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Genetic Determinants of Circulating Interleukin-1 Receptor Antagonist Levels and Their Association With Glycemic Traits

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The proinflammatory cytokine interleukin (IL)-1β is implicated in the development of insulin resistance and β-cell dysfunction, whereas higher circulating levels of IL-1 receptor antagonist (IL-1RA), an endogenous inhibitor of IL-1β, has been suggested to improve glycemia and β-cell function in patients with type 2 diabetes. 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 seven discovery and four replication cohort studies, two single nucleotide polymorphisms (SNPs) were independently associated with circulating IL-1RA concentration (rs4251961 at the *IL1RN* locus [n = 13,955, $P = 2.76 \times 10^{-21}$] and rs6759676, closest gene locus *IL1F10* [n = 13,994, $P = 1.73 \times 10^{-17}$]). 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 levels and lower HOMA insulin resistance. In conclusion, we show that circulating IL-1RA levels are predicted by two independent SNPs at the *IL1RN* and *IL1F10* loci and that genetically raised IL-1RA may be protective against the development of insulin resistance.

The balance between the potent proinflammatory 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 autoinflammatory 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 β-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 β-cell dysfunction on the other (7,8).

Observational studies show that IL-1RA concentrations are increased more than 1 decade before the diagnosis of T2D, and this is accentuated during the 6 years preceding the clinical onset of disease compared with nondiabetic control subjects (9–12). Therefore, IL-1RA shows similarities to cytokines from the transforming growth factor superfamily, including transforming growth factor-β1 and macrophage inhibitory cytokine-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 before the onset of T2D (16–18).

IL-1RA expression and secretion are regulated by proinflammatory cytokines, with IL-1β being the most prominent trigger. In addition, IL-1β expression is 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 these loci. Therefore, the aims of the current study were to *1*) identify novel genetic determinants of circulating IL-1RA in large population-based cohorts and *2*) 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 seven studies for the discovery analysis, totaling 9,285 individuals of European ancestry. Replication included four cohorts comprising 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

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 are available from four phases (phase 1: 1985–1988; phase 3: 1991–1993; phase 5: 1997–1999; and phase 7: 2003–2004). Blood samples for DNA analysis were collected in 2002–2004 from >6,000 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 8,816 individuals who had attended the phase 3 examination. We excluded participants with prevalent T2D at phase 3 (n = 42), missing follow-up data

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on diabetes (n = 552), missing data for key variables (C-reactive protein [CRP] [limited to subjects with CRP <10 mg/L], weight, waist circumference, cholesterol, triglycerides, fasting glucose, fasting insulin) at baseline (n = 2,018) or during follow-up (phases 5 and 7 [n = 3,049]), leading to a cohort of 2,810 subjects.

IL-1RA Measurements

IL-1RA serum concentrations were measured using the Quantikine ELISA Kit (R&D Systems, Wiesbaden, Germany). Mean intra- and interassay coefficients of variation (CVs) were 2.6% and 7.9%, respectively. The limit of detection (LOD) was 14 pg/mL. All samples yielded values above the LOD.

Genotyping and Quality Control

In 2010, 3,413 samples from the WHII study were genotyped using the Illumina MetaboChip (Illumina, San Diego, CA). A subset of these had also previously been genotyped using the Illumina HumanCVD BeadChip array. The combined data used in the current analysis comprised single nucleotide polymorphism (SNP) data for 3,178 Caucasian individuals genotyped on both platforms. After filtering the data for outliers (as identified by multidimensional scaling), cryptic relatedness, ambiguous sex, and sample and SNP call rates (<95%), genetic data for 236,426 SNPs in 3,102 individuals were available for analysis, 2,160 of whom had IL-1RA measurements.

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 concentration was measured with an in-house human insulin radioimmunoassay/ELISA (Dako Cytomation Ltd., Ely, U.K.). CRP level was measured with a high-sensitivity immunonephelometric assay in a BN ProSpec nephelometer (Dade Behring, Milton Keynes, U.K.). HbA_{1c} was measured at phase 7 on a calibrated high-performance liquid chromatography system. Analyzed samples for fasting glucose, 2-h glucose, fasting insulin, 2-h insulin, HOMA insulin resistance (HOMA-IR), HbA_{1c}, and CRP were n = 3,038, n = 3,036, n = 2,866, n = 3,022, n = 2,199, n = 3,040, and n = 2,963, respectively, for rs4251961 and n = 2,992, n = 2,990, n = 2,821, n = 2,976, n = 2,164, n = 2,994, and n = 2,917, respectively, for rs6759676.

National FINRISK Study

Study Population

FINRISK surveys are cross-sectional, population-based studies conducted every 5 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 and linked to national registers of cardiovascular and other health outcomes. Study participants were followed through 31 December 2010. The current study included eligible individuals from FINRISK surveys conducted in 1997 (FINRISK 1997) and 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 5,004 individuals from whom IL-1RA measurements and genotype data were available.

