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. Dec. 2018
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 × 10–⁴), concentrations of interleukin 6 decreased by 0·04 SD (–0·04 to –0·01; –1·7%; p=3·5 × 10–⁴), 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 × 10–⁷) 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.
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 (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-Coronary 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

Figure 1: Study design

CRP=C-reactive protein. GWAS=genome-wide association study. IL-1Ra=interleukin 1 receptor antagonist. IL-6=interleukin 6. RCT=randomised controlled trial. *Refers to the Multiple Tissue Human Expression Resource and Genotype-Tissue Expression project.

See Online for appendix

For the Multiple Tissue Human Expression Resource see http://www.muther.ac.uk

For the Genotype-Tissue Expression project see http://www.gtexportal.org

For UK10K consortium see http://www.uk10k.org

Investigation into Cancer and Nutrition-InterAct;26–30 for abdominal aortic aneurysm, from the Abdominal Aortic Aneurysm Genetics Consortium;31–33 and ischemic 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 consortium34,35 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,36–38 apolipoprotein A1 and B, lipoprotein[a], systolic and diastolic blood pressure, fasting glucose,39 HbA1c,40 fasting insulin,40 2 h glucose,40 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-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.

<table>
<thead>
<tr>
<th>Participants</th>
<th>Assessment method or endpoint definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Interleukin 1 receptor antagonist</strong></td>
<td></td>
</tr>
<tr>
<td>CHS14</td>
<td>3081</td>
</tr>
<tr>
<td><strong>Interleukin 6</strong></td>
<td></td>
</tr>
<tr>
<td>CHS15</td>
<td>2917</td>
</tr>
<tr>
<td>SardNA15</td>
<td>5924</td>
</tr>
<tr>
<td>UK10K consortium</td>
<td>711</td>
</tr>
<tr>
<td><strong>C-reactive protein</strong></td>
<td></td>
</tr>
<tr>
<td>CHS15</td>
<td>3181</td>
</tr>
<tr>
<td>CCHS15</td>
<td>3806</td>
</tr>
<tr>
<td>CGPS15</td>
<td>2228</td>
</tr>
<tr>
<td>EPIC-CVD14</td>
<td>14100</td>
</tr>
<tr>
<td>SardNA15</td>
<td>5716</td>
</tr>
<tr>
<td>UK10K consortium</td>
<td>33911</td>
</tr>
<tr>
<td><strong>Rheumatoid arthritis</strong></td>
<td></td>
</tr>
<tr>
<td>Okada et al16</td>
<td>14361/43923</td>
</tr>
<tr>
<td><strong>Type 2 diabetes</strong></td>
<td></td>
</tr>
<tr>
<td>DIAGRAM and EPIC-InterAct17, 18</td>
<td>18715/61692</td>
</tr>
<tr>
<td><strong>Coronary heart disease</strong></td>
<td></td>
</tr>
<tr>
<td>CAD19</td>
<td>11733/11816</td>
</tr>
<tr>
<td>CARDIoGRAM18</td>
<td>22149/52247</td>
</tr>
<tr>
<td>Studies in CARDioGRAM18 with de-novo genotyping (deCODE,19 GenMIFS I–V,21 and OHGS/CGPS)22</td>
<td>14703/38817</td>
</tr>
<tr>
<td>Studies with de-novo genotyping in a central laboratory (BRAVE,1 CHS,11 OHGS/CGPS,14 EPIC-CVD,14 and PROMIS)23</td>
<td>21947/23494</td>
</tr>
<tr>
<td><strong>Ischaemic stroke</strong></td>
<td></td>
</tr>
<tr>
<td>METAStroke25</td>
<td>12389/62004</td>
</tr>
<tr>
<td><strong>Abdominal aortic aneurysm</strong></td>
<td></td>
</tr>
<tr>
<td>AAA Genetics Consortium26–30</td>
<td>4682/38739</td>
</tr>
</tbody>
</table>

For the Diabetes Genetics Replication and Meta-analysis consortium see http://diagram-consortium.org
For glycaemic traits see http://www.diagram-consortium.org
For anthropometric traits see http://www.broadinstitute.org/collaboration/giant/

