic variants on various clinical outcomes and biomarkers. The *IL6* locus is a relatively well-preserved locus with few genetic variants strongly affecting *IL6*, limiting the power of these analyses unlike for *IL6R*. It is therefore reassuring that the effect direction of inhibition of IL-6 signalling inhibition through either *IL6* or *IL6R* for the expected clinical outcomes (e.g., CAD, RA) was similar. However, this could imply that a number of non-significant concordant associations are simply due to a lack of power. In addition, we have also observed some surprising associations and discrepancies between *IL6* and *IL6R*, and between the main and eQTL-filtered instruments (e.g., the effect on monocytes, platelets, BMI). This is also the first of a number of limitations in our study that warrant further discussion. First, we selected our *IL6* and *IL6R* variants based on the downstream biomarker CRP, which is an indirect estimation of their function. However, IL-6 and IL-6R GWAS are small compared to the CRP GWAS we used and would preclude us from comparing the effects of both targets based on a standardized measurement. Our model framework using an eQTL filter provides an additional layer of evidence, but at the cost of power, since these instruments contain many fewer variants. Second, we included a proxy variant when a variant from our preferred genetic instrument was not available for a specific trait. However, since proxy variants were not always available, the number of variants in some of the analyses was limited. We guarded against weak instrument bias by selecting variants with an *F*-statistic above 10. Third, we did not correct for multiple testing, since this article was meant to be hypothesis-generating to inform the ongoing trial effort. Especially regarding adverse outcomes, we consider it important to report any association we find, as these results can be important for safety analyses in clinical trials. Fourth, while the *cis* focus of our analysis severely limited the potential for horizontal pleiotropy, some variant known to encode IL6 or IL6R were nevertheless in LD with variants affecting neighbouring genes. None of these neighbouring genes for which multiple associations exist with our included variants were, however, related to the considered phenotypes (see Tables S7 and S8 in the Supporting Information), limiting the potential for any LD-based horizontal pleiotropy. Moreover, we have implemented several steps to guard against any inadvertent horizontal pleiotropy bias, hence should any bias remain this is likely minimal. We selected the 100 kb region because approximately 92% of all lead cis-eQTL variants are anticipated to reside within 100 kb of the gene.[23] Fifth, our analyses make use of data derived mainly from European cohorts. Caution is warranted in translating our results to other ethnicities. Last, our model is based on the effect of modest genetic disturbances over the course of a lifetime. Caution is warranted in directly translating these effects towards an anticipated effect of a therapeutic agent that will have a potentially larger effect but over a shorter period of time.

In summary, in this study we show that inhibition of the IL-6 signalling pathway, either by targeting IL-6 or IL-6R is likely to result in

beneficial effects on the risk for CAD, stroke, AF and type 2 diabetes, but the observed association of *IL6R* with pneumonia risk warrants caution and should be evaluated in clinical trials.

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COMPETING INTERESTS

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CONTRIBUTORS

A.J.C., F.W.A., G.K.H. and A.F.S. designed the study. A.J.C. and A.F.S. performed the analyses. A.J.C., F.W.A., P.N., P.M.R., G.K.H. and A.F.S. contributed to the interpretation of the data and the drafting of the manuscript. A.J.C. and A.F.S. had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

DATA AVAILABILITY STATEMENT

The datasets were derived from sources in the public domain and analysed using in-house scripts. A list of the data sources and R code is enclosed in the supplemental materials.

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