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

Freitag, Daniel F.; Butterworth, Adam S.; Willeit, Peter; Howson, Joanna M. M.; Burgess, Stephen; Kaptoge, Stephen; Young, Robin; Ho, Weang Kee; Wood, Angela M.; Sweeting, Michael; Spackman, Sarah; Staley, James R.; Ramond, Anna; Harshfield, Eric; Nielsen, Sune F.; Grande, Peer; Lange, Leslie A.; Bown, Matthew J.; Jones, Gregory T.; Scott, Robert A.; Bevan, Steve; Porcu, Eleonora; Thorleifsson, Gudmar; Zeng, Lingyao; Kessler, Thorsten; Nikpay, Majid; Do, Ron; Zhang, Weihua; Hopewell, Jemma C.; Kleber, Marcus; Delgado, Graciela E.; Nelson, Christopher P.; Goel, Anuj; Bis, Joshua C.; Dehghan, Abbas; Ligthart, Symen; Smith, Albert V.; Qu, Liming; van 't Hof, Femke N. G.; de Bakker, Paul I. W.; Baas, Annette F.; van Rij, Andre; Tromp, Gerard; Kuivaniemi, Helena; Ritchie, Marylyn D.; Verma, Shefali S.; Crawford, Dana C.; Malinowski, Jennifer; de Andrade, Mariza; Kullo, Iftikhar J.

Published in:
The Lancet Diabetes & Endocrinology

DOI:
10.1016/S2213-8587(15)00034-0

2015

Link to publication

Citation for published version (APA):

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 09. Apr. 2019
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 × 10⁻³), concentrations of interleukin 6 decreased by 0·02 SD (–0·04 to –0·01; –1·7%; p=3·5 × 10⁻¹⁵), and concentrations of C-reactive protein decreased by 0·03 SD (–0·04 to –0·02; –3·4%; 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.

Funding UK Medical Research Council, British Heart Foundation, UK National Institute for Health Research, National Institute for Health Research Cambridge Biomedical Research Centre, European Research Council, and European Commission Framework Programme 7.

Copyright © The Interleukin 1 Genetics Consortium. Open Access article distributed under the terms of CC-BY.
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 (eg, MABp1) 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 dual 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-Car 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 1125 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,26–30 for abdominal aortic aneurysm, from the Abdominal Aortic Aneurysm Genetics Consortium,31,32 and ischaemic stroke, from the METASTROKE consortium (appendix pp 26 and 29).25

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 consortia,33,34 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,35,36 apolipoprotein A1 and B, lipoprotein[a], systolic and diastolic blood pressure, fasting glucose,37 HbA1c,38 fasting insulin,39 2 h glucose,37 fasting proinsulin,40 height, BMI, waist circumference, and waist to hip ratio).41,42 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-1β 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 concentration\(^a\) (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 (\(r^2=0.00\); \(D'=0.03\) in 1000 Genomes).\(^b\) 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 disease outcomes, 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.\(^c\) We did analyses separately by study, and pooled \(\beta\) coefficients across studies using fixed-effect inverse-variance-weighted meta-analysis. We assessed heterogeneity with the \(I^2\) 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 assuming a quadratic trend. We also examined a range of other possibilities. We fitted linear additive genetic scores in analyses of coronary heart disease outcomes only. We fitted a quadratic score consisting of two other IL-1Ra-increasing alleles—ie, the I-alleles at rs6743376 and rs1542176 (appendix pp 3 and 42). 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.

Analysis of coronary heart disease outcomes

<table>
<thead>
<tr>
<th>Genetic allele count</th>
<th>Analysis of coronary heart disease outcomes</th>
<th>Analysis of soluble IL-1Ra concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases 8121</td>
<td>Controls 16,960</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>8121</td>
<td>23,029</td>
</tr>
<tr>
<td>Analysis of soluble IL-1Ra concentrations</td>
<td>4119</td>
<td>43,912</td>
</tr>
<tr>
<td>Controls</td>
<td>332</td>
<td>990</td>
</tr>
</tbody>
</table>

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.\(^a\) 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 IL1RN mRNA concentrations in subcutaneous adipose tissue (p=3·9×10⁻⁹) and lymphoblastoid cell lines (p=1·7×10⁻⁴; 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 IL1RN C-allele inherited, IL-1Ra concentrations increased by 0·22 SD (figure 2; appendix p 43). In the Multiple Tissue Human Expression Resource, we noted significant associations between the genetic score and IL1RN mRNA concentrations in subcutaneous adipose tissue (p=3·9×10⁻⁹) and lymphoblastoid cell lines (p=1·7×10⁻⁴; 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).

