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Cardiometabolic effects of genetic upregulation of the interleukin 1 receptor antagonist: a Mendelian randomisation analysis

The Interleukin 1 Genetics Consortium

Summary
Background To investigate potential cardiovascular and other effects of long-term pharmacological interleukin 1 (IL-1) inhibition, we studied genetic variants that produce inhibition of IL-1, a master regulator of inflammation.

Methods We created a genetic score combining the effects of alleles of two common variants (rs6743376 and rs1542176) that are located upstream of IL1RN, the gene encoding the IL-1 receptor antagonist (IL-1Ra; an endogenous inhibitor of both IL-1α and IL-1β); both alleles increase soluble IL-1Ra protein concentration. We compared effects on inflammation biomarkers of this genetic score with those of anakinra, the recombinant form of IL-1Ra, which has previously been studied in randomised trials of rheumatoid arthritis and other inflammatory disorders. In primary analyses, we investigated the score in relation to rheumatoid arthritis and four cardiometabolic diseases (type 2 diabetes, coronary heart disease, ischaemic stroke, and abdominal aortic aneurysm; 453 411 total participants). In exploratory analyses, we studied the relation of the score to many disease traits and to 24 other disorders of proposed relevance to IL-1 signalling (746 171 total participants).

Findings For each IL1RN minor allele inherited, serum concentrations of IL-1Ra increased by 0·22 SD (95% CI 0·18–0·25; 12·5%; p=9·3 × 10−33), concentrations of interleukin 6 decreased by 0·04 SD (–0·07 to –0·01; −1·7%; p=3·5 × 10−14), and concentrations of C-reactive protein decreased by 0·02 SD (–0·04 to –0·01; –1·7%; p=7·7 × 10−14). We noted the effects of the genetic score on these inflammation biomarkers to be directionally concordant with those of anakinra. The allele count of the genetic score had roughly log-linear, dose-dependent associations with both IL-1Ra concentration and risk of coronary heart disease. For people who carried four IL-1Ra-raising alleles, the odds ratio for coronary heart disease was 1·15 (1·08–1·22; p=1·8 × 10−6) compared with people who carried no IL-1Ra-raising alleles; the per-allele odds ratio for coronary heart disease was 1·03 (1·02–1·04; p=3·9 × 10−10). Per-allele odds ratios were 0·97 (0·95–0·99; p=9·9 × 10−4) for rheumatoid arthritis, 0·99 (0·97–1·01; p=0·47) for type 2 diabetes, 1·00 (0·98–1·02; p=0·92) for ischaemic stroke, and 1·08 (1·04–1·12; p=1·8 × 10−5) for abdominal aortic aneurysm. In exploratory analyses, we observed per-allele increases in concentrations of proatherogenic lipids, including LDL-cholesterol, but no clear evidence of association for blood pressure, glycaemic traits, or any of the 24 other disorders studied. Modelling suggested that the observed increase in LDL-cholesterol could account for about a third of the association observed between the genetic score and increased coronary risk.

Interpretation Human genetic data suggest that long-term dual IL-1α/β inhibition could increase cardiovascular risk and, conversely, reduce the risk of development of rheumatoid arthritis. The cardiovascular risk might, in part, be mediated through an increase in proatherogenic lipid concentrations.

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rapid reductions in disease severity and symptoms in inflammatory disorders, they have been too brief or insufficiently powered to assess the effect on cardiovascular and other disease outcomes. A complementary approach is to study genetic variants known to result in IL-1 inhibition. Because genotypes are fixed at conception, human genetic studies could help to predict the effects of long-term IL-1 inhibition.

We aimed to create a genetic score that combines information on rs6743376 and rs1542176, two uncorrelated variants located upstream of ILRN, the gene encoding the IL-1 receptor antagonist (IL-1Ra). These variants are the strongest known genetic determinants of circulating IL-1Ra protein concentrations. IL-1Ra is an endogenous inhibitor of IL-1 that blocks activation of the IL-1 receptor by either IL-1α or IL-1β. This genetic score could thus mimic the effects of IL-1 inhibitors (eg, anakinra) that have the same mechanism of action as IL-1Ra (appendix p 41). By contrast, this genetic score would not necessarily be expected to mimic the effects of drugs that selectively inhibit either IL-1α or IL-1β (eg, canakinumab).