IL-1RA Measurements

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

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 (FINRISK 1997 and DILGOM genome-wide association study [GWAS], respectively; n = 1,146 available for the current study) were genotyped at the Sanger Institute (Cambridge, U.K.) with the Illumina Human610-Quad BeadChip. This set of samples was imputed to the reference panel B36rel22 using MACH v.1.0.16 software. In the imputation, filters of <95% for call rate, <1% for minor allele frequency (MAF), and $<10^{-6}$ for Hardy-Weinberg equilibrium (HWE) P value were used. A subset (n = 3,858 available for the current study) of FINRISK 2007 individuals (DILGOM metabo) was genotyped using the Illumina Cardio-MetaboChip. To control the data quality, sex mismatch and relatedness checks were performed, and any observed discrepancies were removed from both data sets. For the current analysis, the phenotype data were filtered for outliers. In the analysis, thresholds of <95% call rate, $<10^{-6}$ for HWE P value, <1% for MAF, maximum 10% for missingness per SNP, and maximum 10% for missingness per individual were applied for the genotyped data.

Measurement of Metabolic and Immunological Traits

Glucose levels in FINRISK 1997 were measured from serum samples (fasting duration at least 4 h) by glucose hexokinase method (detection range 0.6–44 mmol/L) using an AU400 chemistry analyzer (Olympus, Tokyo, Japan). Insulin levels were determined from serum samples by chemiluminescent microparticle immunoassay (CMIA) (ARCHITECT i2000; Abbott Laboratories, Lake Forest, IL) with an intra-assay CV of 3.05% and an interassay CV of 3.31%. CRP concentration was determined from serum samples by latex immunoassay CRP16 (ARCHITECT c8000, Abbott Laboratories). The

intra-assay and interassay CVs were 0.83% and 0.93%, respectively.

In the FINRISK 2007/DILGOM survey, glucose was measured from fasting plasma samples by glucose hexokinase method (reference range 2.00–20.00, LOD 0.14 mmol/L) using the ARCHITECT ci8200 (Abbott Laboratories). Insulin concentration was determined from fasting serum samples by CMIA (ARCHITECT ci8200), with mean interassay CVs of 3.4%. Serum CRP concentration was determined by immunoturbidimetric method (MULTIGENT CRP Vario; Abbott Laboratories) using the ARCHITECT ci8200. Mean interassay 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 = 4,396, n = 4,412, n = 4,385, and n = 4,451, respectively, for rs4251961 and n = 4,395, n = 4,411, n = 4,384, and n = 4,450, respectively, for rs6759676.

Gene Expression Analysis

From the individuals of the DILGOM GWAS sample, the whole-blood RNA was obtained and hybridized onto Illumina HumanHT-12 BeadChips. In the current study, expression data from 507 individuals were analyzed.

Health 2000

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 from the population aged ≥18 years. The survey included an interview about medical history, health-related lifestyle habits, and a clinical examination (for individuals ≥30 years of age) at which a blood sample was drawn. Study participants were followed through 31 December 2010 and restricted to age ≤80 years at baseline. In the current study, we used the GenMets sample, which includes individuals with metabolic syndrome and matched control subjects drawn from the Health 2000 study. A total sample size of 2,010 individuals from whom IL-1RA measurements and genotype data were available was used.

IL-1RA Measurements

IL-1RA was determined from nonfasting serum using ELISA (R&D Systems, Minneapolis, MN). The intra- and interassay CVs were 3.59% and 5.68%, respectively.

Genotyping and Quality Control

A total of 2,173 individuals from the GenMets cohort have been genotyped with the Illumina Human610-Quad BeadChip at the Sanger Institute. 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 MACH v.1.0.16 software. In the imputation, filters of <95% for call rate, <1% for MAF, and $<10^{-6}$ for HWE P value were used. For the current analysis, the phenotype data were filtered for outliers. Thresholds of 95% call rate and 10^{-6} for the HWE P value for an individual SNP were used.

Measurement of Metabolic and Immunological Traits

Glucose levels were measured from serum samples (fasting duration at least 4 h) by glucose hexokinase method with interassay CVs of 2.1% and 2.3% for mean concentrations of 9.3 and 5.2 mmol/L, respectively, using an AU400 chemistry analyzer (Olympus). Insulin levels were determined from nonfasting serum samples by an automated microparticle enzyme immunosorbent assay on an Abbot IMx analyzer (Abbott Laboratories). Interassay CVs were 4.6% and 4.0% for mean concentrations of 118.7 and 1032.7 pmol/L, respectively. HbA_{1c} was determined by immunoturbidimetric method using the ARCHITECT ci8200. The CVs were 1.8% for an HbA_{1c} of 5.1% and 2.0% for an HbA_{1c} of 10.8%. CRP concentration was determined from serum using latex immunoassay CRP16 (ARCHITECT c8000). The intra- and interassay CVs were 0.83% and 0.93%, respectively. Analyzed samples for fasting glucose, fasting insulin, HOMA-IR, HbA_{1c}, and CRP were n = 2,127, n = 2,071, n = 2,070, n = 2,102, and n = 1,878, respectively, for rs4251961 and n = 2,126, n = 2,070, n = 2,069, n = 2,101, and n = 1,877, respectively, for rs6759676.

Myocardial Infarction Genetics Consortium Study

Study Population

A total of 341 individuals from the National FINRISK Study were sampled into a specific pair-matched case-control sample of myocardial events (Myocardial Infarction Genetics Consortium [MIGen]). In this cohort, the individuals whose main diagnosis or cause of death can be specified with 410 (ICD-9), I21 (ICD-10) and I22 (ICD-10) 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.

IL-1RA Measurements

IL-1RA was measured as described previously for FINRISK 1997.

