GENETIC DETERMINANTS OF CIRCULATING INTERLEUKIN-1 RECEPTOR ANTAGONIST LEVELS AND THEIR ASSOCIATION WITH GLYCEMIC TRAITS

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Tulehdusta välittäviin sytokiineihin kuuluvan interleukiini 1β (IL-1β):n kohonneen systeemisen pitoisuuden on arveltu edesauttavan insuliiniresistenssin kehittymistä ja johtavan haiman β-solujen toimintahäiriöihin. IL-1β:n sisäsyntyisellä vastavaikuttajalla, interleukiini 1 reseptoriantagonistilla (IL-1RA), on puolestaan esitetty olevan suojaava rooli mainittujen fenotyyppien kehittymisessä päinvastaisten vaikutustensa ansiosta.

IL-1RA:n suojaavan roolin havainnollistamiseksi työssä Genetic determinants of circulating interleukin-1 receptor antagonist levels and their association with glycemic traits tunnistettiin veren IL-1RA- pitoisuuteen assosioituvia geneettisiä variantteja, minkä jälkeen selvitettiin näiden yhteyttä glukoosi- ja insuliinimetaboliaan liittyvien muuttujien-, sekä immunologisten muuttujien pitoisuuksiin.

Yhteensä 11 tutkimuskohorttia käsittäneessä genominlaajuisessa assosiaatioanalyysissä ja meta-analyysissä tunnistettiin kaksi toisistaan riippumatonta yhden nukleotidin polymorfismia (SNP), jotka assosioituivat itsenäisesti veren IL-1RA- pitoisuuteen: rs4251961 lokuksessa *IL1RN* (n = 13 955, P = 2,76e^{-21}) ja rs6759676 lokuksen *IL1F10* läheisyydessä (n = 13 994, P = 1,73e^{-17}). Kyseisten varianttien yhteinen selitysosuus IL-1RA:n varianssista oli 2,0 %. Molemmat variantit assosioituivat mataliin C-reaktiivisen proteiinin (CRP) systeemisiin pitoisuuksiin. Tämän lisäksi rs6759676 assosioitui mataliin paastoinsuliinin pitoisuuksiin, sekä matalaan insuliiniresistenssiin (HOMA-IR).

Tutkimuksemme osoittaa, että geneettisesti säädelty IL-1RA:n kohonnut pitoisuus saattaa suojata insuliiniresistenssin kehittymiseltä. Tulokset tukevat myös näkemystä elimistön tulehdusreaktion ja insuliiniresistenssin kehittymisen kausaliteetista, joskin lisätutkimukset ovat tarpeen ilmiön paremmaksi ymmärtämiseksi.

SISÄLLYS

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Genetic determinants of circulating interleukin-1 receptor antagonist levels and their association with glycemic traits

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Abstract

The pro-inflammatory cytokine interleukin (IL)-1β is implicated in the development of insulin resistance and beta-cell dysfunction, whereas higher circulating IL-1 receptor antagonist (IL-1RA – an endogenous inhibitor of IL-1β - has been suggested to improve glycemia and beta-cell function in patients with type 2 diabetes. In order to elucidate the protective role of IL-1RA, this study aimed to identify genetic determinants of circulating IL-1RA concentration and to investigate their associations with immunological and metabolic variables related to cardiometabolic risk. In the analysis of 7 discovery and 4 replication cohort studies, two single nucleotide polymorphisms (SNPs) were independently associated with circulating IL-1RA concentration (rs4251961 at the *IL-1RN* locus, n=13,955, $P=2.76x10^{-21}$; and rs6759676, closest gene locus *IL1F10*, n=13,994, $P=1.73x10^{-17}$). The proportion of the variance in IL-1RA explained by both SNPs combined was 2.0%. IL-1RA-raising alleles of both SNPs were associated with lower circulating C-reactive protein concentration. The IL-1RA-raising allele of rs6759676 was also associated with lower fasting insulin and lower homeostasis model assessment insulin resistance (HOMA-IR). In conclusion, we show that circulating IL-1RA levels are predicted by two independent SNPs at the *IL-1RN* and *IL-1F10* loci and that genetically raised IL-1RA may be protective against the development of insulin resistance.

The balance between the potent pro-inflammatory cytokine interleukin (IL)-1β and its endogenous inhibitor IL-1 receptor antagonist (IL-1RA) is crucial for the regulation of the immune system in health and disease (1-3). Inborn genetic deletion of a region spanning the *IL1RN* gene that encodes IL-1RA leads to severe auto-inflammatory disease (4,5), and recombinant IL-1RA has been used for years to treat inflammatory conditions such as rheumatoid arthritis (3). More recently, a small, randomized clinical trial demonstrated that treatment with recombinant IL-1RA improved glycemic control and beta-cell function in patients with type 2 diabetes (T2D) (6). Although T2D is not characterized by the classical symptoms of inflammation present in diseases such as rheumatoid arthritis, IL-1β has been identified as a proinflammatory cytokine that may underlie the link between metabolic overload leading to glucotoxicity, lipotoxicity, oxidative stress, organelle stress and amyloid deposition on the one hand, and insulin resistance and beta-cell-dysfunction on the other hand (7,8).

Observational studies show that IL-1RA concentrations are increased more than a decade before diagnosis of T2D, and this is accentuated during the six years preceding the clinical onset of disease when compared with non-diabetic controls (9-12). Therefore, IL-1RA shows similarities to cytokines from the transforming growth factor (TGF) superfamily, TGF-β1 and macrophage inhibitory cytokine (MIC)-1, which are also present at elevated levels in individuals who will develop T2D (13-15). In contrast, IL-1RA differs from the anti-inflammatory adipokine, adiponectin, of which expression and release from adipocytes is downregulated prior to the onset of T2D (16-18).

IL-1RA expression and secretion are regulated by pro-inflammatory cytokines with IL-1β being the most prominent trigger. In addition, IL-1β expression is also induced by metabolic stimuli such as glucose and free fatty acids (19-22). Genetic determinants of IL-1RA levels in the *IL1RN* and *IL1F10* loci and the *IL1B* locus encoding IL-1β have been described (23-30).

There is evidence that these variants not only regulate systemic levels of IL-1RA, but also associate with fat mass and concentrations of glucose, insulin and several immune mediators in the circulation (23,24,26-28). So far, it is unknown whether IL-1RA levels are determined by additional gene variants outside the loci above.

Therefore, the aims of the present study were to (i) identify novel genetic determinants of circulating IL-1RA in large population-based cohorts, and (ii) to assess common underlying biological pathways by investigating their associations with gene expression levels and metabolic and immunological variables that contribute to cardiometabolic risk.

RESEARCH DESIGN AND METHODS

Cohorts. We assembled 7 studies for the discovery analysis, totalling up to 9,285 individuals of European ancestry. Replication included 4 cohorts comprising up to 7,938 individuals Local research ethics committees approved all studies and all participants gave informed consent to each original study. Discovery and follow-up cohort characteristics as well as information on genotyping and phenotyping are given by cohort.

Whitehall II Study

(i) Study population: The Whitehall II (WHII) study recruited 10,308 men and women between 1985 and 1989 from 20 London-based civil service departments. Clinical measurements were taken at 5-year intervals. Currently, clinical data is available from 4 phases (phase 1: 1985-1988, phase 3: 1991-1993, phase 5: 1997-1999 and phase 7: 2003-2004). Blood samples for DNA were collected in 2002–2004 from more than 6000 participants. IL-1RA measurements were available from a case-cohort subset in phase 3. Briefly, a random sample was drawn from the source population of 8816 individuals who had attended the phase 3 examination. We excluded participants with prevalent T2D at phase 3 (n=42), missing follow-up data on diabetes (n=552), missing data for key variables (CRP [limited to subjects with CRP<10 mg/l], weight, waist circumference, cholesterol, triglycerides, fasting glucose, fasting insulin) at baseline (n=2018) or during follow-up (phases 5 and 7; n=3049), leading to a case-cohort of 2810 subjects.

(ii) IL-1RA measurements: IL-1RA serum concentrations were measured using the Quantikine ELISA kit (R&D Systems, Wiesbaden, Germany). Mean intra- and inter-assay coefficients of variation (CVs) were 2.6% and 7.9%, respectively. The limit of detection was 14 pg/ml. All samples gave values above the limit of detection.

(iii) Genotyping and quality control: In 2010, 3413 samples from the WHII study were genotyped using the Illumina Metabochip. A subset of these had also previously been genotyped using the Illumina HumanCVD array. The combined data used in this analysis

consisted of SNP data for 3178 Caucasian individuals genotyped on both platforms. After filtering the data for outliers (as identified by multi-dimensional scaling), cryptic relatedness, ambiguous sex, and sample and SNP call rates (<95%), genetic data for 236,426 SNPs in 3102 individuals was available for analysis, of whom 2160 had IL-1RA measurements.

(iv) Measurement of metabolic and immunological traits: Blood samples were collected from participants before and at the end of the 2-h oral glucose tolerance test. Blood glucose was measured with the glucose oxidase method (YSI Corporation, Yellow Springs, OH). Serum insulin was measured with an in-house human insulin radioimmunoassay/ELISA (DakoCytomation Ltd, Ely, UK). C-reactive protein (CRP) was measured with a high-sensitivity immunonephelometric assay in a BN ProSpec nephelometer (Dade Behring, Milton Keynes, UK). HbA1c was measured at phase 7 on a calibrated HPLC system (4). Analysed samples for fasting glucose, 2-h glucose, fasting insulin, 2-h insulin, HOMA-IR, HbA1c and CRP were *n*=3038, *n*=3036, *n*=2866, *n*=3022, *n*=2199, *n*=3040 and *n*=2963, respectively, for rs4251961 and *n*=2992, *n*=2990, *n*=2821, *n*=2976, *n*=2164, *n*=2994 and *n*=2917, respectively for rs6759676.

National FINRISK Study (FINRISK)

(i) Study population: FINRISK surveys are cross-sectional, population-based studies conducted every five years since 1972 to monitor the risk of chronic diseases. For each survey, a representative random sample was selected from 25–74 year-old inhabitants of five geographical regions in Finland. The survey included a questionnaire and a clinical examination, at which a blood sample was drawn, with linkage to national registers of cardiovascular and other health outcomes. Study participants were followed up through 31 December 2010. The current study included eligible individuals from FINRISK surveys conducted in 1997 (FINRISK 1997) and in 2007 (Dietary, Lifestyle, and Genetic determinants of Obesity and Metabolic syndrome [DILGOM] study collected as an extension to the

FINRISK 2007 survey) forming a total sample size of 5004 individuals from whom IL-1RA measurements and genotype data were available.

(ii) IL-1RA measurements: In FINRISK 1997 IL-1RA levels were determined at the laboratory of University of Mainz, Germany, from serum samples by ELISA (R&D Systems). The intra- and inter-assay CVs were 3.59% and 5.68%, respectively. In FINRISK 2007/DILGOM, IL-1RA was determined from serum at the laboratory of the Population Studies Unit of the National Institute for Health and Welfare, Turku, Finland, using the Quantikine ELISA from R&D Systems. The detection limit was 31 pg/ml. Intra- and inter-assay CVs were 2.2% and 10.3%, respectively.

(iii) Genotyping and quality control: FINRISK individuals from the year 1997 and a specific subset of individuals examined more carefully for metabolic traits in the year 2007 (FINRISK1997 and DILGOM GWAS, respectively, total *n* of 1146 available for this study) were genotyped in the Sanger Institute (Cambridge, UK) with the Illumina Human 610K BeadChip. This set of samples was imputed to the reference panel B36rel22 using the software Mach v.1.0.16. In the imputation, filters of <95% for call rate, <1% for MAF and $<10^{-6}$ for HWE *P* value were used. A subset (*n*=3858 available for this study) of FINRISK 2007 individuals (DILGOM metabo) was genotyped using the Illumina Cardiometabochip. To control the data quality, sex mismatch and relatedness checks were performed and any observed discrepancies were removed from both data sets. For this analysis, the phenotype data was filtered for outliers. In the analysis, thresholds of <95% call rate, $<10^{-6}$ for HWE *P* value, <1% for MAF, max. 10% for missingness per SNP and max. 10% for missingness per individual were applied for the genotyped data.

(iv) Measurement of metabolic and immunologic traits: Glucose levels in FINRISK 1997 were measured from serum samples (fasting duration at least four hours) using the glucose hexokinase method with the detection range of 0.6-44 mmol/l. The devise used was Olympus AU400. Insulin levels were determined from serum with chemiluminescent microparticle

immunoassay CMIA (Abbott, Architect i2000) with an intra-assay CV of 3.05% of and an inter-assay CV of 3.31%. CRP was determined from serum by latex immunoassay CRP16 (Abbott, Architect c8000, Abbott Laboratories, Chicago, IL). The intra-assay and inter-assay CVs were 0.83% and 0.93%, respectively.