We investigated this genetic score in relation to rheumatoid arthritis and four cardiovascular disorders (type 2 diabetes, coronary heart disease, ischaemic stroke, and abdominal aortic aneurysm), and, in exploratory analyses, in relation to additional disorders and disease traits.

**Methods**

**Study design and procedures**

Figure 1 summarises the study approach, and the table provides definitions and sources of data used. First, we constructed a score containing two genetic variants (rs6743376 and rs1542176) previously robustly linked with IL-1Ra concentration, and then investigated the biological relevance of the score through analysis of gene expression data (figure 1, appendix pp 1–4). Second, we assessed the effects of our genetic score on circulating concentrations of IL-1Ra, interleukin 6 (IL-6), and C-reactive protein (CRP; table, appendix pp 5–10). We then compared the score’s effects on these inflammation biomarkers with those of anakinra, the recombinant form of IL-1Ra, from existing randomised trial data (appendix p 10). Third, because anakinra is licensed for treatment of rheumatoid arthritis, we assessed the score in patients with rheumatoid arthritis and in healthy controls (table, appendix p 11). Fourth, we assessed the score in relation to type 2 diabetes, coronary heart disease, ischaemic stroke, and abdominal aortic aneurysm (table, appendix pp 11–12) because each of these disorders is either being investigated as an outcome in trials of IL-1 inhibitors, or has been previously robustly linked with a functional genetic variant for inflammation, or both. Fifth, to gain insight into mechanisms that might link IL-1α/β signalling with cardiovascular disorders, we did exploratory analyses of the score in relation to disease traits (appendix pp 13–15). Sixth, to help predict the broad effects of long-term IL-1α/β inhibition, we explored the score in relation to several additional disorders (appendix p 12).

**Samples and data collection**

To investigate the biological relevance of the genetic score, we investigated associations with mRNA concentrations of genes in the vicinity of rs6743376 and rs1542176. We accessed information from the Multiple Tissue Human Expression Resource, which contains information on adipose tissue, skin, and lymphoblastoid cell lines from 850 people, and from the Genotype-Tissue Expression project, which includes information on 13 different tissue types derived from 60–170 people.

In up to 63,442 participants, we quantified the effects of the genetic score on concentrations of IL-1Ra, IL-6, and CRP using data from the Cardiovascular Health Study, Copenhagen City Heart Study, Copenhagen General Population Study, European Prospective Investigation into Cancer and Nutrition-Cardiovascular Disease Study, SardiNIA study, and UK10K consortium (table, appendix pp 5–10, 21–22, and 29).

To compare the effects on inflammation biomarkers of IL-1Ra-raising alleles with those of anakinra, we did a systematic review of published randomised trials, including data for 1,125 patients in eight trials (appendix pp 10, 38, 44). We calculated standardised treatment effects for anakinra doses of 75 mg or 100 mg (the most widely used doses in treatment of rheumatoid arthritis) on concentrations of inflammation biomarkers, and then pooled the results by fixed-effect inverse-variance weighted meta-analysis.

In 453,411 total participants, we investigated the genetic score in relation to rheumatoid arthritis and four cardiovascular disorders (type 2 diabetes, coronary heart disease, ischaemic stroke, and abdominal aortic aneurysm). For each disorder, we sought results from the largest available consortium. For rheumatoid arthritis, we accessed results from Okada and colleagues, for type 2 diabetes, from the Diabetes Genetics Replication and Meta-analysis consortium and European Prospective...
Investigation into Cancer and Nutrition-InterAct;16–18 for abdominal aortic aneurysm, from the Abdominal Aortic Aneurysm Genetics Consortium;19–22 and ischaemic stroke, from the METASTROKE consortium (appendix pp 26 and 29).23