**Table: Definitions and sources of contributing data for main study outcomes**

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\(^4\) (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 \((r^2=0.00; D'=0.03 \text{ in 1000 Genomes}).\) In an analysis of available individual participant data, we used 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.\(^4\) 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 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.
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 (95% CI 0·18 to 0·25; 12·5%; p=9·3×10–³³), IL-6 concentrations decreased by 0·02 SD (–0·04 to –0·01; –1·7%; p=3·5×10–³), and CRP concentrations decreased by 0·03 SD (–0·04 to –0·02; –3·4%; p=7·7×10–¹⁴; figure 3). The effects of the genetic score on these inflammation biomarkers were directionally concordant with those observed in anakinra trials. However, the absolute per-allele effects of the genetic score were much weaker than the effects of anakinra. Per-allele odds ratios with the score 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, 1·03 (1·02–1·04; p=3·9×10–¹⁰) for coronary heart disease, 1·00 (0·98–1·02; p=0·92) for ischaemic stroke, and 1·08 (1·04–1·12; p=1·8×10–⁶) for abdominal aortic aneurysm (figure 4).

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=4·7×10–³]), type 2 diabetes (1·00 [0·98–1·01; p=0·53]), coronary heart disease (1·03 [1·02–1·04; p=3·7×10–⁸]), 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=5·8×10–¹⁰) in total cholesterol concentration, a 0·014 SD (0·007–0·022) increase in LDL cholesterol concentration (0·3%; p=9·5×10–⁴), and a 0·009 SD (0·003–0·015) increase in triglyceride concentration (0·4%; p=2·6×10–¹¹; 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. 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. These complementary approaches yielded broadly concordant findings, suggesting that LDL cholesterol concentration could account for 20–40% of the association between the score and coronary heart disease.

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 differences in the genetic score and risk of coronary heart disease (appendix pp 18–19).

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.15

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 assessed 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.3–6 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)45–47 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.51,52 Hence, our results do not necessarily have implications for trials such as the Canakinumab Anti-inflammatory 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

**Panel: Research in context**

**Systematic review**

We searched Medline for randomised clinical trials published before Feb 18, 2014, without any language restrictions, by combining keywords related to IL-1α/β-blocking drugs and randomised clinical trials—eg, “anakinra”, “riluaccept”, “IL-1R1”, and “AMG108” (the full search strategy is provided in the appendix p 10). We identified 29 randomised, placebo-controlled trials of dual IL-1α/β inhibitors, such as anakinra, which have mainly been done in patients with rheumatoid arthritis and other inflammatory disorders. 22 of these trials reported on serious adverse events, but none specifically assessed cardiovascular safety. Investigators of only a few small trials45–47 have reported on glycosylated haemoglobin values and other metabolic measures, and they have reported conflicting results. Neither of two small trials of anakinra46–47 have reported increases in proatherogenic lipid concentrations, but they did not report numerical results.

**Interpretation**

Our powerful and multilayered analysis of human genetic data has suggested the surprising conclusion that long-term 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, and could have implications for patients taking anakinra and related drugs.
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 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 (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, JMMH, SBu, SKap, AMW, MSw, JRS, AR, DGS, BGN, DS, SGT, and JD. DFF contributed to the study concept and design. PG, MJB, GTJ, AvR, DRC, MAK, GFM, IBW, CDL, LB, SW, AF, SAL, MM-N, SDT, EZ, AS, HH, DFE, TS, HG, RSH, SN, SSA, NS, HSM, NJW, DJR, MK, TA, BTH, VG, AH, OHS, RT, SMP, MF, HW, ASH, NJS, WM, RCI, RCG, JSK, JCC, SKat, RM, JE, AK, HS, KS, UT, JDW, AT, H, DSA, AM, EDA, RCh, BGN, DS, and JD recruited and characterised participants. ASB, JMMH, RW, WHK, SSP, SFG, LAL, MJB, GTJ, RAS, SBE, PIIWB, and JE worked in the laboratory and managed data. DFF, PW, JMMH, SKap, RW, WHK, EH, SFG, LAL, MJB, GTJ, RAS, SBE, EP, GTh, TK, LZ, MN, RD, WZ, JCM, MK, GD, CNP, AG, JCB, AD, SL, AVS, IQ, AFB, FNGvH, GTh, HK, MDR, SSV, DCC, JN, MdA, IJK, PLP, CAM, EPB, OG, DRC, DSC, LR, JAP, JH, NJT, MAK, JK, KGP, AP, MG, YL, NF, MFK, SKG, CDL, LB, MAB, DME, SBE, MAF, HB, SAL, MM-N, JFF, NLS, MSy, EZ, KP, MAN, AS, CP, JPB, HH, DFE, DFT, IPT, MD, KH, GM, TE, HG, JMA, MH, NW, YW, DCh, RSJ, MMI, DTB, MHL, NKH, 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, DGS, BGN, DS, SGT, and JD drafted the manuscript. All authors contributed to, and approved the final version of the report.
The Interleukin 1 Genetics Consortium