In the FINRISK 2007/DILGOM survey, glucose was measured from fasting plasma samples using the glucose hexokinase method with the reference range of 2.00 (min) to 20.00 (max) and a detection limit of 0.14 mmol/l. The devise used was Architect ci8200 (Abbott Laboratories, Abbott Park, IL). Insulin was determined from fasting serum with the chemiluminescent microparticle immunoassay CMIA (Abbott Laboratories) with mean inter-assay CVs of 3.4% using the Architect ci8200 (Abbott Laboratories). Serum CRP was determined with an immunoturbidimetric method (MULTIGENT CRP Vario, Abbott Laboratories) using the Architect ci8200. Mean inter-assay CVs were 3.7%.

In FINRISK 1997, analyzed samples for fasting glucose, fasting insulin, HOMA-IR and CRP were *n*=382, *n*=484, *n*=367 and *n*=504, respectively, for rs4251961 and *n*=382, *n*=484, *n*=367 and *n*=503, respectively, for rs6759676.

In the FINRISK 2007/DILGOM survey analyzed samples for fasting glucose, fasting insulin, HOMA-IR and CRP were *n*=4396, *n*=4412, *n*=4385 and *n*=4451, respectively, for rs4251961 and *n*=4395, *n*=4411, *n*=4384 and *n*=4450, respectively, for rs6759676.

(v) Gene expression analysis: From the individuals of the DILGOM GWAS sample, the whole blood RNA was obtained and hybridized onto Illumina HumanHT-12 BeadChips (Illumina, San Diego, CA). In this study, expression data from 507 individuals was analyzed.

HEALTH 2000

(i) Study population: Health 2000 is a population-based national survey on the health and functional capacity of Finnish individuals (http://www.terveys2000.fi/julkaisut/baseline.pdf). A nationally representative sample of 10,000 individuals was drawn of the population aged 18 years and older. The survey included an interview about medical history, health-related

lifestyle habits, and a clinical examination (for individuals of 30 years of age and older) at which a blood sample was drawn. Study participants were followed up through 31 December 2010 and restricted to be aged ≤80 years at baseline. In this study we used the GenMets sample which includes individuals with metabolic syndrome and matched controls drawn from the Health 2000 study. A total sample size of 2010 individuals from whom IL-1RA measurements and genotype data were available was used.

(ii) IL-1RA measurements: IL-1RA was determined from nonfasting serum using ELISA (R&D Systems, Minneapolis, MN). The intra- and inter-assay coefficients of variation (CVs) were 3.59% and 5.68%, respectively.

(iii) Genotyping and quality control: 2173 individuals from the GenMets cohort have been genotyped with the Illumina Human 610 000 BeadChip in Sanger Institute (Cambridge, UK). To control the data quality, sex mismatch and relatedness checks were performed and any observed discrepancies removed. GenMets has been imputed to the reference panel B36rel22 using the software Mach v.1.0.16. In the imputation, filters of <95% for call rate, <1% for MAF and $<10^{-6}$ for HWE *P* value were used. For this analysis, the phenotype data was filtered for outliers. Thresholds of 95% call rate and 10^{-6} for the Hardy-Weinberg equilibrium *P* value for an individual SNP were used.

(iv) Measurement of metabolic and immunological traits: Glucose levels were measured from serum samples (fasting duration at least four hours) using the glucose hexokinase method with inter-assay CVs of 2.1% and 2.3% for mean concentrations of 9.3 and 5.2 mmol/l, respectively. The devise used was Olympus AU400 (Tokyo, Japan). Insulin levels were determined from nonfasting serum with an automated microparticle enzyme immunosorbent assay (MEIA) on an Abbot IMX analyzer (Abbott Laboratories) with inter-assay CVs of 4.6% and 4.0% for mean concentrations of 118.7 and 1032.7 pmol/l, respectively. HbA1c was determined with an immunoturbidimetric method using the Architect ci8200 (Abbott Laboratories). The CVs were 1.8% for HbA1c of 5.1% and 2.0% for HbA1c of 10.8%. CRP

was determined from serum using latex immunoassay CRP16 (Abbott Laboratories). The intra- and inter-assay CVs were 0.83% and 0.93%, respectively. Analyzed samples for fasting glucose, fasting insulin, HOMA-IR, HbA1c and CRP were *n*=2127, *n*=2071, *n*=2070, *n*=2102 and *n*=1878, respectively, for rs4251961 and *n*=2126, *n*=2070, *n*=2069, *n*=2101 and *n*=1877, respectively for rs6759676.

MIGen Study

(i) Study population: 341 individuals from the National FINRISK Study were sampled into a specific pair-matched case-control sample of myocardial events (MIGen). In this cohort, the individuals whose main diagnosis or cause of death can be specified with 410, I21, I22 codes were defined as cases. These analyses included only individuals from the FINRISK 1997 study. A total sample size of 111 individuals for whom IL-1RA measurements and genotype data were available was used.

(ii) IL-1RA measurements: IL-1RA was measured as described above for FINRISK 1997.

(iii) Genotyping and quality control: Individuals from the MIGen sample were genotyped with the Affymetrix 6.0 platform in the Broad Institute (Cambridge, MA). To control the data quality, sex mismatch and relatedness checks were performed and any observed discrepancies were removed. MIGen data was imputed to the reference panel HapMap2 using the software Mach. In the imputation, filters of <95% for call rate, <1% for MAF and $<10^{-6}$ for HWE *P* value were used. For this analysis, the phenotype data was filtered for outliers.

(iv) Measurement of metabolic and immunological traits: All traits were measured as described above for FINRISK 1997. Analyzed samples for fasting glucose, fasting insulin, HOMA-IR and CRP were *n*=86, *n*=109, *n*=85 and *n*=110, respectively, for both SNPs (rs4251961 and rs6759676).

KORA F4 Study

(i) Study population: The KORA F4 study (2006-2008) is a follow-up survey of the population-based KORA S4 study (1999-2001). A total sample of 6640 men and women aged

25 to 74 years was drawn from the target population in the city of Augsburg (Germany) and two adjacent counties. Of all 4261 participants from the KORA S4 study, 3080 also participated in the follow-up survey KORA F4. Genotype data were available for a subset of 1814 individuals in the age range of 32-81 years at the time of the follow-up.

(ii) IL-1RA measurements: Serum IL-1RA was determined using the Quantikine ELISA kit from R&D Systems (Wiesbaden, Germany) with intra- and inter-assay CVs of 2.8% and 7.0%, respectively. Data from 718 individuals aged 61-82 years were available for this analysis.

(iii) Genotyping and quality control: All samples were genotyped with the Affymetrix Human SNP Array 6.0. Hybridization of genomic DNA was done in accordance with the manufacturer's standard recommendations. Genotypes were determined using Birdseed2 clustering algorithm (Affymetrix Array 6.0). For quality control purposes, we applied a positive control and a negative control DNA every 96 samples. On chip level only subjects with overall genotyping efficiencies of at least 93% were included. In addition the called sex had to agree with the sex in the KORA study database. Imputation of genotypes was performed with the software IMPUTE v0.4.2 based on HapMap II.

(iv) Measurement of metabolic and immunological traits: Blood was collected without stasis and blood was kept at 4°C until centrifugation. All blood parameters, except for 2-hour glucose and 2-hour insulin, were based on fasting blood samples. Serum samples were stored at -80°C until assayed. Serum glucose levels were assessed using the hexokinase method (GLU Flex, Dade Behring). Serum insulin was determined by ELISA (Invitrogen, Karlsruhe, Germany). HbA1c was measured using the HPLC method. Plasma concentrations of CRP were assessed by an immunonephelometric assay on a BN II analyzer (Dade Behring, Marburg, Germany). Sample sizes for the analysis of fasting glucose, 2-hr glucose, fasting insulin, 2-hr insulin, HOMA-IR, HbA1c and CRP were n=1779, n=1598, n=1776, n=713, n=1777, n=723 and n=1777, respectively.

(v) Gene expression analysis: Total RNA was extracted from fasting whole-blood samples that were taken between 8 a.m. and 11 a.m. RNA was reverse transcribed and biotin-UTP-labeled into cRNA using the Illumina TotalPrep-96 RNA Amp Kit (Ambion, Darmstadt, Germany). Gene expression levels were determined using the Illumina Human HT-12 v3 Expression BeadChip (Illumina, San Diego, CA). Expression data was $\log_2$-transformed and normalized by quantile normalization. Data from 718 individuals aged 61-82 years were available for this analysis.

Gutenberg Health Study

(i) Study population: The Gutenberg Health Study (GHS) is designed as a community-based, prospective, observational, single-center cohort study in the Rhine-Main area of Western Germany. The sample was drawn randomly from the governmental local registry offices in the city of Mainz and the district of Mainz-Bingen. The sample was stratified 1:1 for sex and residence (urban and rural) and in equal strata for decades of age. Individuals between 35 and 74 years of age were enrolled. Exclusion criteria were insufficient knowledge of the German language and physical or psychological inability to participate in the examinations at the study center. Baseline examination of 15,000 study participants was performed between 2007 and 2012. Genotype data and IL-1RA levels were available for a subgroup of 4158 individuals.

(ii) IL-1RA measurements: IL-1RA was determined by ELISA (R&D Systems, Wiesbaden, Germany). The inter- and intra-assay CVs were 3.59% and 5.68%, respectively. Data were available for this analysis from 2984 and 1174 individuals from GHS I and GHS II, respectively.

(iii) Genotyping and quality control: Genomic DNA was extracted from buffy coats prepared from EDTA blood samples. Genotyping was performed using the Affymetrix Genome-Wide Human SNP Array 6.0, as described by the Affymetrix user manual. Genotypes were called using the Affymetrix Birdseed-V2 calling algorithm and quality control was performed using GenABEL (http://mga.bionet.nsc.ru/nlru/GenABEL/). Because genotyping was performed in

two successive waves (cohort GHS I (*n*=3500) and cohort GHS II (*n*=1500)), the two cohorts were analyzed separately. Individuals with a call rate below 97% or an autosomal heterozygosity higher than 3 SD around the mean were excluded. After applying standard quality criteria (minor allele frequency 1%, genotype call rate 98% and *P* value of deviation from Hardy-Weinberg equilibrium), 662,405 SNPs in 2996 subjects (GHS I) and 673,914 SNPs in 1179 subjects (GHS II), respectively, remained for analysis. Imputations based on HapMap 2 release 24 were performed separately in GHS I and GHS II using IMPUTE v2.1.0. In total, 2,588,505 (GHS I)/2,586,553 (GHS II) SNPs with a MAF≥1% were available for genetic analyses.

(iv) Measurement of metabolic and immunological traits: Blood sampling was carried out under fasting conditions in lying position. Glucose, insulin, HbA1c and CRP were measured immediately after blood withdrawal by routine laboratory measurements. In GHS I and GHS II, *n*=813 and *n*=1308 individuals, respectively, had a CRP level of <1.0 mg/l which was the limit of detection (LOD). These values were set to 0.5 mg/l (LOD/2). Sample sizes for the analysis of fasting glucose, HbA1c and CRP were *n*=2183, *n*=2969 and *n*=2983, respectively, in GHS I and *n*=880, *n*=1179 and *n*=1179, respectively, in GHS II.

(v) Gene expression analysis: Gene expression analysis was performed using the Illumina HumanHT-12 v3 BeadChip using total monocytic RNA. The integrity of the total RNA was assessed through analysis on an Agilent Bioanalyzer 2100 (Agilent Technologies, Böblingen, Germany). Reverse transcription and cRNA synthesis were performed using the Illumina TotalPrep-96 RNA Amplification Kit (Ambion, Darmstadt, Germany). Data from 1133 individuals were available for this analysis.

Young Finns Study (YFS)

(i) Study population: The Cardiovascular Risk in Young Finns is a population-based 27-year follow-up study (http://med.utu.fi/cardio/youngfinnsstudy/). The first cross-sectional survey was conducted in 1980, when 3596 Caucasian subjects aged 3-18 years participated in the

study. In adulthood, the latest 27-year follow-up study was conducted in 2007 to 2204 participants aged 30-45 years. For 1998 individuals who had participated in the study in 2007 genotype data and IL-1RA measurements were available for this analysis.

(ii) IL-1RA measurements: A magnetic bead-based multiplex assay was used to determine the concentration of IL-1RA in blood. 20 µl aliquots of serum samples (stored at -70 ºC and never thawed before) from 2200 persons were analyzed using Bio-Plex Pro Assays (27-Plex kit including IL-1RA). Intra- and inter-assay CVs were 9.62% and 10.86%, respectively. Lower limit of detection was 10.85 pg/ml.

(iii) Genotyping and quality control: Genotyping of YFS samples was performed at the Sanger Institute (Cambridge, UK) using the custom-built Illumina BeadChip Human670K array. Genotypes were called using Illumina's clustering algorithm. Following quality control filters were applied to the data: MAF 0.01, maximum per-SNP missing 0.05, maximum per-person missing 0.05, and HWE *P* value of 10^{-6}. In addition, sex mismatch and relatedness checks were performed and any observed discrepancies removed. YFS has been imputed to the HapMap2 reference panel using the software Mach v.1.0 (http://www.sph.umich.edu/csg/abecasis/MACH/). For this analysis, the phenotype data was filtered for outliers. Thresholds of 95% call rate and 10^{-6} for Hardy-Weinberg equilibrium *P* value for an individual SNP were used.