For coronary heart disease, we had access to study-level data for 70 532 patients and 126 374 controls. For 98 961 of these participants (36 650 patients and 62 311 controls), we did de-novo genotyping of rs6743376 and rs1542176 (table and appendix pp 5–8). We did genotyping using customised arrays in a central laboratory by technicians masked to the phenotypic status of the participants’ samples for the following five studies contributing participant-level data: the Bangladesh Risk of Acute Vascular Events Study, Copenhagen City Heart Study, Copenhagen Ischaemic Heart Disease/Copenhagen General Population Study, European Prospective Investigation into Cancer and Nutrition-Cardiovascular Disease Study, and Pakistan Risk of Myocardial Infarction Study (appendix pp 9 and 23–25). Similar methods were used in three studies that did de-novo genotyping in local laboratories (deCODE, the German Myocardial Infarction Family Study, and the Ottawa Heart Genomics Study/Cleveland Clinic GeneBank). We supplemented genotyping with existing tabular data from the transatlantic Coronary Artery Disease Genome-wide Replication and Meta-analysis and Coronary Artery Disease Genetics consortia10,20 which enabled us to ascertain cases and controls within each allele count category of the genetic score (appendix p 12). About 90% of patients had myocardial infarction or other major acute coronary events; the remainder had angiographic evidence alone (eg, >50% coronary stenosis; appendix pp 35–36).

In up to 116 937 participants, we did exploratory analyses of the genetic score in relation to different disease traits to gain insight into mechanisms that might link IL-1α/β signalling with cardiometabolic disorders. We first examined 18 conventional cardiometabolic risk factors (total cholesterol, HDL cholesterol, triglycerides, LDL cholesterol,15,16 apolipoprotein A1 and B, lipoprotein[a], systolic and diastolic blood pressure, fasting glucose,17 HbA1c,18 fasting insulin,17 2 h glucose,17 fasting proinsulin,17 height, BMI, waist circumference, and waist to hip ratio).10,20 For each trait, we sought results from the largest available consortium (appendix p 33), supplemented with data from de-novo genotyping from the five studies mentioned previously providing participant-level data (appendix pp 5–8 and 21–22). When we noted suggestive associations between the genetic score and specific proatherogenic lipid concentrations, we extended this exploration to further traits, including metabolic profiles (eg, nuclear magnetic resonance spectroscopy metabolomics), subclinical cardiovascular phenotypes (eg, carotid intima-media thickness), and inflammatory cell subsets (eg, regulatory CD4 T-cell count). Appendix pp 33–34 provide a full account of the biological traits that we explored.

In a total of 205 329 patients and 423 905 controls, we did exploratory analyses of the genetic score in relation to 24 additional disorders of proposed relevance to IL-1 signalling, including autoimmune, degenerative, neoplastic, and infectious diseases. Again, we sought results from the largest available disease-specific consortia. Appendix pp 13–15, 27–28, and 30–34 provide a full account of the disorders that we explored.
Statistical analysis
We constructed a genetic score for IL-1 inhibition by counting the number of IL-1Ra-increasing alleles—ie, the C-alleles at rs6743376 and rs1542176 (appendix p 2). Our objective was to investigate the relevance of a biologically meaningful genetic score to eight prespecified outcomes—ie, three soluble inflammation biomarkers, rheumatoid arthritis, and four cardiometabolic disorders. To reduce the possibility of artifactual results and enable analysis of additional data, we constructed an alternative score consisting of two other IL-1Ra-increasing alleles (rs6759676 and rs4251961, which are each correlated with one of the variants used in the main score) that were identified in a separate genome-wide association study of IL-1Ra concentration4 (appendix p 3). We made allowances for study of eight main outcomes by using a Bonferroni-corrected significance threshold guideline of p=0.006 (ie, 0.05/8). For exploratory analyses (figure 1), we used as guidelines to help interpretation: p=0.003 (0.05/18) for analyses of 18 conventional cardiovascular risk factors and p=0.002 (0.05/24) for analyses of the additional 24 disorders. We could not use data from gene arrays that did not have information on both rs6743376 and rs1542176, or suitable proxies (eg, CardioMetaboChip, Immunochip, ITMAT-Broad-CARE, or Exome array [Illumina, California, USA]). We included information on participants of European or south Asian ancestry, but not on east Asians (rs6743376 and rs1542176 are correlated variants in east Asians, preventing creation of an appropriate genetic score; appendix pp 3 and 42).