Daniel F Freitag PhD, Adam S Butterworth PhD, Peter Willeit MD, Joanna M M Howson PhD, Stephen Burgess PhD, Stephen Kaptoge PhD, Robin Young PhD, Weang Kee Ho PhD, Angela M Wood PhD, Michael Sweeting PhD, Sarah Spackman MMath, James R Staley MSc, Anna Ramond DP harm, Eric Harshfield MPH (University of Cambridge, Cambridge, UK); Sune F Nielsen PhD, Peet Grande MD (Copenhagen University Hospital, University of Copenhagen, Copenhagen, Denmark); Leslie A Lange PhD (University of North Carolina, Chapel Hill, NC, USA); Matthew J Bown FRCS (University of Leicester and National Institute for Health Research Leicester Cardiovascular Biomedical Research Unit, Leicester, UK); Gregory T Jones PhD (University of Otago, Dunedin, New Zealand); Robert A Scott PhD (Medical Research Council Epidemiology Unit, Cambridge, UK); Steve Bevan PhD (University of Cambridge, Cambridge, UK); Eleonora Poreu PhD (Istituto di Ricerca Genetica e Biomedica, Monza, Italy); Gudmarr Thorleifsson PhD (deCODE Geneticae, Reykjavik, Iceland); Lingyu Zeng MSc, Thorsten Kessler MD (Deutsches Herzzentrum München, Technische Universität München, and German Centre for Cardiovascular Research, partner site Munich Heart Alliance, Munich, Germany); Majid Nikpay PhD (Ruddly Canadian Cardiovascular Genetics Centre, University of Ottawa Heart Institute, Ottawa, Canada); Ron Divito PhD, Institute of Cambridge, and Massachusetts General Hospital, Boston, MA, USA); Weihsia Zhang PhD (Imperial College, London, UK); Jemma C Hopewell PhD (University of Oxford, Oxford, UK); Marcus Kleber PhD, Graciela E Delgado MSc (University of Heidelberg, Heidelberg, Germany); Gary S Ibsen PhD (University of Leicester and National Institute for Health Research Leicester Cardiovascular Biomedical Research Unit, Leicester, UK); Anuoj Goel MSc (University of Oxford, Oxford, UK); Joshua C Bis PhD (University of Washington, Seattle, WA, USA); Abbas Dehghan PhD, Symen Ligthart MD (Erasmus Medical Center, University Medical Center Rotterdam, Rotterdam, Netherlands); Albert V Smith PhD (Icelandic Heart Association, Kópavogur, Iceland, and University of Iceland, Reykjavik, Iceland); Liming Qu MS (University of Pennsylvania, Philadelphia, PA, USA); Femke NG van ‘t Hof MD, Paul I w De Bakker PhD, Annette F Baas PhD (University Medical Center Utrecht, Utrecht, Netherlands); Andre van Rij FRACS (University of Otago, Dunedin, New Zealand); Gerard Tromp PhD, Helena Kuivaniemi PhD (Sigtifared and Janet Weis Center for Research, Geisinger Health System, Danville, PA, USA); Marylyn D Ritchie PhD, Shefali S Verma MS (Pennsylvania State University, University Park, PA); Dana C Crawford PhD (Case Western Reserve University, Cleveland, OH, USA); Jennifer Malinowski PhD (Yale University, New Haven, CT, USA); Mariza de Andrade PhD, Ifiikhari J Kullo MD (Mayo Clinic, Rochester, MN, USA); Peggy L Peissig PhD (Marshfield Clinic Research Foundation, Marshall, WI, USA); Catherine A McCarty PhD (Research Division Essentia Institute of Rural Health, Duluth, MN, USA); Erwin P Röttinger MD, Omri Gottesman MD (Kahn School of Medicine Mount Sinai, New York, NY, USA); David R Crosslin PhD (University of Washington, Seattle, WA, USA); David S Carroll PhD (Group Health Research Institute, Seattle, WA, USA); Laura J Rasmussen-Torvik PhD, Jennifer A Pacheco BA (Northwestern University Feinberg School of Medicine, Chicago, IL, USA); European Prospective Investigation into Cancer and Nutrition Cardiovascular Disease study consortium; Aneuryns Consortium; Electronic Medical Records and Genomics Network; Jie Huang MD (University of Cambridge, Cambridge, UK); Jie Huang MD (Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK); Renske van der Meulen MD (University Medical Center Utrecht, Utrecht, Netherlands); Janne Felix PhD (Erasmus Medical Center, University Medical Center Rotterdam, Rotterdam, Netherlands); Nicholas I Psychiatry PhD (University of Washington, Seattle, WA, USA); Cohorts for Heart and Aging Research in Genomic Epidemiology Heart Failure Working Group; Marc Sudman BA, Susan D Thompson PhD (Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA); Consortium for Juvenile Arthritis