(iv) Measurement of metabolic and immunological traits: Fasting serum glucose concentration was determined by the enzymatic hexokinase method (Glucose reagent, Olympus AU400, Olympus, Center Valley, PA). Fasting serum insulin concentration was determined by a microparticle enzyme immunoassay (IMx insulin reagent, Abbott Diagnostics) on an IMx instrument (Abbott). Serum CRP was analyzed using an automated analyzer (Olympus AU400) and a highly sensitive turbidimetric immunoassay kit (CRP-UL-assay, Wako Chemicals, Neuss, Germany). The detection limit was 0.02 mg/l. Inter-assay CVs were 3.33% at the mean level of 1.52 mg/l (*n*=116) and 2.65% at the mean level of 2.51

mg/l (*n*=168). Analyzed samples for fasting glucose, fasting insulin, HOMA-IR and CRP were *n*=1951, *n*=1946, *n*=1938 and *n*=1952, respectively, for rs4251961 and *n*=1950, *n*=1945, *n*=1937 and *n*=1951, respectively for rs6759676.

Statistical analysis. For the discovery cohorts, separate within-cohort linear regression analyses were performed to assess associations between SNPs and systemic IL-1RA levels using an additive genetic model adjusting for age, sex, BMI, waist-hip ratio, smoking (current vs. never/ex smokers) as well as ancestry principal components and field center, as needed. After verifying strand alignment, an inverse variance-weighted fixed effects meta-analysis of the results from the seven studies was conducted. I^2 estimates were used to assess study heterogeneity. Since five of our seven datasets used genome-wide platforms we adopted discovery a *P*-value threshold of $p<5.0x10^{-8}$, in keeping with that generally used in genome-wide association studies. Although five of the studies also used imputed data, the genome-wide significance level of $p<5.0x10^{-8}$ for the number of independent tests was still applicable since imputed SNPs were in linkage disequilibrium (LD) with genotyped SNPs.

Conditional analysis. To explore whether the signals at each locus were independently associated with the phenotype of interest (IL-1RA), we carried out a conditional analysis, where the most significantly associated SNP from the meta-analysis was added to the within-study linear regression model as a covariate, followed by meta-analysis of the resulting conditional estimates. If any SNPs remained significant at the discovery *P* value threshold, the top SNP was again added to the model as covariate. This process was repeated until no more SNPs passed the discovery *P* value threshold.

Replication analysis. The SNPs identified as independent signals in the conditional analysis were then taken forward for replication. In the replication cohorts the same methodological approach was used to obtain an effect estimate for these SNPs as in the discovery cohorts. As study heterogeneity was observed, the summary estimates obtained from all replication

studies were meta-analyzed using a random-effect model. We excluded one study (Young Finns Study (YFS)) from the main analysis for IL-1RA, because a different laboratory method was used to determine IL-1RA levels (bead-based multiplex assay instead of the ELISA method used in all other studies).

The proportion of the variance in IL-1RA explained by rs4251961 and rs6759676 was calculated in the independent population cohort FINRISK1997 (no sample overlap with the discovery cohorts). More recent genotype data in the FINRISK1997 cohort (core-exome chip from Illumina) was imputed using the 1000 Genomes March 2012 release using the IMPUTE software. Imputation information for rs4251961 was 0.97 and for rs6759676 was 0.997. The proportion of variance explained by the two SNPs together was tested with residuals from age, sex, BMI, waist-hip ratio and smoking adjusted ln-transformed IL-1RA levels using linear model: residuals ~ rs4251961 + rs6759676.

Association with metabolic traits. To determine if the independent SNPs associated with IL-1RA levels were also associated with metabolic and immunological traits we examined the association of these SNPs with fasting and 2-hr glucose and insulin, HbA1c, HOMA-IR and CRP levels in all discovery and replication studies where each phenotype was available. Within-study linear regression analysis was carried out for each SNP, adjusting for age and sex in a first model and additionally for BMI, waist-hip ratio, smoking (current vs. never/ex-smokers) as well as ancestry principal components and field centre as needed. Summary estimates obtained from all studies were meta-analyzed using a fixed-effect model, as before, with I^2 estimates used to assess study heterogeneity.

In silico functional analysis. To investigate whether any SNPs could potentially have a functional effect we checked whether each associating SNP were located in any of the ENCODE regulatory regions (31).

Gene expression analysis in blood. Furthermore, the association of the two replicated SNPs with gene expression levels was analyzed in three cohorts (DILGOM GWAS, KORA and

Gutenberg Health Study) for which transcriptomics data were available. In these cohorts, the within-cohort linear regression analyses were performed for each SNP with adjustment for age, sex, BMI and waist-hip ratio and smoking when the data were available. Technical variables were also used for adjustment in KORA and Gutenberg Health Study, as described previously (32).

Analysis of publicly available eQTL data. We used the Genevar software, which allows an integrative analysis and visualization of SNP-gene associations in expression quantitative trait loci (eQTL) studies. We queried eQTL data from adipose tissue collected from 856 healthy female twins (one-third monozygotic and two-thirds dizygotic from the TwinsUK adult registry) of the Multiple Tissue Human Expression Resource (MuTHER) (33). In this study, expression profiling of the samples was performed using Illumina Human HT-12 V3 BeadChips (Illumina), while genotyping was performed with a combination of Illumina HumanHap300, HumanHap610Q, 1M-Duo and 1.2MDuo 1M chips (33). We queried the dataset for any eQTL associations with rs4251961 and rs6759676.

RESULTS

Association between SNPs and circulating IL-1RA

Table 1 provides the characteristics of all discovery and replication cohorts. In the discovery analysis, 54 SNPs passed the discovery *P* value threshold of 5.0×10^{-8} (adjusted for age, sex, BMI, waist-hip ratio and smoking; Figure 1A, Supplementary Table 1). All these SNPs reside within the same region on chromosome 2 spanning the *IL1F10* and *IL1RN* genes.

Two SNPs remained significant at the discovery threshold after a first conditional analysis on the most significant SNP, rs4251961. The most significant SNP in the conditional analysis was rs6759676 with $P = 1.5 \times 10^{-10}$. When the meta-analysis in the discovery cohorts was repeated with both SNPs rs4251961 and rs6759676 as covariates, no additional SNPs remained significant at the discovery *P*-value threshold. Therefore, these 2 SNPs were considered to be the only robust, independent signals in the chromosome 2 region and were taken forward for replication (Figures 1B and 1C). The proportion of the variance in IL-1RA explained by rs4251961 and rs6759676 together was 2.0%.

Both SNPs were also clearly associated with IL-1RA levels in the replication cohorts, with the combined association $P<0.05$ (Table 2). Results of the meta-analyses including the YFS data are given in Supplementary Table 2.

In silico analysis of rs6759676 and rs4251961

The rs6759676 SNP is in strong LD with rs6761276 ($R^2=0.9$; based on 1000 Genome CEU population). The latter is a non-synonymous coding SNP within the *IL1F10* gene, which has been previously reported to be associated with IL-1RA (25). However, PolyPhen-2 (34) predicts this SNP to be 'benign' with no effect on protein structure/function. Based on ENCODE data rs6759676 falls within a region enriched for the H3K27Ac histone acetylation mark (often found near active regulatory elements) in epidermal keratinocytes and human mammary epithelial cells; within a DNase hypersensitive region (characteristic of open

chromatin regions) in multiple cell lines; and also within STAT transcription factor binding sites (Supplementary Figure 1). This suggests that the rs6759676 SNP could influence expression of nearby genes.

The rs4251961 SNP upstream (5') of the *IL1RN* gene also falls within a region enriched for the H3K27Ac histone acetylation mark in epidermal keratinocytes cells and human mammary epithelial cells (Supplementary Figure 2). It is in close proximity to regions enriched for transcription factor binding sites and indicative of open chromatin, suggesting that variants in this region could affect gene expression of *IL1RN* (Supplementary Figure 2).

Association between significant SNPs, immunological and glycemic traits

The minor allele of rs4251961 was inversely associated with circulating IL-1RA concentrations, whereas a positive association was observed for rs6759676 (Table 3, Figures 2A, 2B). The IL-1RA-decreasing alleles were also significantly associated with higher CRP levels for both SNPs (Figures 2C, 2D). These analyses were adjusted for age, sex, BMI, waist-hip ratio and smoking.

While no associations were found between rs4251961 and fasting glucose, 2-hr glucose, fasting insulin, 2-hr insulin and HOMA-IR, the IL-1RA-increasing allele of rs6759676 was associated with lower fasting insulin (P=0.010) and lower HOMA-IR (P=0.006) (Figures 2E-2H). These associations were nominally significant, but became non-significant after Bonferroni adjustment for multiple testing.

Association between significant SNPs and expression of IL-1 family genes in/near the IL-1RN locus

Associations between rs4251961, rs6759676 and transcript levels were first assessed for *IL1RN*/IL-1RA. We found no associations for either SNP with IL-1RA mRNA levels in peripheral blood in the KORA F4 Study (N=718, Table 4). In line with this finding, no associations were found in the Gutenberg Health Study (GHS I, N=1133) for either SNP (P=0.58 and P=0.89 for transcript ILMN_1774874, respectively) in isolated monocytes.

In KORA, we also assessed the potential impact of rs4251961 and rs6759676 on the expression of additional genes of the IL-1 family near the *IL1RN* locus in order to exclude pleiotropic effects beyond *IL-1RN*/IL-1RA (Table 4). Data were available for *IL1A*, *IL1B*, *IL1F7*/*IL37*, *IL1F9*/*IL36G*, *IL1F6*/*IL36A*, *IL1F8*/*IL36B*, *IL1F5*/*IL36RN* and *IL1F10* from 723 participants of the KORA F4 study. After adjustment for age, sex, BMI, waist-hip ratio, smoking and technical variables, we found a nominally significant association between rs4251961 and one transcript of *IL1F8*/*IL36B* (P=0.03) and another nominally significant association between rs6759676 and one transcript of *IL1F7*/*IL37* (P=0.04). However, these associations were not statistically significant after adjusting for multiple testing.

In the DILGOM GWAS sample (N=507), the eQTL analysis for rs4251961 and rs6759676 adjusted for age, sex, BMI and WHR showed no association with IL-1RA mRNA expression level (*IL1RN*) after Bonferroni correction for multiple testing leading to a significance level of $P=4.6\text{x}10^{-4}$. When testing the association of our variants with the expression loci located within 1 Mb with the *IL1RN* locus used as a midpoint, a significant association of rs4251961 with the expression of the gene *SLC20A1* (solute carrier family 20 (phosphate)) was observed ($P=2.4\text{x}10^{-4}$). Moreover, rs6759676 was significantly associated ($P=9.8\text{x}10^{-6}$) with the expression of *PAX8* (paired box 8).

Analysis of publicly available eQTL data

Grundberg *et al.* (33) used a per-tissue false discovery rate (FDR) of 1% to identify *cis*-eQTLs, corresponding to $P<5.0\times10^{-5}$ in adipose tissue, and a GWAS threshold of $P<5\text{x}10^{-8}$ for *trans*-eQTLs. Although rs6759676 showed nominal association ($0.0001<P<0.001$) with two probes (ILMN_1774874 and ILMN_1689734) of the *IL1RN* gene, none of those associations passed the specified significance thresholds for the 2 SNPs.

DISCUSSION

Our study presents four key findings regarding genetic determinants of circulating IL-1RA concentration and their associations with gene expression levels, metabolic and immunological variables associated with cardiometabolic disease risk: First, two independent SNPs in the *IL1RN* and *IL1F10* loci (rs4251961 and rs6759676) were significantly associated with IL-1RA levels. Second, these associations were independent of associations of the SNPs with gene expression of IL-1RA or other IL-1 family members in whole blood or monocytes. Third, alleles of both SNPs which were associated with elevated IL-1RA were associated with lower circulating CRP concentration. Fourth, the IL-1RA-raising and CRP-lowering allele of rs6759676 was also associated with lower fasting insulin and lower HOMA-IR.

Genetic determinants of circulating IL-1RA

Previous studies reported significant associations between several SNPs in or near the *IL1RN* locus and circulating IL-1RA (24,29), but it was not clear whether these represented independent associations. Our study shows for the first time that at least two independent genetic determinants of circulating IL-1RA are located in the vicinity of this locus. The first is marked by rs4251961 and has been described previously in European and African American ancestry populations (24,29,35). Most published SNPs that were previously reported to be associated with IL-1RA are in LD with rs4251961 (r^2 0.4-0.7, assessed with SNAP version 2.2, http://www.broadinstitute.org/mpg/snap/ldsearchpw.php). The second genetic region marked by rs6759676 in the *IL1F10* locus appears to represent a novel independent effect which does not correlate with rs4251961 (r^2=0.106), but shows some correlation to the recently described rs6743376 (30).

Associations between gene variants within or near the *IL1RN* locus have been reported with fat mass (23,27). However, our observations were independent of indices of obesity as the analyses were adjusted for body mass index and waist-hip ratio.

