SUPPLEMENTAL METHODS

CARDIA study participants

The Coronary Artery Risk Development in Young Adults (CARDIA) Study is a prospective cohort study of the development of cardiovascular risk factors in young adults [1]. Beginning in 1985, 5,115 participants aged 18–30 years were recruited from four clinical sites located in Birmingham, Alabama; Chicago, Illinois; Minneapolis, Minnesota; and Oakland, California. The initial CARDIA study population was approximately balanced with respect to age, sex, and ethnicity. Participants were contacted every 6 months and re-examined at six follow-up examinations (year 2 in 1987-88, year 5 in 1990-91, year 7 in 1992-93, year 10 in 1995-96, year 15 in 2000-01, and year 20 in 2005-06). Overall retention rates among surviving participants were 91% at year 2, 86% at year 5, 81% at year 7, 79% at year 10, and 74% at year 15, and 72% at year 20. For the current study, eligible participants included 1,932 self-identified white individuals who consented to isolation of genomic DNA at year 10 and for whom adequate DNA samples was available (92% of those examined at year 10). CRP and fibrinogen concentration were measured at University of Vermont using stored plasma participants attending the year 7 and year 5 exams by immunonephelometry (BNII Nephelometer 100 Analyzer; Dade Behring, Deerfield, IL, USA) and the Clauss clotting rate assay [2], respectively. We further excluded from all analyses 165 participants with >25% missing genotypes and 140 with missing data for CRP or fibrinogen, resulting in a final sample of n=1,627.

Candidate gene and SNP selection and genotyping in CARDIA

We selected 16 genes to screen for association with plasma CRP levels in the CARDIA sample. Using genomic re-sequencing data from the SeattleSNPs Program for Genomics Applications (http://pga.mbt.washington.edu/), we identified a set of 121 SNPs that efficiently tag common

patterns of nucleotide variation across the 16 inflammation-related genes. TagSNPs were selected using the LDselect algorithm of Carlson et al [3] such that all known common variants (defined as MAF ≥5%) among European-Americans are correlated with a tagSNP with r^2 of >0.64. The candidate genes and number of tagSNPs per locus considered were *IFNG*, 4; *IL10*, 5; *IL1B*, 10; *IL1RN*, 15; *IL4*, 9; *IL6*, 8; *IL8*, 2; *LTA*, 6; *LTB*, 1; *NFKBIA*, 5; *PPARA*, 14; *RELA*, 5; *STAT3,* 10; *TNF*, 2; *TNFRSF1A*, 5; *TNFRSF1B*, 20. TagSNP genotyping was performed for *CEBPB*, *IFNG*, *IL10*, *IL1RN*, *IL4, IL8*, *NFKBIA*, *PPARA*, *RELA*, *STAT3, TNFRSF1A*, and *TNFRSF1B*, using the Illumina GoldenGate platform. *IL1B*, *IL6*, *LTA*, *LTB*, and *TNF* genotyping was performed on a Taqman system using Assays By Design on an ABI 7900 real time thermal cycler under standard conditions (TaqMan® SNP Genotyping Assays Protocol, Rev. B, Part #4332856b, Applied Biosystems, Foster City, CA). The SeattleSNPs nomenclature, *rs* numbers, genotype and minor allele frequencies, missing rates, and Hardy-Weinberg p-values for the 121 SNPs assayed are shown in **Supplemental Table 1**. All 121 SNPs had Hardy Weinberg equilibrium p-values >0.001. The median missing data rate per SNP was 0.3% (range 0% to 36%). Blind duplicate concordance rates were >99%.

CLEAR Study

The Carotid Lesion Epidemiology and Risk (CLEAR) study is a Seattle-based case-control study of severe carotid artery disease (CAAD). Study participants were recruited from the University of Washington, Virginia Mason Medical Center, and Veterans Affairs Puget Sound Health Care System and were predominantly EA men ranging in age from 37 to 89 years [4,5]. The number of non-Caucasians was insufficient for ethnically stratified analysis, so those samples were excluded. Other exclusion criteria included autosomal dominant familial hypercholesterolemia or coagulopathy. The CAAD cases (N=499) had ≥80% internal carotid artery stenosis, unilaterally

or bilaterally, or had undergone a carotid endarterectomy for symptomatic disease. Controls (N=646) had no known vascular disease and subsequently were shown to have less than 15% internal carotid artery stenosis, bilaterally, by duplex ultrasound. The remaining affected subjects (N = 63) had intermediate internal carotid stenosis levels, between 50% and 79% stenosis by ultrasound. Plasma CRP (n=1,208) and fibrinogen levels (n=857) were measured by immunonephelometry (BNII Nephelometer 100 Analyzer; Dade Behring) and Clauss clotting rate assay [2], respectively, at the Northwest Lipid Metabolism and Diabetes Research Laboratories (Seattle, WA). Genotyping was performed using the Illumina GoldenGate platform at the University of Washington Department of Genome Sciences.

CHS cohort

The Cardiovascular Health Study (CHS) is a prospective population-based cohort study of older adult men and women recruited from four U.S. field centers: Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Pittsburgh, Pennsylvania [6]. Those eligible to participate included all persons 65 years of age or older living in the household of each individual sampled. The original cohort (n=5,201) was recruited from 1989 to 1990. A second minority cohort (n= 687) was recruited between 1992 and 1993. Of the total CHS cohort (n=5,888), 4,925 participants identified themselves as EA, 924 as AA, and 39 as "other." The CHS baseline evaluation included demographic, lifestyle and medical histories, physical examination, and fasting blood collection. Baseline CRP was measured using a high-sensitivity ELISA [7]. Baseline plasma fibrinogen levels were measured using the Clauss assay [2]. Baseline IL-6 was measured from stored serum samples using a high sensitivity ELISA (Quantikine HS Human IL-6 Immunoassay; RD Systems, Minneapolis, MN. Participants for whom DNA was not collected, who did not consent to the use of their DNA, or who reported

race as "other" were excluded, along with those for missing plasma biomarker data. In CHS, the *IL1RN* tagSNPs were typed using the Illumina GoldenGate platform by the Center for Inherited Disease Research (CIDR).