To analyse summary-level data from consortia, we did a fixed-effect meta-analysis of the separate effects of rs6743376 and rs1542176 because these two variants are independent in European and south Asian ancestry populations (r²=0.00; D’=0.03 in 1000 Genomes). In an analysis of available individual participant data, we natural log transformed values of inflammation biomarkers and other continuous traits that had skewed distributions. We regressed standardised trait values on the genetic score, adjusting for age, sex, and ancestry-informative principal components. To help comparisons across markers, we primarily expressed associations as SD differences in concentrations and, secondarily, as percentage differences. For log-transformed variables, we obtained percentage changes as the exponent of the pooled log-transformed differences. For non-log-transformed variables, we obtained percentage change with reference to the pooled mean of each variable across studies. For estimates derived from consortia, the mean value and SD corresponded to that of the largest study. For all analyses, we used complete participant analysis—ie, we excluded participants with missing data.

To assess associations of the genetic score with dichotomous outcome diseases, we used logistic regression models that adjusted for age, sex, and ancestry-informative principal components. In the analysis of tabular data, we used logistic regression models to estimate the per-allele odds ratio, or calculated odds ratios within each score category compared with the reference category. We assessed dose–response relations for the genetic score with IL-1Ra concentrations or coronary heart disease risk, irrespective of an arbitrarily chosen reference group by attributing a floating variance estimate to each category, including the reference group, based on Plummer’s method.5 We did analyses separately by study, and pooled β coefficients across studies using fixed-effect inverse-variance-weighted meta-analysis. We assessed heterogeneity with the I² statistic. To test for deviation from a linear dose–response association of the genetic score with coronary heart disease risk, we compared the fit of models that assumed a linear trend of the genetic score with those that made no assumption about the shape of the association, using a likelihood ratio test. We used Stata 13.1 for statistical analyses.

Role of the funding source
The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. DF and JD had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Figure 2: Relation of the genetic score allele count with soluble IL-1Ra concentrations and risk of coronary heart disease
Box sizes correspond to the number of coronary heart disease cases or participants with IL-1Ra concentration measurements contributing to analyses. Error bars represent 95% CIs, calculated with the method of Plummer.4 Estimates are derived from fixed-effect meta-analyses of study-specific estimates. Contributing studies are listed in the appendix pp 35–36. The underlying study-specific results used to construct this figure are provided in the appendix p 48, which includes study-specific tabulations of cases and controls within each score category, study-specific odds ratios, and the pooling of these results across contributing studies. IL-1Ra=interleukin 1 receptor antagonist.
Results

The frequency of IL-1Ra-raising alleles was about 30% for rs6743376 and 50% for rs1542176. Analysis of the Encyclopedia of DNA Elements (ENCODE) suggested that the locus around rs6743376 and rs1542176 is a gene-regulatory region (appendix p 42). In the Multiple Tissue Human Expression Resource, we noted significant associations between the genetic score and ILIRN mRNA concentrations in subcutaneous adipose tissue (p = 3.9 x 10⁻⁸) and lymphoblastoid cell lines (p = 1.7 x 10⁻⁴; appendix pp 20 and 42–43). However, in these tissues (and in about ten further tissues we studied in the Genotype-Tissue Expression project; appendix p 4), we did not note significant associations between this score and mRNA concentrations of other genes in this region (data not shown).