Associations between rs4251961, rs6759676 and gene expression

The most probable mechanism linking both SNPs with circulating IL-1RA levels would be the regulation of *IL1RN* transcription. Accordingly, our *in silico* analysis suggested that both SNPs may regulate gene expression because of the density of transcription factor binding sites in their vicinity. However, this was not confirmed in our directly observed analyses of whole blood or monocytes, as neither rs4251961 nor rs6759676 had any substantial impact on expression levels of *IL1RN*. The analysis of publicly available eQTL data indicated, however, that such an effect cannot be ruled out for rs6759676 in adipose tissue (33), and effects on transcription may be possible in other IL-1RA-producing cell types and tissues. In this context it is notable that one study reported an association between rs4251961 and several other SNPs with the peptidoglycan-induced production of the IL-1RA protein in whole-blood samples (26) suggesting a potential role for this SNP in the regulation of IL-1RA in response to inflammatory stimuli.

To examine pleiotropic effects of both SNPs we also assessed their associations with gene expression levels of other IL-1 family members encoded near the *IL1RN* locus, because an indirect effect via the regulation of the expression of IL-1β with subsequent upregulation of IL-1RA is conceivable. However, we found no convincing evidence for such an indirect effect.

Overall, our results are consistent with the regulation of circulating IL-1RA by post-transcriptional mechanisms influenced by genotype at rs4251961 and rs6759676. However the possibility that both SNPs could be linked with gene expression levels in tissues other

than whole blood cannot be excluded based on our work and needs to be explored in future studies.

Associations between rs4251961, rs6759676 and C-reactive protein levels

Given the anti-inflammatory properties of IL-1RA, it is possible that genetically determined levels of IL-1RA are associated with other markers of subclinical inflammation. The most frequently measured such marker is CRP, which we also included in our study. As for circulating IL-1RA, our findings of associations of two independent SNPs with systemic CRP levels are novel and extend the current literature because previous reports focused only on rs4251961 (26) or identified rs6734238 in the *IL1F10* locus, which is in LD with rs4251961 (r^2=0.613; r^2<0.1 with rs6759676), as a determinant of CRP levels in a GWAS (36). The association between rs6734238 in *IL1F10* and CRP was confirmed at genome-wide significance in African American women, but not in Hispanic American women (37). The associations between rs6759676 and CRP concentration has not been previously described. Notably, our observation that IL-1RA-raising alleles of both SNPs were associated with lower circulating CRP levels is in line with the aforementioned randomized clinical trial, in which treatment with recombinant IL-1RA not only improved glycemic control and beta-cell function in patients with T2D, but also decreased systemic CRP levels (6).

Taken together, these data indicate that even modest genetically determined elevations of circulating IL-1RA throughout life counteract systemic subclinical inflammation as reflected by circulating CRP. Further work should investigate the association of IL-1RA-associated variants and a wider range of inflammatory markers to corroborate this conclusion. For example, we have previously reported an association of rs425196 with IL-6 (38). This is of interest as recent Mendelian randomization analyses suggest that IL-6 signalling is causally associated with cardiovascular disease (39).

Associations between rs4251961, rs6759676 and parameters of glucose metabolism

If subclinical inflammation, and higher IL-1β in particular, are causally related to the development of T2D, the genetic upregulation of IL-1RA should be associated with more favorable metabolic control. Associations between SNPs in/near the *IL1RN* locus and parameters of glucose metabolism have been investigated before. However evidence from studies smaller than our meta-analysis report no significant associations between rs4251961 and 6 other SNPs not in LD with either of our strongest signals with and fasting glucose or fasting insulin (24,28). A third study reported an association between rs3213448 (r^2 with rs4251961 and rs6759676 <0.1) and incident T2D in men (but not in women) in HEALTH 2000, but no association in FINRISK97 (12).

Our study represents the largest study to date to investigate associations between genetic determinants of IL-1RA and measures of glucose metabolism. We observed that the IL-1RA-increasing allele of rs6759676 is associated with lower fasting insulin, and HOMA-IR, thus indicating that this SNP is associated with higher insulin sensitivity. However, we found this association only for rs6759676, whereas it was not statistically significant for rs4251961, although the associations between both SNPs and circulating IL-1RA were comparable. In order to explain this difference, gene expression data from other insulin-responsive tissues would be desirable to investigate whether both SNPs act mainly via the regulation of IL-1RA levels or whether one of them or even both also have pleiotropic effects by regulating other IL-1 family members that could represent mediators of the relationship between genetic variation, immunological and metabolic effects.

Our findings with genetic data mirror those from an intervention study. A recent clinical trial showed that daily subcutaneous injections of recombinant IL-1RA (which raised circulating IL-1RA levels) reduced HbA1c levels in patients with T2D (6), although it was not clear to what extent IL-1RA acted on insulin sensitivity or beta-cell function. Our findings are biologically plausible because the only known function of IL-1RA in humans is to block IL-

1β-mediated signalling. Importantly, this anti-inflammatory effect has pleiotropic metabolic consequences because IL-1β interferes with insulin signalling in adipocytes and hepatocytes and also suppresses insulin-induced glucose uptake, inhibits lipogenesis and decreases the release of adiponectin from adipocytes (40-42).

Our findings appear not to be in agreement with previous observational data from the Whitehall II study and Finnish cohort studies which suggest that an upregulation of IL-1RA in the circulation was linked to an increased risk of T2D (9-12). We hypothesize that the upregulation of IL-1RA before the clinical manifestation of T2D represents a counterregulation to proinflammatory and/or metabolic stimuli and can mainly be interpreted as a futile response to the presence of multiple diabetes risk factors which does not confer a sufficient degree of protection against the onset of the disease.

In the present study, our findings reflect the impact of a genetically determined and lifelong upregulation of IL-1RA without effects of potentially confounding factors on the association between genotype and metabolic traits. The data indicate that the persistent genetically determined upregulation of IL-1RA may attenuate diabetes-promoting effects of IL-1β and thus support the notion that subclinical inflammation and insulin resistance are causally related. However, our data have to be interpreted with caution because we observed significant effects for only one of the two IL-1RA-related SNPs, and our findings were only nominally significant.

Strengths and limitations

Our study has several strengths: it is the largest study so far to search for genetic determinants of IL-1RA levels and their immunological and pleiotropic effects; and the genetic approach of the potential causal impact of IL-1RA levels on metabolic traits is more robust against confounding than observational studies based on circulating IL-1RA only. However, our analyses were limited in scope by gene-centric genotyping platforms in some cohorts.

Therefore, the existence of further genetic determinants of IL-1RA levels with comparable effect sizes cannot be ruled out. We had only data for HOMA-IR as surrogate measure of insulin resistance, and dynamic measures of beta-cell function were not available in our analysis. Finally, we observed differences between studies with IL-1RA measured by ELISA and one study that measured IL-1RA with a bead-based multiplex assay, which led to the exclusion of this latter study from the main analysis. Unfortunately, we were not able to perform comparisons of different laboratory methods for IL-1RA measurement to further elucidate the underlying reasons for this observation.

Conclusions

Taken together, we identified one novel genetic determinant of circulating IL-1RA levels in the *IL1F10* locus which exerts systemic anti-inflammatory effects. Furthermore, we provide preliminary evidence that genetically raised IL-1RA concentrations by this SNP may protect against the development of insulin resistance. Thus, our data are in line with modest therapeutic benefits reported for novel IL-1β-targeting treatment strategies.

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Author contributions:

C.H., M.L.N., S.S., D.R.W., K.L., Me.Ku., M.P., and V.S. conceived and designed the study. C.H., M.L.N., S.B., E.J.B., M.C., C.G., H.G., A.J., Mi.Ka., Mi.Ki., W.K., C.L., T.L., K.L., Ca.Ma., A.P., H.P., O.R., W.R., M.S., D.S., A.G.T., B.T., N.W., P.S.W., T.Z., A.D.H., and V.S. contributed/researched data. M.L.N., S.S., Ch.Mü., J.K., K.K., and K.S. analyzed data. C.H., M.L.N., T.L., M.R., Me.Ku., M.P., and V.S. contributed to discussion. C.H., M.L.N., and S.S. wrote the manuscript. All authors reviewed/edited the manuscript and approved of the final version.

Me.Ku. and V.S. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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DUALITY OF INTEREST

The authors have no potential conflicts of interest relevant to this article to report.

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TABLE1. Cohort characteristics

	Discovery Cohorts							Replication Cohorts			
	WHII	**FR1997**	**DILGOM (FR07) GWAS**	**DILGOM (FR07) metabochip**	**HEALTH2000 GenMets cases**	**HEALTH2000 GenMets controls**	**FIN MIGen**	**KORA**	**GHS1**	**GHS2**	**YFS**
N	2160	468	678	3858	979	1031	111	719	2984	1174	1998
Age (years)	48.9 (6.0)	56.1 (12.5)	53.5 (13.4)	52.4 (13.5)	51.4 (11.1)	49.9 (10.9)	56.1 (8.8)	60.9 (8.9)	55.9 (10.9)	55.1 (10.9)	37.7 (5.0)
Sex (% males)	77.1	47.6	48.8	45.9	50.5	47.1	62.2	48.65	51.4	50.1	45.2
BMI (kg/m²)	25.4 (3.7)	26.5 (4.3)	26.6 (4.5)	26.9 (4.7)	29.5 (4.2)	25.1 (3.5)	28.1 (4.7)	28.14 (4.78)	27.1 (4.7)	27.3 (5.0)	26.0 (4.8)
Waist-hip ratio	0.9 (0.08)	0.9 (0.09)	0.9 (0.09)	0.9 (0.09)	0.9 (0.08)	0.9 (0.08)	0.9 (0.09)	0.89 (0.08)	0.93 (0.09)	0.92 (0.1)	0.9 (0.09)
Current smokers (%)	12.2	27.1	18.9	17.1	28.8	30.2	21.6	14.53	18.2	21.2	27.2
Fasting glucose (mmol/l)	5.3 (0.5)	5.07 (0.64)	5.90 (0.72)	5.87 (0.72)	5.51 (0.50)	5.22 (0.42)	5.20 (0.86)	5.60 (1.10)	5.29 (1.05)	5.26 (0.85)	5.29 (0.56)
2-hr glucose (mmol/l)	5.6 (1.7)	NA	NA	NA	NA	NA	NA	6.56 (2.21)	NA	NA	NA
Fasting insulin (μU/ml)	6.5 (5.7)	6.73 (4.17)	7.24 (8.30)	6.73 (5.09)	10.85 (6.13)	5.98 (2.96)	7.90 (4.76)	8.83 (24.31)	NA	NA	8.66 (6.67)
2-hr insulin (μU/ml)	46.5 (39.1)	NA	NA	NA	NA	NA	NA	71.64 (79.22)	NA	NA	NA
HOMA-IR	0.9 (0.58)	1.51 (1.04)	1.92 (2.46)	1.80 (1.57)	2.67 (1.58)	1.41 (0.74)	1.85 (1.19)	2.33 (6.45)	NA	NA	2.09 (1.80)
HbA1c (%)	5.3 (0.7)	NA	NA	NA	5.32 (0.33)	5.13 (0.31)	NA	5.74 (0.68)	5.47 (0.74)	5.48 (0.67)	NA
HbA1c (mmol/mol)	34 (7.7)	NA	NA	NA	35 (3.6)	33 (3.4)	NA	39 (7.4)	36 (8.1)	36 (7.3)	NA
CRP (mg/l)	1.5 (1.8)	2.65 (3.77)	2.14 (3.07)	2.03 (2.78)	2.42 (3.62)	1.63 (2.77)	2.94 (4.19)	2.48 (5.08)	2.88 (4.63)	2.86 (4.95)	1.63 (2.32)
IL-1RA (pg/ml)	286.3 (205.3)	251.4 (149.0)	291.6 (146.0)	312.3 (161.8)	433.9 (247.3)	323.4 (169.8)	295.0 (154.4)	335.03 (160.51)	353.5 (189.1)	392.4 (210.2)	256.7 (141.0)

Genotyping platform	Metabochip 50k cardiochip	Illumina Human 610K BeadChip	Illumina Human 610K BeadChip	Metabochip	Illumina Human 610K BeadChip	Illumina Human 610K BeadChip	Affymetrix 6.0 platform	Affymetrix Human SNP Array 6.0	Affymetrix Human SNP Array 6.0	Affymetrix Human SNP Array 6.0	Illumina BeadChip Human670 K array

For all variables, mean (SD) or % is shown. The number of study participants refers to the individuals for whom genotype data and IL-1RA measurements were available. Sample sizes may vary for the other traits as described in the section Research design and Methods.

TABLE 2. Meta-analysis results for the SNPs independently associated with circulating IL-1RA levels.