Genotyping and Quality Control

Individuals from the MIGen sample were genotyped with the Affymetrix 6.0 platform at 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 were imputed to the reference panel HapMap II using MACH v1.0.16 software. In the imputation, filters of <95% for call rate, <1% for MAF, and $<10^{-6}$ for HWE *P* value were used. For the current analysis, the phenotype data were filtered for outliers.

Measurement of Metabolic and Immunological Traits

All traits were measured as described previously 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).

Cooperative Health Research in the Region of Augsburg F4 Study

Study Population

The Cooperative Health Research in the Region of Augsburg (KORA) F4 study (2006–2008) is a follow-up survey of the population-based KORA S4 study (1999–2001). A total sample of 6,640 men and women aged 25–74 years was drawn from the target population in the city of Augsburg (Germany) and two adjacent counties. Of all 4,261 participants from the KORA S4 study, 3,080 also participated in the KORA F4. Genotype data were available for a subset of 1,814 individuals aged 32–81 years at the time of the follow-up.

IL-1RA Measurements

Serum IL-1RA was determined using the Quantikine ELISA kit with intra- and interassay CVs of 2.8% and 7.0%, respectively. Data from 718 individuals aged 61–82 years were available for the current analysis.

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 the 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 IMPUTE v.0.4.2 software based on HapMap II.

Measurement of Metabolic and Immunological Traits

Blood was collected without stasis and kept at 4°C until centrifugation. All blood parameters, except for 2-h glucose and 2-h insulin, were based on fasting blood samples. Serum samples were stored at −80°C until assayed. Serum glucose levels were assessed by hexokinase method (GLU Flex; Dade Behring). Serum insulin was determined by ELISA (Invitrogen, Karlsruhe, Germany). HbA_{1c} was measured using high-performance liquid chromatography. 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-h glucose, fasting insulin, 2-h insulin, HOMA-IR, HbA_{1c}, and CRP were n = 1,779, n = 1,598, n = 1,776, n = 713, n = 1,777, n = 723, and n = 1,777, respectively for both SNPs (rs4251961 and rs6759676).

Gene Expression Analysis

Total RNA was extracted from fasting whole-blood samples taken between 8:00 A.M. and 11:00 A.M. RNA was reverse transcribed and biotin-UTP-labeled into complementary RNA using the Illumina TotalPrep-96 RNA Amplification Kit (Ambion, Darmstadt, Germany). Gene expression levels were determined using the Illumina HumanHT-12 v3 Expression BeadChip. Expression data were $\log_2$-transformed and normalized by quantile normalization. Data from 718 individuals aged 61–82 years were available for the current analysis.

Gutenberg Health Study

Study Population

The Gutenberg Health Study (GHS) is 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 one to one 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 4,158 individuals.

IL-1RA Measurements

IL-1RA was determined by ELISA (R&D Systems). The inter- and intra-assay CVs were 3.59% and 5.68%, respectively. Data were available for the current analysis from 2,984 and 1,174 individuals from GHS I and GHS II, respectively.

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 = 3,500] and cohort GHS II [n = 1,500]), the two cohorts were analyzed separately. Individuals with a call rate <97% or an autosomal heterozygosity >3 SDs around the mean were excluded. After applying

standard quality criteria (MAF 1%, genotype call rate 98%, and *P* value of deviation from HWE), 662,405 SNPs in 2,996 subjects (GHS I) and 673,914 SNPs in 1,179 subjects (GHS II) remained for analysis. Imputations based on HapMap II release 24 were performed separately in GHS I and GHS II using IMPUTE v.2.1.0 software. In total, 2,588,505 (GHS I) and 2,586,553 (GHS II) SNPs with a MAF ≥1% were available for genetic analyses.

Measurement of Metabolic and Immunological Traits

Blood sampling was carried out under fasting conditions while lying supine. Glucose, insulin, HbA_{1c}, and CRP levels were measured immediately after blood withdrawal by routine laboratory measurements. In GHS I and GHS II, n = 813 and n = 1,308 individuals, respectively, had a CRP level of <1.0 mg/L, which was the LOD. These values were set to 0.5 mg/L (LOD / 2). Sample sizes for the analysis of fasting glucose, HbA_{1c}, and CRP concentration were n = 2,183, n = 2,969, and n = 2,983, respectively, in GHS I and n = 880, n = 1,179, and n = 1,179, respectively, in GHS II.

Gene Expression Analysis

Gene expression analysis was performed with the Illumina HumanHT-12 v3 BeadChip using total monocytic RNA. The integrity of the total RNA was assessed with an Agilent Bioanalyzer 2100 (Agilent Technologies, Böblingen, Germany). Reverse transcription and complementary RNA synthesis were performed using the Illumina TotalPrep-96 RNA Amplification Kit. Data from 1,133 individuals were available for the current analysis.

Young Finns Study

Study Population

The Cardiovascular Risk in Young Finns Study (YFS) 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 3,596 Caucasian subjects aged 3–18 years participated in the study. In adulthood, the latest 27-year follow-up study was conducted in 2007 in 2,204 participants aged 30–45 years. For 1,998 individuals who had participated in the study in 2007, genotype data and IL-1RA measurements were available for the current analysis.