We noted a roughly log-linear, dose-dependent association of the genetic score with IL-1Ra concentration (figure 2; appendix p 43). For each ILIRN C-allele inherited, IL-1Ra concentrations increased by 0.22 SD (figure 2; appendix p 43). For each association of the genetic score with IL-1Ra concentration (data not shown).

![Figure 3](https://example.com/figure3.png)

**Figure 3:** Effects of inflammation biomarkers of the genetic score compared with administration of 75 mg or 100 mg anakinra in eight randomised trials

Error bars show 95% CIs. To enable comparison of the magnitude of associations across several different markers, we did analyses with standardised units of measurement for each marker. Associations are presented as per 75 mg or 100 mg dose of anakinra compared with placebo, or per-allele change in the biomarker expressed as SDs. Study descriptions, individual study estimates, and meta-analysis results are provided for trial results in the appendix pp 16, 38, and 45. Genetic analyses are also provided in the appendix p 46. CRP=C-reactive protein. IL-1Ra=interleukin 1 receptor antagonist. IL-6=interleukin 6.
For the table of associations of the genetic score with intermediate traits see http://www.phpc.cam.ac.uk/ceu/il-1-genetics-consortium

we observed between the score and risk of coronary heart disease (appendix pp 18–19).

We did not observe clear evidence of associations between the score and apolipoprotein B concentration, perhaps because available data on apolipoprotein B were only about a fifth as great as those for total cholesterol. Similarly, we did not observe clear evidence of associations between the score and several glycaemic traits. Perhaps because we generally had low power to study some traits, we did not note any clear associations of the genetic score with a range of proatherogenic lipid subclasses, metabolites, and other intermediate traits (eg, carotid intima-media thickness, presence of carotid plaque, or carotid-femoral pulse wave velocity; appendix p 37). For these traits, we typically analysed data for about a tenth of the number of participants we studied for total cholesterol.

We did not observe clear evidence of associations between the score and any of the 24 additional disorders that we studied, including a range of autoimmune, neoplastic, degenerative, and infectious disease outcomes.
However, we noted substantial variation in the amount of available data (and, hence, power to detect associations) across the different disorders that we explored (appendix pp 54–56).

Discussion

Drugs such as anakinra that produce dual IL-1α/β inhibition are licensed for treatment of inflammatory disorders, but the long-term effects of such treatment on cardiovascular and other outcomes remain unknown (panel). Our powerful and multilayered analysis of human genetic data has suggested the surprising conclusion that sustained dual IL-1α/β inhibition could increase the risk of cardiovascular diseases, in part via increased proatherogenic lipids. These findings provide new insights into the clinical and biological effects of IL-1α/β signalling.

We anchored our investigation in a genetic score that was associated with upregulation of IL-1Ra and that produced effects on soluble biomarkers consistent with IL-1α/β inhibition. The score’s biological relevance was suggested by its exclusive association with IL1RN mRNA concentrations in two tissues, by its roughly log-linear dose–response relation with soluble IL-1Ra concentration, and by its effects on soluble inflammation biomarkers that were directionally concordant with those of anakinra. We studied this genetic score in relation to rheumatoid arthritis, a disorder already treated with anakinra. We noted that our score was associated with a decreased risk of rheumatoid arthritis, a new finding that reinforces the relevance of our score to the known clinical effects of IL-1α/β inhibition. This finding raises the possibility that long-term IL-1α/β inhibition could prevent (or at least delay) development of rheumatoid arthritis. Moreover, this finding provides a further example of the overlap noted between targets related to genes implicated in rheumatoid arthritis and treatments already approved for it.6,51

Contrary to expectation, however, several of our findings suggest that long-term IL-1α/β inhibition could increase the risk of cardiovascular diseases. First, our genetic score was associated with an increased risk of coronary heart disease in a roughly log-linear and dose-dependent manner, analogous to the association we observed between the same score and IL-1Ra concentration. However, even taken together, investigators of trials of anakinra have recorded fewer than 40 coronary heart disease outcomes (appendix pp 16 and 39–40), and none have specifically addressed cardiovascular safety. Thus, we could not compare our results on cardiovascular risk with those from randomised trials.