						Discovery						
Marker name	**Chr**	**Position (HG18)**	**Gene**	**Minor (coded) allele**	**Major allele**	**Mean MAF**	***N***	**Effect**	**SE**	***P***	***I^2 (%)***	**Meta-analysis model**
rs4251961	2	113590938	*IL1RN*	C	T	0.32	9092	-0.078	0.006	2.19E-34	39.8	FE
rs6759676	2	113552819	closest *IL1F10*	C	T	0.44	9131	0.063	0.006	6.12E-27	0.0	FE

						Replication						
Marker name	**Chr**	**Position (HG18)**	**Gene**	**Minor (coded) allele**	**Major allele**	**Mean MAF**	***N***	**Effect**	**SE**	***P***	***I^2 (%)***	**Meta-analysis model**
rs4251961	2	113590938	*IL1RN*	C	T	0.32	4863	-0.086	0.023	2.00E-04	78.90	RE
rs6759676	2	113552819	closest *IL1F10*	C	T	0.44	4863	0.099	0.019	3.54E-07	71.91	RE

						Combined						
Marker name	**Chr**	**Position (HG18)**	**Gene**	**Minor (coded) allele**	**Major allele**	**Mean MAF**	***N***	**Effect**	**SE**	***P***	***I^2 (%)***	**Meta-analysis model**
rs4251961	2	113590938	*IL1RN*	C	T	0.32	13955	-0.082	0.009	2.76E-21	53.84	RE
rs6759676	2	113552819	closest *IL1F10*	C	T	0.44	13994	0.075	0.009	1.73E-17	60.45	RE

TABLE 3. Association of rs4251961and rs6759676 with quantitative immunological and glycaemic traits.

SNP	Trait*	beta	SE	*P*	Number of studies	*N*	I^2 (%)
rs4251961	IL-1RA (pg/ml)	**-0.082**	0.009	**2.76e-21**	10	13955	53.84
	CRP (mg/l)	0.0601	0.0108	**2.95e-08**	11	17797	0.79
	Fasting glucose (mmol/l)	0.0005	0.0012	0.6960	11	16822	0.0
	2-hr glucose (mmol/l)	-0.0068	0.0060	0.2637	2	4634	0.0
	HbA1c (%)	0.0011	0.0013	0.3964	6	10013	0.0
	Fasting insulin (µU/ml)	0.0099	0.0067	0.1390	9	13664	0.0
	2-hr insulin (µU/ml)	-0.0129	0.0186	0.4881	2	3735	0.0
	HOMA-IR	0.0071	0.0068	0.2992	9	12821	0.0
rs6759676	IL-1RA (pg/ml)	**0.075**	0.009	**1.73E-17**	10	13994	60.45
	CRP (mg/l)	-0.0284	0.0103	**0.0055**	11	17747	0.0
	Fasting glucose (mmol/l)	-0.0012	0.0011	0.2603	11	16773	12.5
	2-hr glucose (mmol/l)	0.0039	0.0060	0.5140	2	4588	0.0
	HbA1c (%)	-0.0007	0.0012	0.5295	6	9966	0.0
	Fasting insulin (µU/ml)	-0.0159	0.0061	**0.0096**	9	13616	20.2
	2-hr insulin (µU/ml)	-0.0271	0.0182	0.1381	2	3689	0.0
	HOMA-IR	-0.0173	0.0063	**0.0063**	9	12783	0.0

*All traits were ln-transformed. Analyses were adjusted for age, sex, BMI, WHR and smoking (additive model, major allele as reference).

TABLE 4. Associations between rs4251961, rs6759676 and mRNA expression of further transcripts of the IL-1 family near the *IL1RN* locus in the KORA F4 Study ($N = 718$)

Transcript	Gene	Beta	SE	*P*
rs4251961				
ILMN_1806249	*IL1RN*	0.0012	0.0085	0.9989
ILMN_1689734	*IL1RN*	-0.0278	0.0300	0.3527
ILMN_1774874	*IL1RN*	-0.0063	0.0244	0.7974
ILMN_1658483	*IL1A*	-0.0020	0.0070	0.7727
ILMN_1775501	*IL1B*	-0.0099	0.0261	0.7045
ILMN_2353936	*IL37*	-0.0064	0.0079	0.4179
ILMN_1718275	*IL37*	0.0033	0.0074	0.6518
ILMN_1697710	*IL37*	0.0012	0.0185	0.9464
ILMN_2158713	*IL36G*	-0.0045	0.0070	0.5234
ILMN_1704000	*IL36A*	0.0088	0.0075	0.2404
ILMN_1761927	*IL36B*	0.0026	0.0068	0.7009
ILMN_1754002	*IL36B*	0.0018	0.0075	0.8119
ILMN_1799519	***IL36B***	**0.0155**	**0.0070**	**0.0269**
ILMN_1759141	*IL36RN*	0.0077	0.0075	0.3015
ILMN_1804901	*IL36RN*	0.0123	0.0106	0.2434
ILMN_1790556	*IL1F10*	-0.0023	0.0071	0.7507
ILMN_2359733	*IL1F10*	-0.0068	0.0074	0.3547
rs6759676				
ILMN_1806249	*IL1RN*	-0.0144	0.0084	0.0869
ILMN_1689734	*IL1RN*	0.0217	0.0296	0.4627
ILMN_1774874	*IL1RN*	0.0117	0.0242	0.6292
ILMN_1658483	*IL1A*	-0.0031	0.0069	0.6579
ILMN_1775501	*IL1B*	-0.0037	0.0257	0.8851
ILMN_2353936	*IL37*	0.0151	0.0078	0.0523
ILMN_1718275	***IL37***	**-0.0146**	**0.0073**	**0.0448**
ILMN_1697710	*IL37*	0.0291	0.0182	0.1107
ILMN_2158713	*IL36G*	-0.0110	0.0069	0.1120
ILMN_1704000	*IL36A*	-0.0035	0.0074	0.6340
ILMN_1761927	*IL36B*	-0.0043	0.0067	0.5207
ILMN_1754002	*IL36B*	-0.0093	0.0074	0.2067
ILMN_1799519	*IL36B*	-0.0101	0.0069	0.1480
ILMN_1759141	*IL36RN*	-0.0068	0.0074	0.3593
ILMN_1804901	*IL36RN*	-0.0121	0.0105	0.2466
ILMN_1790556	*IL1F10*	0.0020	0.0071	0.7777
ILMN_2359733	*IL1F10*	0.0019	0.0073	0.7992

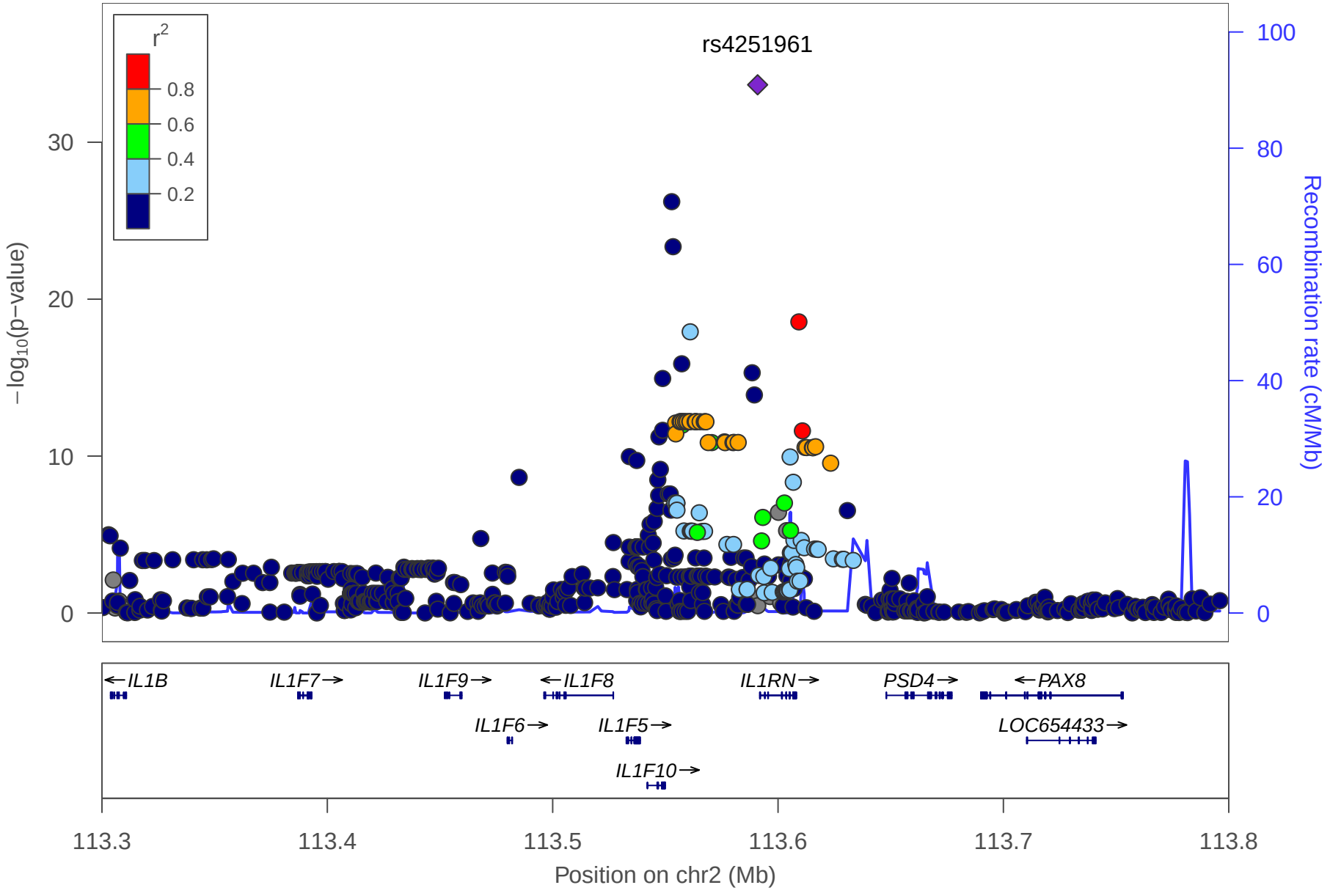
Analyses were adjusted for age, sex, BMI, WHR, smoking and technical variables. The first column gives Illumina transcript IDs. Alternative nomenclature: *IL36RN* = *IL1F5*, *IL36A* = *IL1F6*, *IL37* = *IL1F7*, *IL36B* = *IL1F8*, *IL36G* = *IL1F9*. Nominally significant associations are highlighted by bold print.

Fig. 1. Association between rs4251961, rs6759676 and IL-1RA in the discovery analysis.

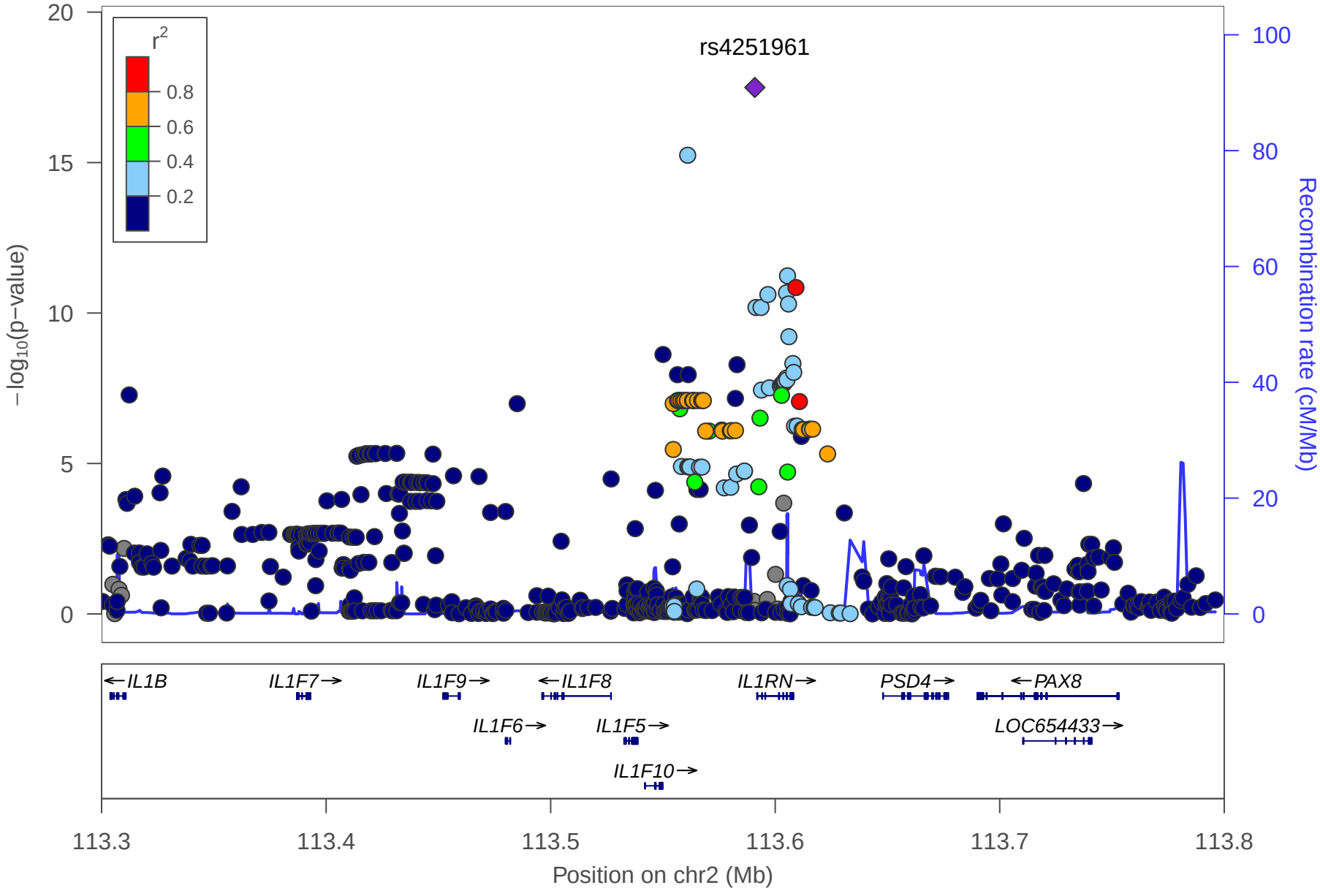
Figure 1 shows GWA P values for all the variants that were tested in IL-1RA association analysis and that were located in the IL1RN gene cluster on the chromosome 2. A. Rs4251961 shows the strongest association with IL-1RA in the discovery analysis. B. Rs4251961 remains as an independent hit when conditioning on rs6759676. C. Rs6759676 remains as an independent hit when conditioning on rs4251961.