IL-1RA Measurements

A magnetic bead–based multiplex assay was used to determine the concentration of IL-1RA in blood. Twenty microliter aliquots of serum samples (stored at −70°C and never thawed before) from 2,200 persons were analyzed using Bio-Plex Pro assays (27-plex kit, including IL-1RA). Intra- and interassay CVs were 9.62% and 10.86%, respectively. The lower LOD was 10.85 pg/mL.

Genotyping and Quality Control

Genotyping of YFS samples was performed at the Sanger Institute using the custom-built Illumina BeadChip Human670K array. Genotypes were called by using Illumina's clustering algorithm. The 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 $P = 10^{-6}$. In addition, sex mismatch and relatedness checks were performed and any observed discrepancies removed. YFS has been imputed to the HapMap II reference panel using MACH v.1.0 software (http://www.sph.umich.edu/csg/abecasis/MACH). For the current analysis, the phenotype data were filtered for outliers. Thresholds of 95% call rate and 10^{-6} for HWE *P* value for an individual SNP were used.

Measurement of Metabolic and Immunological Traits

Fasting serum glucose concentration was determined by the enzymatic hexokinase method (glucose reagent, AU400 chemistry analyzer, Olympus). Fasting serum insulin concentration was determined by a microparticle enzyme immunoassay (IMx insulin reagent) on an IMx instrument (Abbott). Serum CRP concentration was analyzed using an AU400 chemistry analyzer (Olympus) and a highly sensitive turbidimetric immunoassay kit (CRP-UL-assay, Wako Chemicals, Neuss, Germany). The LOD was 0.02 mg/L. Interassay 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 concentrations were n = 1,951, n = 1,946, n = 1,938, and n = 1,952, respectively, for rs4251961 and n = 1,950, n = 1,945, n = 1,937, and n = 1,951, 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-to-hip ratio, and 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. Because five of the seven data sets used genome-wide platforms, we adopted discovery a *P* value threshold of $<5.0 \times 10^{-8}$ in keeping with that generally used in GWASs. Although five of the studies also used imputed data, the genome-wide significance level of $P < 5.0 \times 10^{-8}$ for the number of independent tests was still applicable because 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 a 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 a 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. Because study heterogeneity was observed, the summary estimates obtained from all replication studies underwent meta-analysis using a random-effects model. We excluded one 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 ELISA as used in the other studies).

The proportion of the variance in IL-1RA explained by rs4251961 and rs6759676 was calculated in the independent population cohort FINRISK 1997 (no sample overlap with the discovery cohorts). More recent genotype data in the FINRISK 1997 cohort (core-exome chip from Illumina) was imputed with the 1000 Genomes March 2012 release using IMPUTE software. Imputation information was 0.97 and 0.997 for rs4251961 and rs6759676, respectively. The proportion of variance explained by the two SNPs together was tested with residuals from age-, sex-, BMI-, waist-to-hip ratio–, and smoking-adjusted ln-transformed IL-1RA levels using the following linear model: residuals $\sim$ rs4251961 + rs6759676.

Association With Metabolic Traits

To determine whether 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-h glucose and insulin, HbA_{1c}, 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-to-hip ratio, and smoking (current vs. never/ex-smokers) as well as ancestry principal components and field center as needed. Summary estimates obtained from all studies underwent meta-analysis using a fixed-effects 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 was located in any of the ENCODE (Encyclopedia of DNA Elements) 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 GHS) 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, waist-to-hip ratio, and smoking when the data were available. Technical variables were also used for adjustment in KORA and GHS, as described previously (32).

Analysis of Publicly Available Expression Quantitative Trait Loci Data

We used 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 MuTHER (Multiple Tissue Human Expression Resource) (33). In this study, expression profiling of the samples was performed using Illumina HumanHT-12 v3 BeadChips, whereas genotyping was performed with a combination of Illumina HumanHap300, HumanHap610Q, 1M-Duo, and 1.2M-Duo 1M chips (33). We queried the data set 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-to-hip ratio, and smoking) (Fig. 1*A* and 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 SNP rs4251961 and rs6759676 as covariates, no additional SNPs remained significant at the discovery *P* value threshold. Therefore, these two SNPs were considered the only robust, independent signals in the chromosome 2 region and were taken forward for replication (Fig. 1*B* and *C*). 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 1.

In Silico Analysis of rs6759676 and rs4251961

The rs6759676 SNP is in strong LD with rs6761276 (R^2 = 0.9 based on the 1000 Genomes CEU population [Utah residents with Northern and Western European ancestry]). The latter is a nonsynonymous coding SNP

Table 1—Cohort characteristics

	Discovery cohorts							Replication cohorts			
	WHII	FR1997	DILGOM (FR07) GWAS	DILGOM (FR07) Metabo-Chip	Health 2000 GenMets cases	Health 2000 GenMets controls	FIN MIGen	KORA	GHS I	GHS II	YFS
n	2,160	468	678	3,858	979	1,031	111	719	2,984	1,174	1,998
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)
Male sex (%)	77.1	47.6	48.8	45.9	50.5	47.1	62.2	48.65	51.4	50.1	45.2
BMI (kg/m^2)	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)	28.1 (4.8)	27.1 (4.7)	27.3 (5.0)	26.0 (4.8)
WHR	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	14.5	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-h 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-h 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)
HbA_{1c} (%)	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
HbA_{1c} (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	Metabo-Chip 50k cardiochip	Illumina Human 610-Quad BeadChip	Illumina Human 610-Quad BeadChip	Metabo-Chip	Illumina Human 610-Quad BeadChip	Illumina Human 610-Quad BeadChip	Affymetrix 6.0 platform	Affymetrix Human SNP Array 6.0	Affymetrix Human SNP Array 6.0	Affymetrix Human SNP Array 6.0	Illumina Human 670K BeadChip

Data are mean (SD) or %. 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 RESEARCH DESIGN AND METHODS. FR07, FINRISK 2007; FR1997, FINRISK 1997; NA, not available; WHR, waist-to-hip ratio.