Bayesian regression and imputation analysis

As an additional way of pooling data across studies, we used the "Bayesian IMputation-Based Association Mapping" or BIMBAM method of Servin and Stephens [8] to combine typed SNP genotype and inflammation phenotype data from CARDIA, CLEAR, and CHS with information on multi-marker linkage disequilibrium (LD) from the SeattleSNPs re-sequencing panel to assess the strength of evidence for phenotypic association with the 7 "typed" and 149 "untyped" SNPs located within or near the *IL1RN* gene. BIMBAM quantifies the strength of evidence for association by computing Bayes Factors (BFs) for each typed and untyped SNP. Quantile normalized mean covariate-adjusted CRP values were used as the quantitative phenotype for the BIMBAM analysis. BFs were computed under the linear model

$$Y_i = \mu + aX_i + dI(X_i = 1) + \varepsilon_i$$

where Y_i denotes the CRP phenotype for individual i, X_i denotes the genotype for individual i (coded as 0, 1 or 2), a denotes the additive effect, d denotes the dominance effect, and ε_i denotes an error term. The BFs were computed using the prior D2,[7] averaging over $\sigma_a = 0.05$, 0.1, 0.2, 0.4 and $\sigma_d = \sigma_a/4$. Single-SNP p values were obtained from these BFs by permutation [8]. In the combined imputation analysis, we computed single-SNP BF and permutation p-values for each untyped SNP using the posterior mean genotype as the estimated genotype [8].

Analysis of *IL1RN* gene expression and IL-1RA protein production in whole blood *ex vivo*

Non-smoking, European-American individuals between the ages of 18-65 years of age were recruited from the metropolitan Seattle area (n=285). The study was approved by the Division of Human Subjects Research, University of Washington. Exclusions to enrollment included recent antibiotic use, symptoms consistent with infection, a history of auto-immune disease, immunodeficiency, use of immunosuppressive medications, cancer, or pregnancy.

Measurement of whole blood IL-1RA levels

Blood was drawn from fasting subjects before 10:00 AM and was anti-coagulated with pyrogen-free citrate (0.1 M, pH 7.2), mixed 1:1 with RPMI 1640 and incubated at 37^{o}C with media alone or the innate immune stimulus *S. aureus* peptidoglycan (PGN) (100 μg/mL) (Fluka/Sigma-Aldrich, Saint Louis, MS). After 4 hours of incubation an aliquot of the blood:RPMI mixture was removed placed in Nucleic Acid Purification Lysis Solution (Applied Biosystems, Foster City, CA) for RNA purification. After 6 hours of incubation, supernatants were isolated by centrifugation. IL-1RA levels in the supernatants were determined using an immunoassay (R&D Systems, Minneapolis, MN) quantitated on a cytometric bead-based system (Luminex, Austin, TX) as previously described [9,10]. Total and differential leukocyte counts were performed on each blood sample in the Harborview Medical Center clinical laboratory. The IL-1RA levels for each subject were normalized to the neutrophil count measured in that subject.

IL1RN genotyping

IL1RN genotyping was performed on genomic DNA from the healthy volunteers using a BeadStation Laboratory System (Illumina Inc., San Diego, CA) paramagnetic microbead array as previously described [11]. Genotypes were called using the Illumina GenCall software package. We replicated 2% of the samples for quality control.

RNA purification and IL1RN mRNA quantitation

Total RNA was purified from PGN-stimulated whole blood samples obtained from subjects with known *IL1RN*$_{C1018T}$ genotypes (CC, n=19; CT, n=19; TT, n=19) using an ABI Prism 6100 Nucleic Acid PrepStation (Applied Biosystems, Foster City, CA) per the manufacturer's protocol. IL1RN mRNA was determined using quantitative real-time RT-PCR. Reverse transcription was performed using a one-step kit (SensiMix, Bioline, Taunton, MA) followed by quantitative real-time PCR for *IL1RN* and an endogenous control (*GAPDH*) using primer-probe sets obtained from the Applied Biosystems (Foster City, CA) repository (IL1RN, #Hs00277299_m1, GAPDH, #Hs99999905_m1) and TaqMan-based real-time PCR, as previously described [12], on an ABI PRISM 7900 (Applied Biosystems, Foster City, CA) with the following conditions; 95^{o}C for 15 sec, 60^{o}C for 1 min repeated for 40 cycles. Ct values observed for each sample were normalized to the level of GAPDH mRNA detected in each sample and a relative quantity (RQ) obtained by dividing the normalized value for each sample against a normalized value for a reference sample. These RQ values were then normalized to the neutrophil count associated with each whole blood sample.

Statistical analysis

Observed genotype frequencies were compared with expected frequencies to test for deviations from Hardy-Weinberg equilibrium. IL-1RA values normalized to neutrophil count for each subject were logarithmically ($\log_{10}$) transformed prior to analysis. The relationship between the copy number of each SNP and IL1-RA production (neutrophil normalized and $\log_{10}$ transformed) was analyzed assuming a co-dominant or additive effect using linear regression. *IL1RN* relative gene expression normalized to blood neutrophil counts (RQ/PMN) was compared between subjects with different *IL1RN*$_{C1018T}$ genotypes also using linear regression. All analyses were performed using the STATA 9.0 statistical package (StataCorp LP, College Station, TX).

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