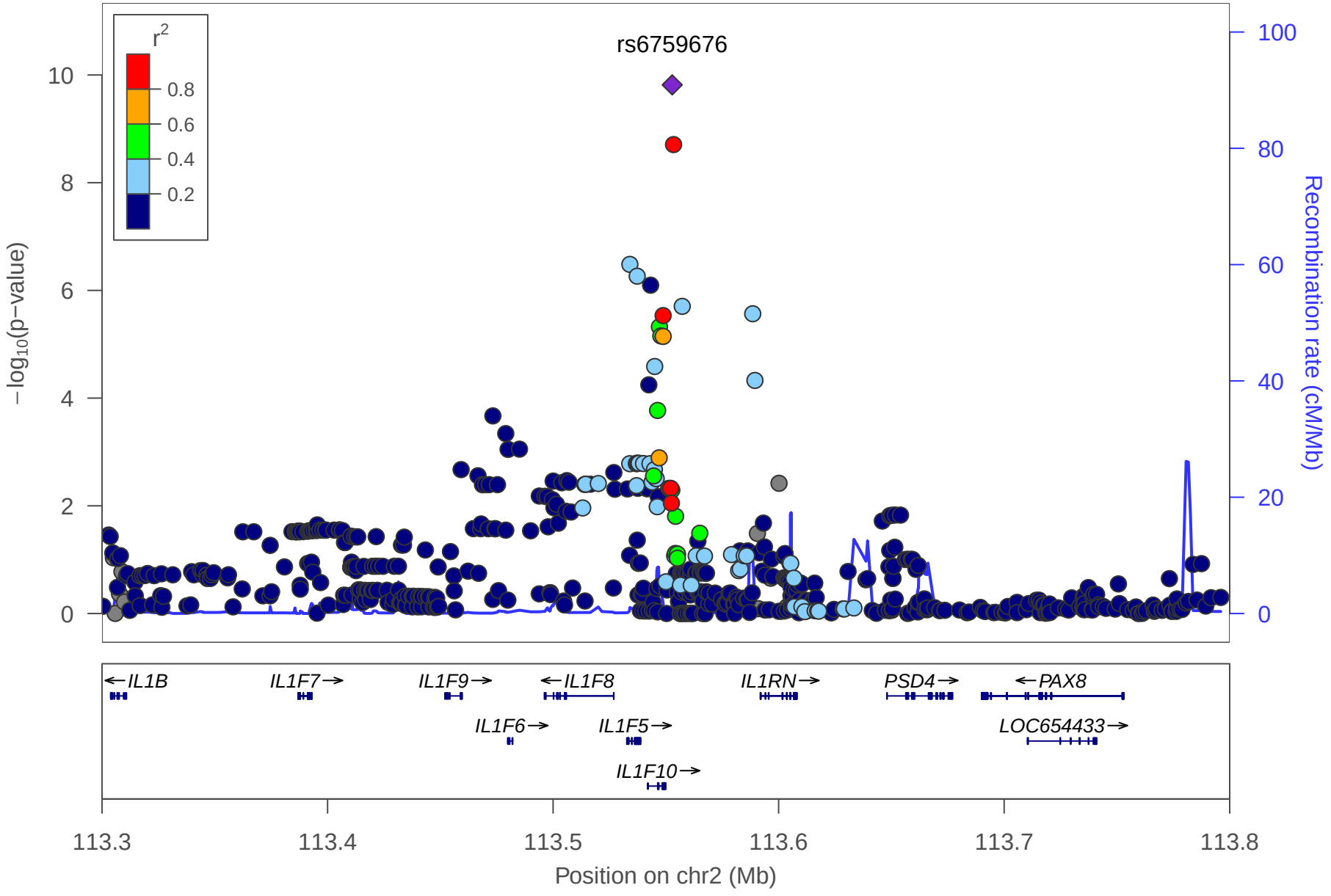
Fig. 2. Association of rs4251961 and rs6759676 with circulating IL-1RA levels, CRP, insulin and HOMA-IR in individual studies included in the discovery and replication analysis.

A, B: IL-1RA; C, D: CRP; E, F: insulin; G, H: HOMA-IR.
All analyses were adjusted for age, sex, BMI, waist-to-hip ratio and smoking.

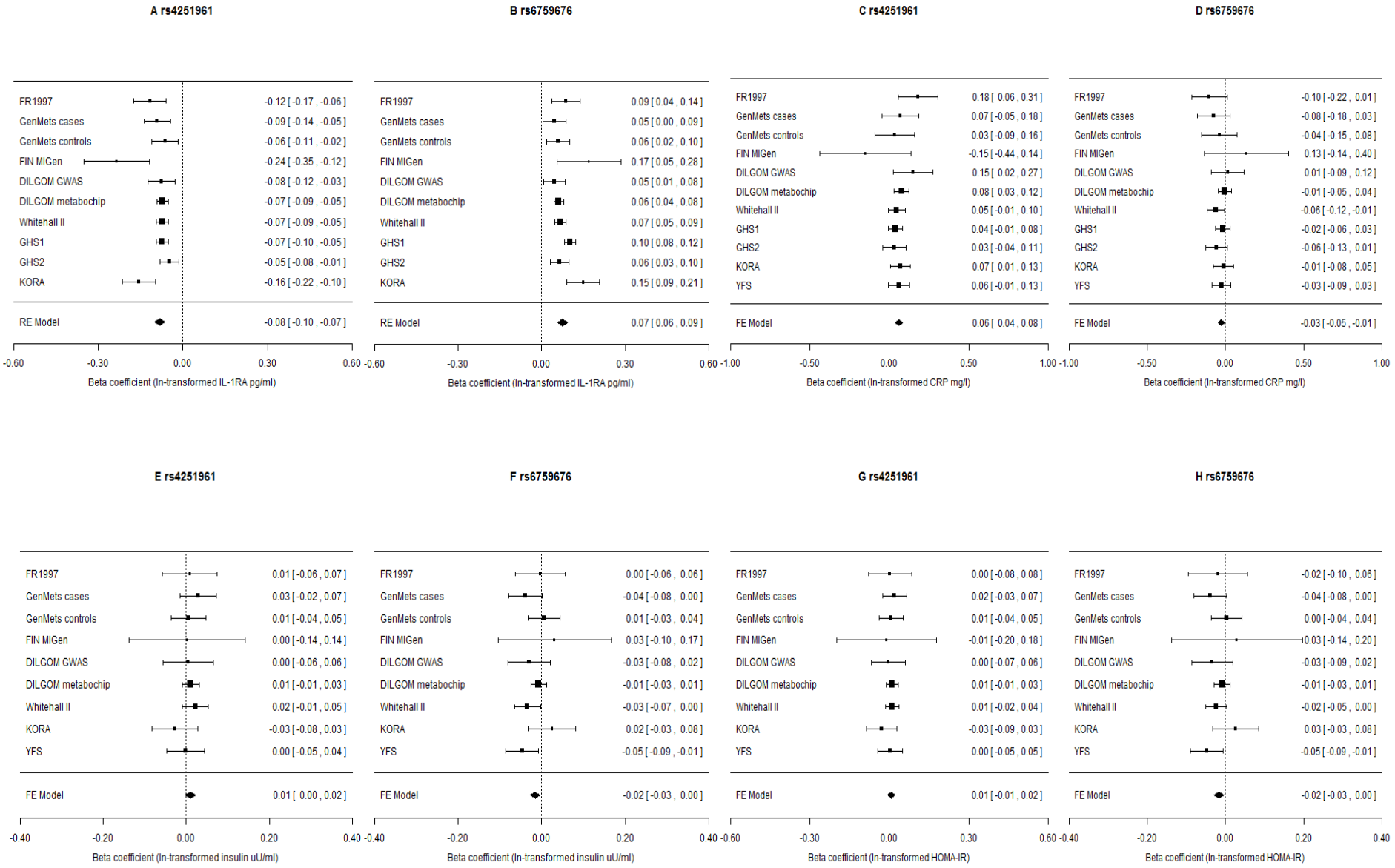

rs4251961
r2
0.8
0.6
0.4
0.2
−log10(p−value)
30
20
10
0
100
80
60
40
20
0
Recombination rate (cM/Mb)
←IL1B
IL1F7→
IL1F9→
←IL1F8
IL1RN→
PSD4→
←PAX8
IL1F6→
IL1F5→
LOC654433→
IL1F10→
113.3
113.4
113.5
113.6
113.7
113.8
Position on chr2 (Mb)

Diabetes


rs4251961
r2
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
IL1B
IL1F7
IL1F9
IL1F8
IL1RN
PSD4
PAX8
IL1F6
IL1F5
LOC654433
IL1F10
Position on chr2 (Mb)


rs6759676
r2
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
IL1B
IL1F7
IL1F9
IL1F8
IL1RN
PSD4
PAX8
IL1F6
IL1F5
LOC654433
IL1F10
Position on chr2 (Mb)