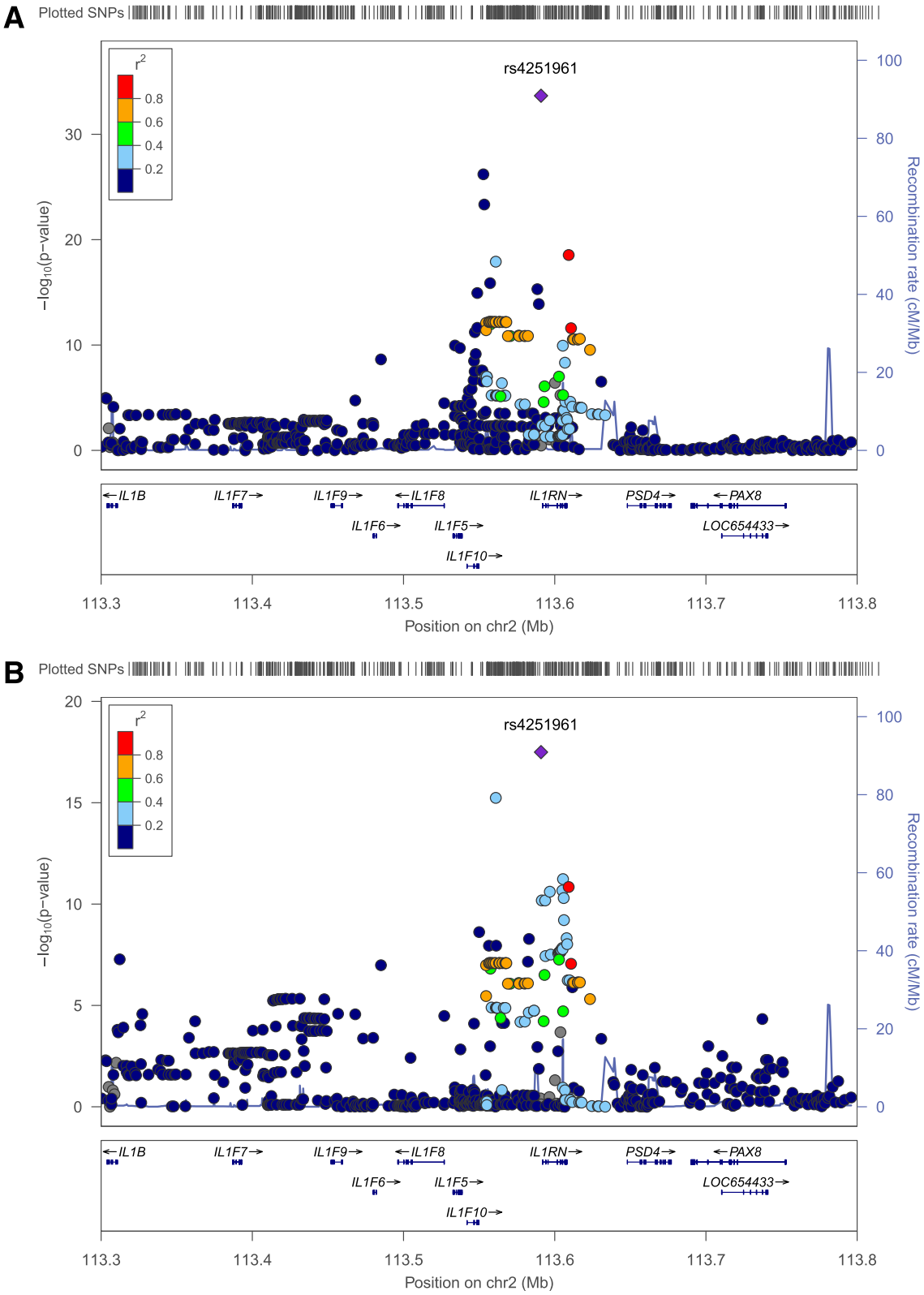

Figure 1—Association between rs4251961 and rs6759676 and IL-1RA in the discovery analysis. Shown are genome-wide association *P* values for all variants that were tested in the IL-1RA association analysis and located in the *IL1RN* gene cluster on the chromosome 2 (chr2). *A*: SNP rs4251961 allele shows the strongest association with IL-1RA in the discovery analysis. *B*: SNP rs4251961 remains an independent hit when conditioning on rs6759676. *C*: SNP rs6759676 remains an independent hit when conditioning on rs4251961.