Genetics; Eleftheria Zeggini PhD, Kalliope Fanoutsopoulou PhD (Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK); Arthritis Research UK Osteoarthritis Genetics consortium; Mike A Nalls PhD, Andrew Singleton PhD (National Institute on Aging, National Institutes of Health, Bethesda, MD, USA); International Parkinson's Disease Consortium; Constantin Polychronakos MD (McGill University Health Centre, Montreal, QC, Canada); Jonathan P Bradfield PhD, Hakan Hakonarson MD (Children's Hospital Philadelphia, Philadelphia, PA, USA); Douglas F Easton PhD, Deborah Thompson PhD (University of Cambridge, Cambridge, UK); Breast Cancer Association Consortium; Ian P Tomlinson MD (Wellcome Trust Centre for Human Genetics, Oxford, UK); Malcolm Dunlop FMedSci (University of Edinburgh, and Medical Research Council Human Genetics Unit, Edinburgh, UK); Kari Hemminki MD (German Cancer Research Centre, Heidelberg, Germany, and Lund University, Malmö, Sweden); Gareth Morgan FRCP (University of Arkansas for Medical Sciences, Little Rock, AR, USA); Timothy Eisen FRCP (Cambridge University Health Partners, Cambridge, UK); Hartmut Goldschmidt MD (Heidelberg University, and National Center for Tumor Diseases, Heidelberg, Germany); James M Allan PhD (Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, UK); Marc Henrion PhD, Nicola Whiffin MA, Yufei Wang PhD, Daniel Chubb PhD (Institute of Cancer Research, Sutton, UK); Richard S Houlston FMedSci (Institute of Cancer Research, London, UK); Mark M Iles PhD, D Timothy Bishop PhD (Leeds Cancer Research UK Centre, University of Leeds, Leeds, UK); Matthew H Law PhD, Nicholas K Hayward PhD (Queensland Institute of Medical Research-Berghofer Medical Research Institute, Brisbane, QLD, Australia); Melanoma Genetics consortium; Yang Luo PhD (Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK); Sergey Nejentsev PhD (University of Cambridge, Cambridge, UK); Maja Babalic PhD (University of Knock Health Science Centre, Houston, TX, USA); Cohorts for Heart and Aging Research in Genomic Epidemiology inflammation working group; David Crossman FRCP (University of St Andrews, St Andrews, UK); Serena Sanna PhD (Istituto di Ricerca Genetica e Biomedica, Monza, Italy); Nicole Soranzo PhD (Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK); Hugh S Markus FRCP (University of Cambridge, Cambridge, UK); Charis Choy PhD, Sue S Layer PhD, David J Reilly MD, Andrew Haynes MD (Queensland Institute of Medical Research-Berghofer Medical Research Institute, Brisbane, QLD, Australia); Melbourne Asthma Genetics Consortium; Carmen Figueiredo PhD (University of Edinburgh, Edinburgh, UK); Xinjing Li PhD (University of Edinburgh, Edinburgh, UK); Samuel Sankaran PhD (University of Cambridge, Cambridge, UK).
Articles

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

Daniel J Rader MD, Muzerak 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 Peasy 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 Mörz MD (Synlabil Academy, Synlab Services GmbH, Mannheim, Germany); Robert Clarke FRCP, Rory Collins FMedSci (University of Oxford, Oxford, UK); Jaspal S Kooser 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 FRCP(C) (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 Herzzentrum München, Technische Universität München, and German Centre for Cardiovascular Research, partner site Munich Heart Alliance, Munich, Germany); Kari Stefansson MD, Gunnar 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); Abdullah Al Shaib Majumder FRCP (National Institute of Cardiovascular Diseases, Dhaka, Bangladesh); Emanuele Di Angelantonio MD, Rajiv Chowdhury MD (University of Cambridge, Cambridge, UK); Børge G Nordestgaard MD (Copenhagen University Hospital, University of Copenhagen, Copenhagen, Denmark); Danish Saleehan 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, IRS, 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 (268834), and European Commission Framework Programme 7 (HEALTH-F2-2012-279213). Funding for the component studies in this analysis is provided in the appendix. Murray Clarke, Philip De Jager, 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: 2331–39.