Diabetes


A rs4251961
FR1997 -0.12 [-0.17 , -0.06]
GenMets cases -0.09 [-0.14 , -0.05]
GenMets controls -0.06 [-0.11 , -0.02]
FIN MIGen -0.24 [-0.35 , -0.12]
DILGOM GWAS -0.08 [-0.12 , -0.03]
DILGOM metabochip -0.07 [-0.09 , -0.05]
Whitehall II -0.07 [-0.09 , -0.05]
GHS1 -0.07 [-0.10 , -0.05]
GHS2 -0.05 [-0.08 , -0.01]
KORA -0.16 [-0.22 , -0.10]
RE Model -0.08 [-0.10 , -0.07]
-0.60 -0.30 0.00 0.30 0.60
Beta coefficient (ln-transformed IL-1RA pg/ml)
B rs6759676
FR1997 0.09 [0.04 , 0.14]
GenMets cases 0.05 [0.00 , 0.09]
GenMets controls 0.06 [0.02 , 0.10]
FIN MIGen 0.17 [0.05 , 0.28]
DILGOM GWAS 0.05 [0.01 , 0.08]
DILGOM metabochip 0.06 [0.04 , 0.08]
Whitehall II 0.07 [0.05 , 0.09]
GHS1 0.10 [0.08 , 0.12]
GHS2 0.06 [0.03 , 0.10]
KORA 0.15 [0.09 , 0.21]
RE Model 0.07 [0.06 , 0.09]
-0.60 -0.30 0.00 0.30 0.60
Beta coefficient (ln-transformed IL-1RA pg/ml)
C rs4251961
FR1997 0.18 [0.06 , 0.31]
GenMets cases 0.07 [-0.05 , 0.18]
GenMets controls 0.03 [-0.09 , 0.16]
FIN MIGen -0.15 [-0.44 , 0.14]
DILGOM GWAS 0.15 [0.02 , 0.27]
DILGOM metabochip 0.08 [0.03 , 0.12]
Whitehall II 0.05 [-0.01 , 0.10]
GHS1 0.04 [-0.01 , 0.08]
GHS2 0.03 [-0.04 , 0.11]
KORA 0.07 [0.01 , 0.13]
YFS 0.06 [-0.01 , 0.13]
FE Model 0.06 [0.04 , 0.08]
-1.00 -0.50 0.00 0.50 1.00
Beta coefficient (ln-transformed CRP mg/l)
D rs6759676
FR1997 -0.10 [-0.22 , 0.01]
GenMets cases -0.08 [-0.18 , 0.03]
GenMets controls -0.04 [-0.15 , 0.08]
FIN MIGen 0.13 [-0.14 , 0.40]
DILGOM GWAS 0.01 [-0.09 , 0.12]
DILGOM metabochip -0.01 [-0.05 , 0.04]
Whitehall II -0.06 [-0.12 , -0.01]
GHS1 -0.02 [-0.06 , 0.03]
GHS2 -0.06 [-0.13 , 0.01]
KORA -0.01 [-0.08 , 0.05]
YFS -0.03 [-0.09 , 0.03]
FE Model -0.03 [-0.05 , -0.01]
-1.00 -0.50 0.00 0.50 1.00
Beta coefficient (ln-transformed CRP mg/l)
E rs4251961
FR1997 0.01 [-0.06 , 0.07]
GenMets cases 0.03 [-0.02 , 0.07]
GenMets controls 0.01 [-0.04 , 0.05]
FIN MIGen 0.00 [-0.14 , 0.14]
DILGOM GWAS 0.00 [-0.06 , 0.06]
DILGOM metabochip 0.01 [-0.01 , 0.03]
Whitehall II 0.02 [-0.01 , 0.05]
KORA -0.03 [-0.08 , 0.03]
YFS 0.00 [-0.05 , 0.04]
FE Model 0.01 [0.00 , 0.02]
-0.40 -0.20 0.00 0.20 0.40
Beta coefficient (ln-transformed insulin uU/ml)
F rs6759676
FR1997 0.00 [-0.06 , 0.06]
GenMets cases -0.04 [-0.08 , 0.00]
GenMets controls 0.01 [-0.03 , 0.04]
FIN MIGen 0.03 [-0.10 , 0.17]
DILGOM GWAS -0.03 [-0.08 , 0.02]
DILGOM metabochip -0.01 [-0.03 , 0.01]
Whitehall II -0.03 [-0.07 , 0.00]
KORA 0.02 [-0.03 , 0.08]
YFS -0.05 [-0.09 , -0.01]
FE Model -0.02 [-0.03 , 0.00]
-0.40 -0.20 0.00 0.20 0.40
Beta coefficient (ln-transformed insulin uU/ml)
G rs4251961
FR1997 0.00 [-0.08 , 0.08]
GenMets cases 0.02 [-0.03 , 0.07]
GenMets controls 0.01 [-0.04 , 0.05]
FIN MIGen -0.01 [-0.20 , 0.18]
DILGOM GWAS 0.00 [-0.07 , 0.06]
DILGOM metabochip 0.01 [-0.01 , 0.03]
Whitehall II 0.01 [-0.02 , 0.04]
KORA -0.03 [-0.09 , 0.03]
YFS 0.00 [-0.05 , 0.05]
FE Model 0.01 [-0.01 , 0.02]
-0.60 -0.30 0.00 0.30 0.60
Beta coefficient (ln-transformed HOMA-IR)
H rs6759676
FR1997 -0.02 [-0.10 , 0.06]
GenMets cases -0.04 [-0.08 , 0.00]
GenMets controls 0.00 [-0.04 , 0.04]
FIN MIGen 0.03 [-0.14 , 0.20]
DILGOM GWAS -0.03 [-0.09 , 0.02]
DILGOM metabochip -0.01 [-0.03 , 0.01]
Whitehall II -0.02 [-0.05 , 0.00]
KORA 0.03 [-0.03 , 0.08]
YFS -0.05 [-0.09 , -0.01]
FE Model -0.02 [-0.03 , 0.00]
-0.40 -0.20 0.00 0.20 0.40
Beta coefficient (ln-transformed HOMA-IR)

Supplementary Table 1. Meta-analysis results for SNPs associated with IL-1RA in the discovery cohorts.

MarkerName	Chr	Position (HG18)	Gene	Minor (Coded) Allele	Major Allele	Mean MAF	Discovery			
							N	Effect	SE	*P*
rs4251961	**2**	**113590938**	***IL1RN***	**C**	**T**	**0.32**	**9092**	**-0.078**	**0.006**	**2.19E-34**
rs6759676	**2**	**113552819**	**closest *IL1F10***	**C**	**T**	**0.44**	**9131**	**0.063**	**0.006**	**6.12E-27**
rs10186133	2	113553415	closest *IL1F10*	G	T	0.42	9126	0.060	0.006	4.52E-24
rs315949	2	113609245	closest *IL1RN*	A	G	0.37	5306	-0.070	0.008	2.80E-19
rs6731551	2	113561024	closest *IL1F10*	C	T	0.47	9133	-0.052	0.006	1.20E-18
rs10199363	2	113557262	closest *IL1F10*	G	A	0.18	9134	0.065	0.008	1.31E-16
rs315921	2	113588522	closest *IL1RN*	A	G	0.17	9096	0.065	0.008	4.89E-16
rs6761276	2	113548783	*IL1F10*	T	C	0.43	5310	0.061	0.008	1.13E-15
rs315920	2	113589489	closest *IL1RN*	T	C	0.19	9095	0.059	0.008	1.26E-14
rs10176274	2	113557046	closest *IL1F10*	G	C	0.34	3209	-0.085	0.012	6.37E-13
rs10188292	2	113556914	closest *IL1F10*	T	A	0.34	3209	-0.085	0.012	6.37E-13
rs6743171	2	113556529	closest *IL1F10*	C	G	0.34	3209	-0.085	0.012	6.37E-13
rs13398728	2	113558977	closest *IL1F10*	C	T	0.34	3209	-0.085	0.012	6.37E-13
rs13410964	2	113559754	closest *IL1F10*	A	G	0.34	3209	-0.085	0.012	6.37E-13
rs6722922	2	113557986	closest *IL1F10*	T	C	0.34	3209	-0.085	0.012	6.37E-13
rs6750559	2	113558003	closest *IL1F10*	A	G	0.34	3209	-0.085	0.012	6.37E-13
rs4496335	2	113560946	closest *IL1F10*	T	C	0.34	3209	-0.085	0.012	6.37E-13
rs6741180	2	113560855	closest *IL1F10*	A	G	0.34	3209	-0.085	0.012	6.37E-13
rs12328766	2	113563209	closest *IL1F10*	G	A	0.34	3209	-0.085	0.012	6.38E-13
rs12329129	2	113563478	closest *IL1F10*	A	G	0.34	3209	-0.085	0.012	6.38E-13
rs12328368	2	113563615	closest *IL1F10*	G	C	0.34	3209	-0.085	0.012	6.38E-13
rs6730516	2	113565432	closest *IL1RN*	T	C	0.34	3209	-0.085	0.012	6.44E-13
rs1446510	2	113567988	closest *IL1RN*	T	C	0.34	3209	-0.085	0.012	6.47E-13
rs1446509	2	113567630	closest *IL1RN*	T	A	0.34	3209	-0.085	0.012	6.47E-13

rs6738239	2	113567263	closest *IL1RN*	A	C	0.34	3209	-0.085	0.012	6.47E-13
rs13409360	2	113554573	closest *IL1F10*	A	G	0.34	3209	-0.084	0.012	7.78E-13
rs6734238	2	113557501	closest *IL1F10*	G	A	0.34	3209	-0.084	0.012	1.06E-12
rs6743376	2	113548804	*IL1F10*	C	A	0.37	5303	0.056	0.008	2.28E-12
rs315943	2	113610809	closest *IL1RN*	G	A	0.34	3209	-0.082	0.012	2.46E-12
rs13409371	2	113554616	closest *IL1F10*	A	G	0.40	3209	-0.083	0.012	3.83E-12
rs4145013	2	113547159	*IL1F10*	G	A	0.35	5306	0.055	0.008	5.91E-12
rs7574159	2	113576232	*IL1RN*	A	G	0.33	3209	-0.082	0.012	1.24E-11
rs17207494	2	113580481	*IL1RN*	C	A	0.33	3209	-0.082	0.012	1.34E-11
rs10171849	2	113582305	*IL1RN*	C	A	0.33	3209	-0.082	0.012	1.35E-11
rs13382561	2	113580007	*IL1RN*	G	A	0.33	3209	-0.082	0.012	1.37E-11
rs13424580	2	113569035	closest *IL1RN*	A	G	0.33	3209	-0.081	0.012	1.37E-11
rs6746979	2	113570591	closest *IL1RN*	A	T	0.33	3209	-0.081	0.012	1.38E-11
rs7574427	2	113576509	*IL1RN*	A	G	0.33	3209	-0.082	0.012	1.39E-11
rs11885498	2	113616648	closest *IL1RN*	G	A	0.36	3209	-0.078	0.012	2.53E-11
rs2902452	2	113611987	closest *IL1RN*	A	C	0.36	3209	-0.078	0.012	2.80E-11
rs6754298	2	113612684	closest *IL1RN*	A	T	0.36	3209	-0.078	0.012	2.85E-11
rs1374281	2	113615260	closest *IL1RN*	G	C	0.36	3209	-0.078	0.012	2.86E-11
rs895496	2	113615496	closest *IL1RN*	T	C	0.36	3209	-0.078	0.012	2.93E-11
rs1530551	2	113534037	*IL36RN*	T	C	0.38	5307	0.050	0.008	1.08E-10
rs380092	2	113605371	*IL1RN*	T	A	0.36	5308	0.052	0.008	1.13E-10
rs2472188	2	113537285	*IL36RN*	C	G	0.38	5304	0.049	0.008	1.89E-10
rs11123167	2	113623340	closest *IL1RN*	C	G	0.36	3209	-0.074	0.012	2.82E-10
rs3827763	2	113547760	*IL1F10*	A	G	0.30	5310	0.052	0.008	6.99E-10
rs1867828	2	113485091	closest *IL1F10*	A	G	0.26	5310	-0.052	0.009	2.27E-09
rs3811050	2	113546644	*IL1F10*	T	C	0.18	5310	-0.062	0.011	3.22E-09
rs315952	2	113606775	*IL1RN*	C	T	0.35	5300	0.047	0.008	4.62E-09
rs11678375	2	113552162	closest *IL1F10*	T	C	0.43	3209	0.060	0.011	2.58E-08
rs13386602	2	113551291	closest *IL1F10*	A	C	0.43	3209	0.060	0.011	2.61E-08
rs12469822	2	113547034	*IL1F10*	G	A	0.44	3209	0.060	0.011	3.22E-08

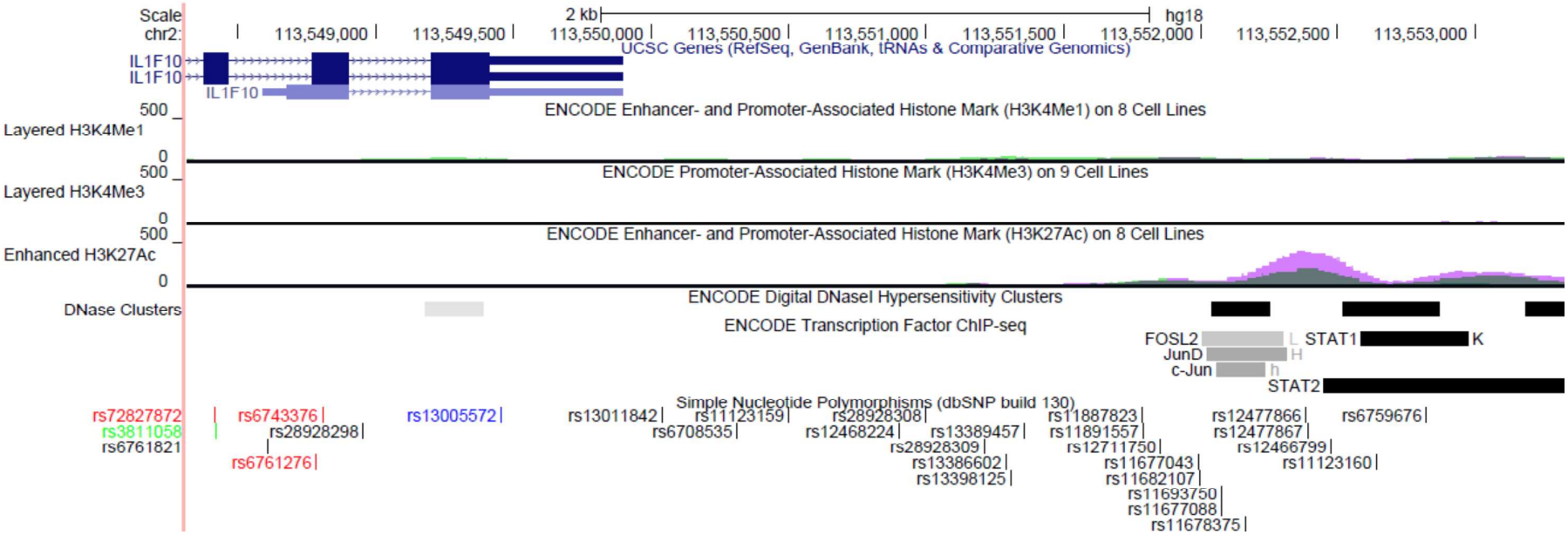
Variants that passed the discovery *P* value threshold of 5.0×10^{-8} are shown. The two most significantly associated independent SNPs from the discovery meta-analysis (marked in bold) were then taken forward for replication.

Supplementary Table 2. Replication and combined meta-analysis results for the SNPs independently associated with circulating IL-1RA levels with YFS included in analyses.