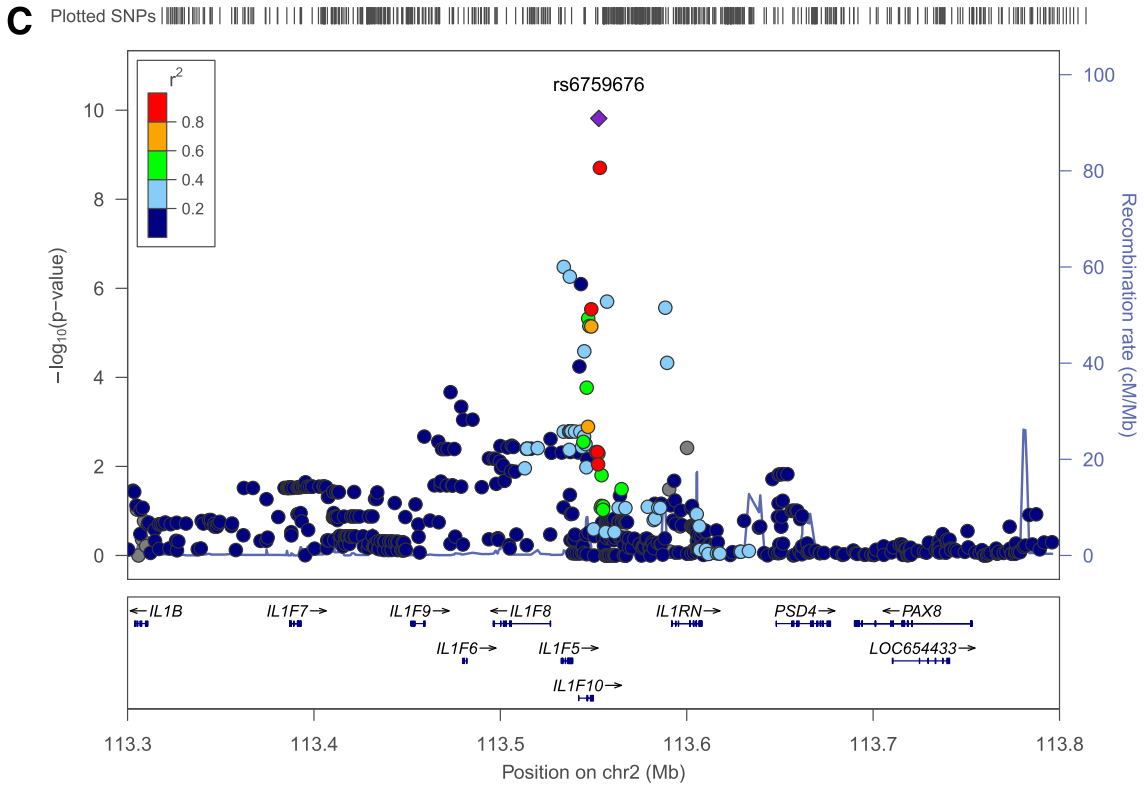

Figure 1—*Continued*.

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 within STAT transcription factor binding sites (Supplementary Fig. 1). This suggests that the rs6759676 SNP could influence the 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 Fig. 2). It is in 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 Fig. 2).

Association Between Significant SNPs and 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 and Fig. 2*A* and *B*). The IL-1RA–decreasing alleles were also significantly associated with higher CRP levels for both SNPs (Fig. 2*C* and *D*). These analyses were adjusted for age, sex, BMI, waist-to-hip ratio, and smoking.

Although no associations were found between rs4251961 and fasting glucose, 2-h glucose, fasting insulin, 2-h 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) (Fig. 2*E*–H). These associations were nominally significant but became nonsignificant after Bonferroni adjustment for multiple testing.

Association Between Significant SNPs and Expression of IL-1 Family Genes Within/Near the IL-1RN Locus

Associations between rs4251961 and 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 GHS I (n = 1,133) 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 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-to-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 waist-to-hip ratio showed no association with IL-1RA mRNA expression level (*IL1RN*) after Bonferroni correction for multiple testing, leading to a significance level of $P = 4.6 \times 10^{-4}$. When testing the association of the current study 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 ($P = 2.4 \times 10^{-4}$). Moreover, rs6759676 was significantly associated ($P = 9.8 \times 10^{-6}$) 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 of 1% to identify *cis*-eQTLs corresponding to $P < 5.0 \times 10^{-5}$ in adipose tissue and a GWAS threshold of $P < 5 \times 10^{-8}$ for *trans*-eQTLs. Although rs6759676 showed a 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 two SNPs.

DISCUSSION

This study presents four key findings regarding genetic determinants of circulating IL-1RA concentration and their associations with gene expression levels and 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 the 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 associated with elevated IL-1RA were associated with lower circulating CRP concentration. Finally, the IL-1RA–raising and CRP-lowering allele of rs6759676 was also associated with lower fasting insulin concentrations and lower HOMA-IR.

Genetic Determinants of Circulating IL-1RA

Previous studies reported significant associations between several SNPs within or near the *IL1RN* locus and circulating IL-1RA (24,29), but it was not clear whether these represented independent associations. The current study shows for the first time in our knowledge 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).