In subsidiary analyses, we noted that rs6743376 and rs1542176 each had similar-sized effects, both on IL-1Ra concentration and coronary heart disease risk (appendix pp 46–47). We used an alternative score described in the methods section consisting of two further SNPs (rs6759676 and rs4251961, which each are correlated with one of the SNPs used in our main score) (appendix pp 17 and 50–51). Per-allele odds ratios with the alternative score were similar to those in the primary score for rheumatoid arthritis (odds ratio 0·96 [0·94–0·98; p=4·7×10⁻⁴]), type 2 diabetes (1·00 [0·98–1·01; p=0·53]), coronary heart disease (1·03 [1·02–1·04; p=3·7×10⁻⁴]), and abdominal aortic aneurysm (1·04 [1·01–1·08; p=0·011]; appendix pp 50–51).

We noted a roughly log-linear and dose-dependent association between the score and coronary heart disease risk (figure 2), with no evidence to support the existence of a non-linear association (p=0·59). For the 3% of 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). Results were similar in analyses restricted to studies of myocardial infarction (appendix p 49). We did not attempt dose–response analyses for other main outcomes studied because we did not have sufficient power.

We noted that, per allele, the genetic score was associated with a 0·016 SD (0·009–0·022) increase (0·3%; p=5·8×10⁻⁶) in total cholesterol concentration, a 0·014 SD (0·007–0·022) increase in LDL cholesterol concentration (0·3%; p=9·5×10⁻⁷), and a 0·009 SD (0·003–0·015) increase in triglyceride concentration (0·4%; p=2·6×10⁻³; figure 5; appendix p 18). To estimate how much of the association we observed between the genetic score and coronary heart disease could be accounted for by LDL cholesterol concentration, we estimated the causal effect of life-long change in LDL cholesterol concentration in two ways. First, our genetic estimation used published reports that identified variants that were robustly and exclusively associated with LDL cholesterol concentrations.6 Second, our phenotypic estimation used individual participant data for long-term average serum lipid concentrations from 302 430 participants in the Emerging Risk Factors Collaboration.6 These complementary approaches yielded broadly concordant findings, suggesting that LDL cholesterol concentration could account for 20–40% of the association.

### Table: Standardised effect of genetic score perturbations

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Perturbation</th>
<th>Standardised effect on biomarker, per 75 or 100 mg anakinra or per genetic score allele</th>
<th>Standardised effect (95% CI)</th>
<th>p value</th>
<th>Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1Ra</td>
<td>Anakinra</td>
<td>-0·17 (0·21 to -0·14)</td>
<td>4·22 (3·04 to 5·40)</td>
<td>2·7×10⁻¹⁰</td>
<td>63 442</td>
</tr>
<tr>
<td></td>
<td>Genetic score</td>
<td>-0·03 (-0·04 to -0·02)</td>
<td>0·22 (0·18 to 0·25)</td>
<td>9·3×10⁻¹¹</td>
<td>3081</td>
</tr>
<tr>
<td>IL-6</td>
<td>Anakinra</td>
<td>-0·23 (-0·39 to -0·07)</td>
<td>0·22 (0·18 to 0·25)</td>
<td>9·3×10⁻¹¹</td>
<td>3081</td>
</tr>
<tr>
<td></td>
<td>Genetic score</td>
<td>-0·02 (-0·04 to -0·01)</td>
<td>-0·02 (-0·04 to -0·01)</td>
<td>4·7×10⁻⁵</td>
<td>278</td>
</tr>
<tr>
<td>CRP</td>
<td>Anakinra</td>
<td>-0·17 (-0·21 to -0·14)</td>
<td>-0·17 (-0·21 to -0·14)</td>
<td>4·0×10⁻⁷</td>
<td>1125</td>
</tr>
<tr>
<td></td>
<td>Genetic score</td>
<td>-0·03 (-0·04 to -0·02)</td>
<td>-0·03 (-0·04 to -0·02)</td>
<td>7·7×10⁻⁷</td>
<td>63 442</td>
</tr>
</tbody>
</table>

Figure 3: Effects on 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.
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.