Second, we noted potential associations between the genetic score and increased concentrations of LDL cholesterol and triglycerides. Our modelling analysis suggested that such associations could explain about a third of the observed association between the score and increased coronary heart disease risk.56,49 However, we were again unable to compare our findings meaningfully with results from relevant randomised trials. Findings from two small anakinra trials (together consisting of fewer than 100 participants)46,47 showed no significant increases in proatherogenic lipid concentrations, but the magnitude of lipid increases detectable in these trials is unknown because the studies did not report numerical results. By contrast, investigators of a trial of canakinumab (a selective IL-1β inhibitor) in about 500 participants50 reported significant elevations in triglyceride (but not LDL cholesterol) concentrations. However, the relevance of our genetic score to canakinumab is uncertain because although our score should mimic the effects of dual IL-1α/β inhibition, it does not necessarily mirror selective IL-1β inhibition. Indeed, we note that suitable genetic scores do not exist that distinguish the effects of long-term IL-1α/β inhibition from those of long-term inhibition of IL-1β, and the two cytokines seem distinct and non-redundant.4,51 Hence, our results do not necessarily have implications for trials such as the Canakinumab Anti-infl ammatory Thrombosis Outcome Study, which is designed to test selective IL-1β inhibition in secondary prevention of cardiovascular disease.4,52

Third, our genetic score was associated with increased risk of abdominal aortic aneurysm. Whereas our findings show that inhibition of IL-1α/β signalling could increase the risk of coronary heart disease and abdominal aortic aneurysm, findings from previous human genetic studies (including our own) have shown that inhibition...
of IL-6 signalling could actually reduce the risk of these disorders. Together, these findings highlight the complexity of inflammatory pathways underlying cardiovascular diseases. Studies are needed to help to understand mechanisms that account for the divergent effects of these two interrelated inflammation pathways.

Our findings also challenge studies in animals that have reported reductions in atherosclerosis, or slowing of aneurysm growth, after pharmacological dual IL-1α/β inhibition or genetic deletion of components of the IL-1α/β system. For example, findings from some studies of mice without the IL-1 receptor have suggested a protective role of IL-1α/β signalling in atherosclerosis. The contrast between most previous animal studies and the results from our study of human genetic data might be due, at least in part, to the limited ability of model organisms to fully represent the human immune system and human cardiovascular diseases.

Fourth, we noted no evidence of associations between our genetic score and the risk of type 2 diabetes, insulin sensitivity, other glycaemic traits, blood pressure, or adiposity. By comparison, investigators of three small randomised trials of anakinra (collectively comprising about 150 participants) reporting conflicting results in relation to effects on HbA1c values. Furthermore, findings from a trial of canakinumab in about 500 participants did not show significant reductions in HbA1c values. Nevertheless, our genetic study had substantially less power to assess type 2 diabetes and measures of glycaemia than it did to assess coronary heart disease and proatherogenic lipids. Hence, although our results suggest that long-term IL-1α/β inhibition is unlikely to prevent type 2 diabetes or improve metabolic features associated with the disease, further studies might be needed to assess any moderate effects. Similar considerations apply to the null association between our genetic score and the risk of ischaemic stroke.

Our study had major strengths and potential limitations. One strength was that we used a prespecified analysis plan. We also replicated our main findings using an alternative genetic score that contained different variants from those used in our main score. We accessed results from about 1 million people in worldwide consortia of relevant diseases and traits, which we supplemented with data from de-novo genotyping in nearly a further 100 000 participants. Because we showed that our genetic score was exclusively associated with ILIRN mRNA concentrations in adipose tissue and lymphoblastoid cell lines, the associations that we observed of the genetic score with cardiovascular diseases and traits were unlikely to be driven by neighbouring genes or variants. However, we did not have access to data for other potentially relevant tissues (eg, primary leucocytes or hepatocytes). Because our genetic score should provide information about the effects of lifelong IL-1α/β inhibition, reduced IL-1α/β signalling from early life could lead to compensatory changes that affect cardiovascular risk. However, our genetic score was associated with reduced concentrations of both IL-6 and CRP in adults, consistent with the expected downstream effects of uncompensated IL-1 inhibition.