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

Cohort and marker	Chr	Position (HG18)	Gene	Minor (coded) allele	Major allele	Mean MAF	*n*	Effect	SE	*P* value	I^2 (%)	Meta-analysis model
Discovery												
rs4251961	2	113590938	*IL1RN*	C	T	0.32	9,092	−0.078	0.006	2.19E-34	39.8	FE
rs6759676	2	113552819	Closest *IL1F10*	C	T	0.44	9,131	0.063	0.006	6.12E-27	0.0	FE
Replication												
rs4251961	2	113590938	*IL1RN*	C	T	0.32	4,863	−0.086	0.023	2.00E-04	78.90	RE
rs6759676	2	113552819	Closest *IL1F10*	C	T	0.44	4,863	0.099	0.019	3.54E-07	71.91	RE
Combined												
rs4251961	2	113590938	*IL1RN*	C	T	0.32	13,955	−0.082	0.009	2.76E-21	53.84	RE
rs6759676	2	113552819	Closest *IL1F10*	C	T	0.44	13,994	0.075	0.009	1.73E-17	60.45	RE

Chr, chromosome; FE, fixed effects; HG, human genome; RE, random effects.

Table 3—Association of rs4251961and rs6759676 with quantitative immunological and glycemic traits

SNP and trait*	β	SE	*P* value	Number of studies	*n*	I^2 (%)
rs4251961						
IL-1RA (pg/mL)	-0.082	0.009	2.76e-21	10	13,955	53.84
CRP (mg/L)	0.0601	0.0108	2.95e-08	11	17,797	0.79
Fasting glucose (mmol/L)	0.0005	0.0012	0.6960	11	16,822	0.0
2-h glucose (mmol/L)	−0.0068	0.0060	0.2637	2	4,634	0.0
HbA_{1c} (%)	0.0011	0.0013	0.3964	6	10,013	0.0
Fasting insulin (μU/mL)	0.0099	0.0067	0.1390	9	13,664	0.0
2-h insulin (μU/mL)	−0.0129	0.0186	0.4881	2	3,735	0.0
HOMA-IR	0.0071	0.0068	0.2992	9	12,821	0.0
rs6759676						
IL-1RA (pg/mL)	0.075	0.009	1.73E-17	10	13,994	60.45
CRP (mg/L)	−0.0284	0.0103	0.0055	11	17,747	0.0
Fasting glucose (mmol/L)	−0.0012	0.0011	0.2603	11	16,773	12.5
2-h glucose (mmol/L)	0.0039	0.0060	0.5140	2	4,588	0.0
HbA_{1c} (%)	−0.0007	0.0012	0.5295	6	9,966	0.0
Fasting insulin (μU/mL)	−0.0159	0.0061	0.0096	9	13,616	20.2
2-h insulin (μU/mL)	−0.0271	0.0182	0.1381	2	3,689	0.0
HOMA-IR	−0.0173	0.0063	0.0063	9	12,783	0.0

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

Most published SNPs reported to be associated with IL-1RA are in LD with rs4251961 (r^2 = 0.4–0.7 as 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 that 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, the current observations were independent of indices of obesity because the analyses were adjusted for BMI and waist-to-hip ratio.

Associations Between rs4251961 and rs6759676 and Gene Expression

The most probable mechanism linking both SNPs with circulating IL-1RA levels would be the regulation of *IL1RN* transcription. Accordingly, the present in silico analysis suggests 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 the directly observed analyses of whole blood or monocytes because 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 through 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, the results are consistent with the regulation of circulating IL-1RA by posttranscriptional 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 and rs6759676 and CRP 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 this study. As for circulating IL-1RA, the 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 levels 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. Of note, the current observation that IL-1RA–raising alleles of both SNPs were associated with lower circulating CRP