**Figure 4:** Associations of the genetic score with rheumatoid arthritis and four cardiometabolic disorders

Box sizes correspond to the number of cases contributing to analyses. Error bars show 95% CI. Summary statistics for individual single-nucleotide polymorphisms in the genetic score are provided in the appendix p 56. Coronary heart disease analyses are based on fixed-effects meta-analyses of study-level tabular data, assuming a per-allele model. Individual study estimates and meta-analysis results are provided in the appendix p 47. The I² value for between-study heterogeneity for coronary heart disease analyses was 28.2% (p=0.113). For the other endpoints, however, a measure of between-study heterogeneity could not be calculated because each of the odds ratios derive from a single result provided by a consortium. Analyses of ischaemic stroke subtypes are provided in the appendix p 52. IL-1-interleukin 1. NA—not applicable. *Contributing data include, in part, rs6761276[T] as a proxy for rs6743376[C]. †Not applicable because effect estimates derive from a single result.

**Figure 5:** Associations of the genetic score with cardiovascular and metabolic risk factors

Box sizes correspond to the number of participants contributing to analyses. Error bars show 95% CI. Summary statistics for individual single-nucleotide polymorphisms in the genetic score are provided in the appendix p 56. Coronary heart disease analyses are based on fixed-effects meta-analyses of study-level tabular data, assuming a per-allele model. Individual study estimates and meta-analysis results are provided in the appendix p 47. The I² value for between-study heterogeneity for coronary heart disease analyses was 28.2% (p=0.113). For the other endpoints, however, a measure of between-study heterogeneity could not be calculated because each of the odds ratios derive from a single result provided by a consortium. Analyses of ischaemic stroke subtypes are provided in the appendix p 52. IL-1-interleukin 1. NA—not applicable. *Contributing data include, in part, rs6761276[T] as a proxy for rs6743376[C]. †Not applicable because effect estimates derive from a single result.

For the table of associations of the genetic score with intermediate traits see http://www.phpc.cam.ac.uk/ceu/il-1-genetics-consortium
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.13

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.17,40 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 participants40 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 inhibition of IL-1α from those of long-term inhibition of IL-1β, and the two cytokines seem distinct and non-redundant.6,51 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.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) reported 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 IL1RN 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 IL1Ra 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 (LDLR and HMGCR) 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, JMHH, Sbu, Skap, AMW, Msw, Jrs, AR, DcG, BGN, DS, SGT, and JD. DFF, ASB, PW, Sbu, Skap, JD contributed to the study concept and design. PG, MB, DRC, MA-K, GFM, IBW, CDL, LB, SW, AF, SAL, MM-N, Sdt, EZ, AS, HH, DFE, TE, HG, Rsh, SN, Ssa, NS, HSM, NJW, DJR, MR, TA, TBH, VG, AH, OIF, RT, Bmp, MF, HW, ASH, NJS, WM, Rcl, Rgs, Jsk, Jcc, Skat, BM, JE, Ak, HS, KS, Ut, JdW, Abh, Dsa, AM, EDA, Rch, BGN, DS, and JD recruited and characterized participants. ASB, JMHH, RW, Whk, Ssp, SFN, LAL, MjB, GTJ, RAS, SBE, Ptwgb, and Je worked in the laboratory and managed data. DFF, PW, JMHH, Skap, RW, WHK, EH, SFN, LAL, MjB, GTJ, RAS, SBE, EP, Gth, TK, LZ, MN, RD, WZ, JCk, MK, GED, CPN, AG, Jcb, AD, Sl, AvS, Iq, AFb, FngVh, Gtr, HK, Mdr, Ssv, Dcc, JM, Mda, Ikj, Plp, Cam, Epb, Og, Drc, Dsc, IJr, Jap, HJ, Njt, Maks, Kk, Gp, AP, MG, Yli, NF, MFk, Skg, Cdl, LB, MAB, Dme, Sbu, MAF, HB, SAL, MM-N, Jff, Nls, M5u, Ez, KP, Man, AS, CP, IpB, HH, Dfe, Df, DfE, Ipt, MD, KH, GM, TE, HG, Jma, MH, NW, Yw, Dch, Rsh, Mmi, Dtb, Mhl, Nkh, YLu, SN, and Mb contributed results. DFF, ASB, JMHH, Sbu, Skap, and SGT were responsible for data and statistical analyses. DFF, ASB, PW, JMHH, Sbu, Skap, AMW, Msw, Jrs, AR, DcG, BGN, DS, SGT, and JD drafted the manuscript. All authors contributed to, read, and approved the final version of the report.
Articles

of Cambridge, Cambridge, UK); Nicholas J Wareham FRCP (Medical Research Council Epidemiology Unit, Cambridge, UK);