One limitation of our study is that its findings can suggest only qualitative concordance of the effects on inflammation biomarkers of our genetic score and anakinra. Genetic and pharmacological IL-1 inhibition differ with respect to the magnitude and duration of inhibition, shown by the 5–10-times weaker effects that we observed of our genetic score on inflammation biomarkers compared with those of anakinra. Few people in randomised trials of anakinra have had IL-1Ra concentrations measured. In addition, whereas anakinra has mainly been studied in trials of people with pre-existing inflammatory disorders, we related our genetic score to inflammation biomarkers mainly in healthy people.

For the aforementioned reasons, the data in this report are difficult to use to estimate the magnitude of potential cardiovascular hazard associated with dual IL-1α/β inhibition. Nevertheless, the robust but moderate associations that we identified in this study between genetic IL-1α/β inhibition and cardiovascular risk do not preclude a substantial clinical effect because the size of an odds ratio conferred by natural variation in a particular gene bears no necessary relation to the size of hazard or benefit that might accrue from intervention directed at the pathway that the gene identifies. For example, statins confer substantial reductions in cardiovascular risk, despite slight associations between common variants in genes that are the target of statins and coronary heart disease. In summary, our study—which has introduced the concept of use of a wide-angle genetic approach to predict the broad phenotypic effects of perturbation of a biological pathway—has provided new insights into the clinical and biological effects of dual IL-1α/β signalling in relation to several cardiometabolic disorders and disease traits.

Contributors
The writing committee was composed of DFF, ASB, PW, JMMH, Sbu, Skap, AMW, MSw, JRS, AR, DGo, BGN, MS, DGT, JD, and JD. DFF, ASB, PW, Sbu, Skap, SGT, and JD contributed to the study concept and design. PG, MJ, GTJ, AvR, DRC, MA, K, GFM, IBW, CDL, LB, SW, AF, SAL, MM-N, S, ED, AS, HH, DFE, Te, HG, RSH, S, SSa, NS, NS, HSM, NJW, DJR, JR, TA, TBH, VA, AH, OH, RT, B, MP, HW, ASH, NJS, WM, RCI, Rgi, JJS, JCC, JCA, JBM, J, AK, HS, KS, UT, JD, AT, H, DSA, AM, EDA, RCH, BGN, DS, and JD recruited and characterised participants. ASB, JMMH, RW, HK, SSp, SFN, LAL, MJB, GTJ, RAS, SBE, PIVGB, and JEP worked in the laboratory and managed data. DFF, PW, JMMH, Sbu, Skap, RW, HK, EH, SFN, LAL, MJB, GTJ, RAS, SBE, EP, GTH, TK, LZ, MN, RD, W, JCH, MK, GED, CPN, AG, JCA, AD, SL, AVS, I, LQ, AFB, FNG, GT, HK, MDR, SSD, DCC, J, MdA, IKJ, PLP, CAM, EPB, OC, DRC, DSC, U, J, JAP, JH, NJT, MAK, K, GFM, AP, MG, YLI, NF, MFK, SKG, CDL, LB, MAB, DME, Sbu, MB, BB, SAL, MM-N, JFF, NLS, Msw, EZ, KP, MAN, AS, CP, IB, HH, DFE, DF, JFP, J, IPT, MD, KH, GM, TE, HG, JMA, MH, NW, Y, A, DCh, RSH, MMI, MDT, MHL, NKK, YLu, SN, and MB contributed results. DFF, ASB, JMMH, Sbu, Skap, and SGT were responsible for data and statistical analyses. DFF, ASB, PW, JMMH, Sbu, Skap, AMW, MSw, JRS, AR, DGo, BGN, DS, SG, and JD drafted the manuscript. All authors contributed to, read, and approved the final version of the report.
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Declaration of interests

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