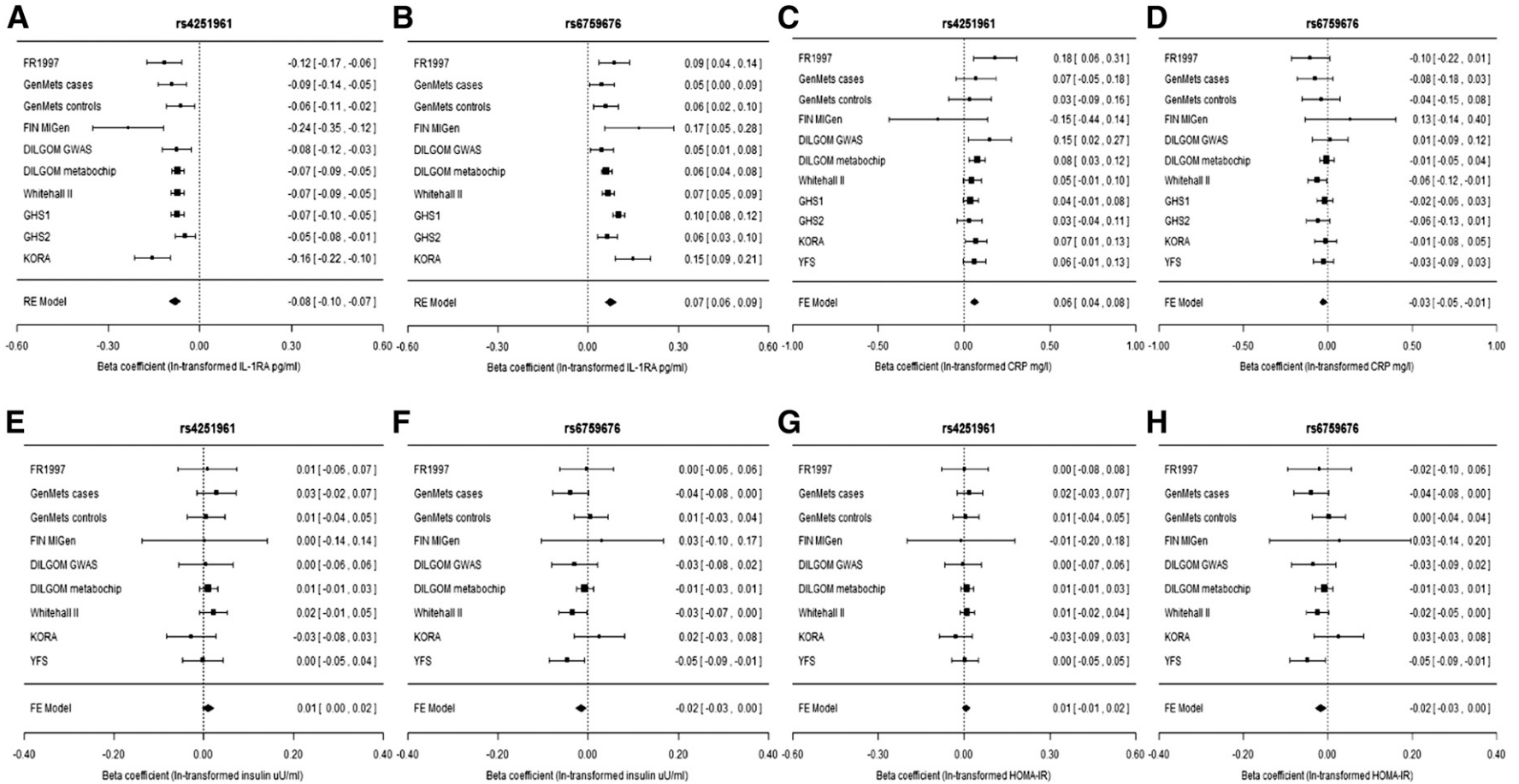

Figure 2—Association of rs4251961 and rs6759676 with circulating IL-1RA, CRP, insulin levels, and HOMA-IR in individual studies included in the discovery and replication analysis. *A* and *B*: IL-1RA. *C* and *D*: CRP. *E* and *F*: Insulin. *G* and *H*: HOMA-IR. All analyses were adjusted for age, sex, BMI, waist-to-hip ratio, and smoking. FE, fixed effects; FR1997, FINRISK 1997; RE, random effects.

Table 4—Associations between rs4251961 and 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	β	SE	*P* value
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, waist-to-hip ratio, smoking, and technical variables. The first column shows the Illumina transcript identifications. Alternative nomenclature: *IL36RN* = *IL1F5*, *IL36A* = *IL1F6*, *IL37* = *IL1F7*, *IL36B* = *IL1F8*, and *IL36G* = *IL1F9*. Boldface indicates nominally significant associations.

levels is in line with the aforementioned randomized clinical trial in which treatment with recombinant IL-1RA not only improved glycemic control and β-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 previously reported an association of rs4251961 with IL-6 (38). This is of interest because Mendelian randomization analyses suggested that IL-6 signaling is causally associated with cardiovascular disease (39).

Associations Between rs4251961 and 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 within/near the *IL1RN* locus and parameters of glucose metabolism have been investigated before. However, for fasting glucose and insulin studies smaller than the current meta-analysis, no significant associations were reported between rs4251961 and six other SNPs not in LD with either of the strongest signals we found (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 FINRISK 1997 (12).

The current study represents the largest to date investigating 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, indicating that this SNP is associated with higher insulin sensitivity. However, we found this association only for rs6759676, as it was not statistically significant for rs4251961, although the associations between both SNPs and circulating IL-1RA were comparable. To explain this difference, gene expression data from other insulin-responsive tissues would be desirable to investigate whether both SNPs act mainly through the regulation of IL-1RA levels or whether one or even both also have pleiotropic effects by regulating other IL-1 family members that could represent mediators of the relationship between genetic variation and immunological and metabolic effects.

We found that the genetic data mirror those from a clinical trial showing that daily subcutaneous injections of recombinant IL-1RA (which raised circulating IL-1RA levels) reduced HbA_{1c} levels in patients with T2D (6), although it was not clear to what extent IL-1RA acted on insulin sensitivity or β-cell function. The current findings are biologically plausible because the only known function of IL-1RA in humans is to block IL-1β–mediated signaling. Of note, this anti-inflammatory effect has pleiotropic metabolic consequences because IL-1β interferes with insulin signaling in adipocytes and hepatocytes and suppresses insulin-induced glucose uptake, inhibits lipogenesis, and decreases the release of adiponectin from adipocytes (40–42).

The findings appear not to agree with previous observational data from the WHII study and Finnish cohort studies, which suggested that an upregulation of IL-1RA in the circulation is 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.

The current study 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, the data must be interpreted with caution because we observed significant effects for only one of the two IL-1RA–related SNPs, and the findings were only nominally significant.

Strengths and Limitations

This 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, the 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 only had data for HOMA-IR as a surrogate measure of insulin resistance, and dynamic measures of β-cell function were not available for this analysis. Finally, we observed differences between studies with IL-1RA measured by ELISA, and one study 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 various laboratory methods for measuring IL-1RA to further elucidate the underlying reasons for this observation.

Conclusions

We identify one novel genetic determinant of circulating IL-1RA levels in the *IL1F10* locus that 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, the data are in line with modest therapeutic benefits reported for novel IL-1β–targeting treatment strategies.

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