Daniel J Rader MD, Murredch Reilly MD (Institute for Translational Medicine and Therapeutics, and Cardiovascular Institute, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA); Themistocles Assimes MD (Stanford University, Stanford, CA, USA); Tamara B Harris MD (Laboratory of Epidemiology and Population Sciences, National Institute on Aging, Bethesda, MD, USA); Albert Hofman MD, Oscar H Franco MD (Erasmus Medical Center, University Medical Center Rotterdam, Rotterdam, Netherlands); Vilmundur Gudnason MD (Icelandic Heart Association, Kópavogur, Iceland, and University of Iceland, Reykjavik, Iceland); Russell Tracy PhD (University of Vermont College of Medicine, Burlington, VT, USA); Bruce M Peatty MD (University of Washington, Seattle, WA, USA); Martin Farrall PhD, Hugh Watkins FMedSci (University of Oxford, Oxford, UK); Alastair S Hall FRCP (Leeds Institute of Genetics, Health and Therapeutics, University of Leeds, Leeds, UK); Nilesh J Samani FRCP (University of Leicester and National Institute for Health Research Leicester Cardiovascular Biomedical Research Unit, Leicester, UK); Winfried März MD (Sylnab Academy, Sylnab Services GmbH, Mannheim, Germany); Robert Clarke FRCP, Rory Collins FMedSci (University of Oxford, Oxford, UK); Jaspal S Kooper FRCP, John C Chambers PhD (Imperial College, London, UK); Myocardial Infarction Genetics Consortium; Sekar Kathiresan MD (Broad Institute, Cambridge and Massachusetts General Hospital, Boston, MA, USA); Ruth McPherson FRCPC (Ruddy Canadian Cardiovascular Genetics Centre, University of Ottawa Heart Institute, Ottawa, Canada); Jeanette Erdmann PhD (Institute for Integrative and Experimental Genomics, University of Lübeck, and German Research Centre for Cardiovascular Research, Lübeck, Germany); Adnan Kastrati MD, Heribert Schunkert MD (Deutsches Herzcentrum München, Technische Universität München, and German Centre for Cardiovascular Research, partner site Munich Heart Alliance, Munich, Germany); Kári Stefánsson MD, Úrurur Thorsteinsdottir PhD (deCODE Genetics/Amgen, and University of Iceland, Reykjavik, Iceland); Jeremy D Walston MD (Johns Hopkins University School of Medicine, Baltimore, MD, USA); Anne Tybjærg-Hansen MD (Copenhagen University Hospital, University of Copenhagen, Copenhagen, Denmark); Dewan S Alam PhD (Centre for Control of Chronic Diseases, International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh); Abdulla Al Shaif Majumder FRCP (National Institute of Cardiovascular Diseases, Dhaka, Bangladesh); Emanuele Di Angelantonio MD, Rajiv Chowdhury MD (University of Cambridge, Cambridge, UK); Birge G Nordestgaard MD (Copenhagen University Hospital, University of Copenhagen, Copenhagen, Denmark); Danish Salehuddin MD (University of Pennsylvania, Philadelphia, USA, and University of Cambridge, Cambridge, UK); Simon G Thompson FMedSci, and John Danesh FRCP (University of Cambridge, Cambridge, UK)

Declaration of interests

The work of DFF, ASB, SGT, JRS, AR, JMMH, SKap, AMW, SB, and JD has been supported by grants awarded to JD from the UK Medical Research Council, British Heart Foundation, UK National Institute for Health Research, National Institute for Health Research Cambridge Biomedical Research Centre, European Research Council Framework Programme 7 (HEALTH-F2-2012-279213), Funding for the component studies in this analysis is provided in the appendix. Murray Clarke, Philip De Jagter, Peter Libby, Ziad Mallat, Nadeem Sarwar, and John Todd provided helpful comments on an earlier version of the manuscript. Collaborators are listed in the appendix pp 65–86. A full set of acknowledgments is provided in the appendix pp 87–96.

References


13 Kamstrup PR, Tybjærg-Hansen A, Steffensen R, Nordestgaard BG. Genetically elevated lipoprotein(a) and increased risk of myocardial infarction. JAMA 2009; 301: 2311–19.


www.thelancet.com/diabetes-endocrinology Vol 3 April 2015 253 Articles