						Replication						
Marker name	**Chr**	**Position (HG18)**	**Gene**	**Minor (coded) allele**	**Major allele**	**Mean MAF**	***N***	**Effect**	**SE**	***P***	***I^2 (%)***	**Meta-analysis model**
rs4251961	2	113590938	*IL1RN*	C	T	0.32	6802	-0.064	0.027	0.016	90.74	RE
rs6759676	2	113552819	closest *IL1F10*	C	T	0.44	6802	0.073	0.034	0.031	94.94	RE

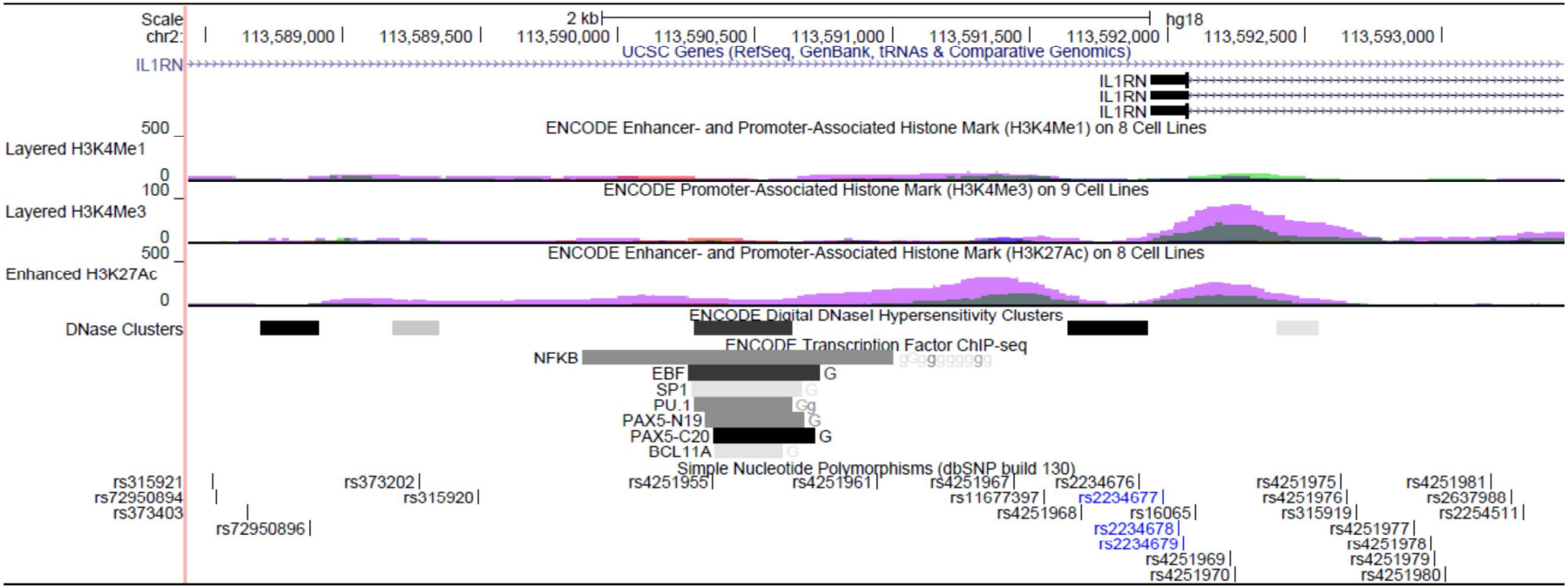
						Combined						
Marker name	**Chr**	**Position (HG18)**	**Gene**	**Minor (coded) allele**	**Major allele**	**Mean MAF**	***N***	**Effect**	**SE**	***P***	***I^2 (%)***	**Meta-analysis model**
rs4251961	2	113590938	*IL1RN*	C	T	0.32	15894	-0.077	0.012	9.46E-11	79.72	RE
rs6759676	2	113552819	closest *IL1F10*	C	T	0.44	15933	0.068	0.013	9.14E-08	84.69	RE

Supplementary Figure 1. In silico functional analysis for rs6759676.



A snapshot of the UCSC Genome Browser shows that rs6759676 falls within an region enriched for H3K27Ac histone acetylation (often found near active regulatory elements) in epidermal keratinocytes cells (pink peak) and human mammary epithelial cells (grey peak); within a DNase hypersensitive region (characteristic of open chromatin regions) in multiple cell lines; and also within STAT transcription factor binding sites.

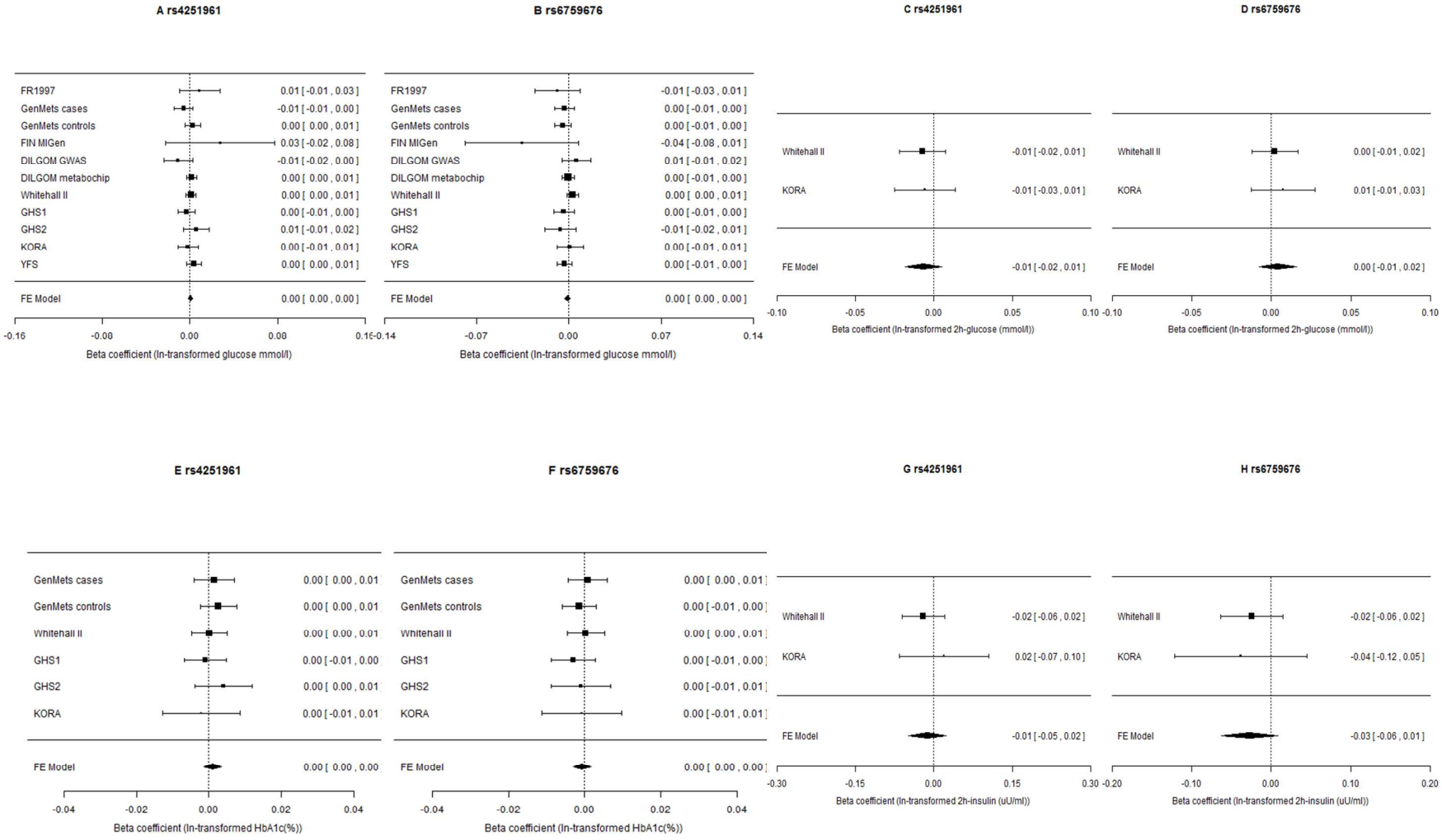
Supplementary Figure 2. In silico functional analysis for rs4251961.



A snapshot of the UCSC Genome Browser shows that rs4251961 falls within a region enriched for the H3K27Ac histone acetylation mark (often found near active regulatory elements) in epidermal keratinocytes cells (pink peak) and human mammary epithelial cells (grey peak) (Supplementary Figure 2), upstream of the IL1RN gene. It is very close to regions enriched for transcription factor binding and DNase hypersensitivity, indicative of open chromatin.

Supplementary Figure 3. Association of rs4251961 and rs6759676 with circulating fasting glucose, 2-hr glucose, HbA1c and 2-hr insulin in individual studies included in the discovery and replication analysis.

A, B: fasting glucose; C, D: 2-hr glucose; E, F: HbA1c; G, H: 2-hr insulin.
All analyses were adjusted for age, sex, BMI, waist-to-hip ratio and smoking.


A rs4251961
FR1997 0.01 [-0.01 , 0.03]
GenMets cases -0.01 [-0.01 , 0.00]
GenMets controls 0.00 [0.00 , 0.01]
FIN MIGen 0.03 [-0.02 , 0.08]
DILGOM GWAS -0.01 [-0.02 , 0.00]
DILGOM metabochip 0.00 [0.00 , 0.01]
Whitehall II 0.00 [0.00 , 0.01]
GHS1 0.00 [-0.01 , 0.00]
GHS2 0.01 [-0.01 , 0.02]
KORA 0.00 [-0.01 , 0.01]
YFS 0.00 [0.00 , 0.01]
FE Model 0.00 [0.00 , 0.00]
-0.16 -0.08 0.00 0.08 0.16
Beta coefficient (ln-transformed glucose mmol/l)
B rs6759676
FR1997 -0.01 [-0.03 , 0.01]
GenMets cases 0.00 [-0.01 , 0.00]
GenMets controls 0.00 [-0.01 , 0.00]
FIN MIGen -0.04 [-0.08 , 0.01]
DILGOM GWAS 0.01 [-0.01 , 0.02]
DILGOM metabochip 0.00 [-0.01 , 0.00]
Whitehall II 0.00 [0.00 , 0.01]
GHS1 0.00 [-0.01 , 0.00]
GHS2 -0.01 [-0.02 , 0.01]
KORA 0.00 [-0.01 , 0.01]
YFS 0.00 [-0.01 , 0.00]
FE Model 0.00 [0.00 , 0.00]
-0.14 -0.07 0.00 0.07 0.14
Beta coefficient (ln-transformed glucose mmol/l)
C rs4251961
Whitehall II -0.01 [-0.02 , 0.01]
KORA -0.01 [-0.03 , 0.01]
FE Model -0.01 [-0.02 , 0.01]
-0.10 -0.05 0.00 0.05 0.10
Beta coefficient (ln-transformed 2h-glucose (mmol/l))
D rs6759676
Whitehall II 0.00 [-0.01 , 0.02]
KORA 0.01 [-0.01 , 0.03]
FE Model 0.00 [-0.01 , 0.02]
-0.10 -0.05 0.00 0.05 0.10
Beta coefficient (ln-transformed 2h-glucose (mmol/l))
E rs4251961
GenMets cases 0.00 [0.00 , 0.01]
GenMets controls 0.00 [0.00 , 0.01]
Whitehall II 0.00 [0.00 , 0.01]
GHS1 0.00 [-0.01 , 0.00]
GHS2 0.00 [0.00 , 0.01]
KORA 0.00 [-0.01 , 0.01]
FE Model 0.00 [0.00 , 0.00]
-0.04 -0.02 0.00 0.02 0.04
Beta coefficient (ln-transformed HbA1c(%))
F rs6759676
GenMets cases 0.00 [0.00 , 0.01]
GenMets controls 0.00 [-0.01 , 0.00]
Whitehall II 0.00 [0.00 , 0.01]
GHS1 0.00 [-0.01 , 0.00]
GHS2 0.00 [-0.01 , 0.01]
KORA 0.00 [-0.01 , 0.01]
FE Model 0.00 [0.00 , 0.00]
-0.04 -0.02 0.00 0.02 0.04
Beta coefficient (ln-transformed HbA1c(%))
G rs4251961
Whitehall II -0.02 [-0.06 , 0.02]
KORA 0.02 [-0.07 , 0.10]
FE Model -0.01 [-0.05 , 0.02]
-0.30 -0.15 0.00 0.15 0.30
Beta coefficient (ln-transformed 2h-insulin (uU/ml))
H rs6759676
Whitehall II -0.02 [-0.06 , 0.02]
KORA -0.04 [-0.12 , 0.05]
FE Model -0.03 [-0.06 , 0.01]
-0.20 -0.10 0.00 0.10 0.20
Beta coefficient (ln-transformed 2h-insulin (uU/ml))

Supplementary Figure 4. Association of rs4251961 and rs6759676 with circulating IL-1RA levels in individual discovery and replication studies while YFS is included in the replication analysis.

All analyses were adjusted for age, sex, BMI, waist-to-hip ratio and smoking.