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Polymorphisms of the IL1-receptor antagonist gene (*IL1RN*) are associated with multiple markers of systemic inflammation

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Abstract

Background—Circulating levels of acute phase reactant proteins such as plasma C-reactive protein (CRP) are likely influenced by multiple genes regulating the innate immune response.

Methods—We screened a set of 16 inflammation-related genes for association with CRP in a large, population-based study of healthy young adults (n=1,627). Results were validated in two independent studies (n=1,208 and n=4,310), including a pooled analysis of all 3 studies.

Results—In the pooled analysis, the minor allele of *IL1RN* 1018 (rs4251961) within the gene encoding interleukin-1 receptor antagonist (IL-1RA) was significantly associated with higher mean plasma log(CRP) level ($p < 1 \times 10^{-4}$). The same *IL1RN* 1018 allele was associated with higher mean plasma log(IL-6) levels (p=0.004). In the pooled analysis, the minor allele of *IL1RN* 13888 (rs2232354) was associated with higher fibrinogen, ($p = 0.001$). The *IL1RN* 1018 and 13888 variant alleles tag a clade of *IL1RN* haplotypes linked to allele 1 of a 86 bp VNTR polymorphism. We confirmed that the *IL1RN* 1018 variant (rs4251961) was associated with decreased cellular IL-1RA production *ex vivo*.

Conclusions—Common functional polymorphisms of the *IL1RN* gene are associated with several markers of systemic inflammation.

Keywords

IL-1receptor antagonist; C-reactive protein; inflammation; fibrinogen

INTRODUCTION

C-reactive protein (CRP) is a systemic marker of inflammation in humans [1]. Basal plasma CRP levels correlate with age, sex, ethnicity, smoking and body mass index (BMI). High basal levels of CRP predict future cardiovascular disease (CVD) events in otherwise healthy adults [2]. CRP, fibrinogen, and other acute phase proteins are produced primarily in the liver. CRP

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biosynthesis is largely under transcriptional control of interleukin (IL)-6, but other cytokines such as IL-1β and tumor necrosis factor (TNF)-α also contribute [3,4]. IL-6 and IL-1 activate the transcription factors STAT3, C/EBP, and the NF-κB/Rel family, which interact synergistically to induce maximal CRP gene transcription [5].

Recently, several polymorphisms of the *CRP* gene were associated with plasma CRP concentration [6,7], but these account for only a small proportion of the reported overall 35–50% heritability [8–11]. The remaining genetic determinants of CRP level are largely unknown. We hypothesized that gene variants in pathways related to cytokine signaling and the acute phase response influence CRP levels. We tested this by using a single nucleotide polymorphism (SNP) tagging approach to assess common genetic variation within a set of 16 candidate genes in young, healthy adults from the population-based Coronary Artery Risk Development in Young Adults (CARDIA) study. We validated our initial CARDIA findings of association between *IL1RN* genotypes and plasma CRP in two additional population studies of older adults, and also tested for association with other pro-inflammatory biomarkers (fibrinogen and interleukin-6). Finally, we assessed the functional influence of the associated *IL1RN* polymorphisms on cellular IL-1RA production.

METHODS

Initial screening of candidate gene SNPs and plasma CRP levels was performed in younger European-American men and women from the community-based CARDIA study. Subsequent replication of genetic association findings with inflammation biomarkers was performed within 2 studies of older adults, the Carotid Lesion Epidemiology and Risk (CLEAR) study and the Cardiovascular Health Study (CHS). In all studies, participants provided written informed consent.

CARDIA study participants and candidate gene genotyping

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. 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. Based on our hypothesis that common SNPs within candidate genes involved in cytokine signaling and acute phase response are associated with plasma CRP levels, we typed 121 tagSNPs across 16 genes to screen for association in CARDIA (Supplemental Table I), as described under Supplemental Methods (please see http://atvb.ahajournals.org.). For the current study, we included a subset of n=1,627 European-American participants who had genotyping performed and had CRP and fibrinogen concentration measured at the year 7 and year 5 exams, respectively.

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. The study subjects in the current analysis are European-American men ranging in age from 37 to 89 years, including 499 CAAD cases, 646 controls, and 63 individuals with intermediate internal carotid stenosis levels (see Supplemental Methods). Five of seven *IL1RN* tag SNPs were successfully typed in CLEAR: 1018 (rs4251961), 10257 (rs4252001), 13888 (rs2232354), 15132 (rs432014), and 15453 (rs380092), with numbering based on GenBank accession number AY196903 (Supplemental Figure I).

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CHS cohort

The Cardiovascular Health Study (CHS) is a prospective population-based cohort study of 5,888 men and women aged 65 and older recruited from four U.S. field centers: Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Pittsburgh, Pennsylvania. At study entry, CHS participants underwent assessment of CVD risk factors and measurement of plasma biomarkers, including CRP, fibrinogen, and interleukin-6, as described under Supplemental Methods. The final sample for the current study included 4,310 European-American men and women who consented to DNA testing. In CHS, all 7 *IL1RN* tagSNPs were typed as in CARDIA: 1018 (rs4251961), 2765 (rs315919), 5848 (rs3213448), 10257 (rs4252001), 13888 (rs2232354), 15132 (rs432014), and 15453 (rs380092) (Supplemental Figure I).

Statistical analysis

Allelic correlation as a measure of linkage disequilibrium (LD) between pairs of polymorphic sites was calculated as r^2. Haplotypes across the *IL1RN* gene were inferred from the unphased tagSNP genotype data using the haplotype reconstruction algorithm, fastPHASE [12].

Associations between SNP genotypes and quantitative CRP, fibrinogen, or IL-6 levels were assessed using multiple linear regression. CRP and IL-6 were log-transformed to reduce skewness. Linear regression models were adjusted for age, gender, field center, BMI, smoking status (and CAAD status in the CLEAR study). Covariate-adjusted SNP-specific change in estimated mean plasma biomarker level was estimated from the regression coefficients (β), separately for heterozygote and rare homozygote genotypes, using the homozygous wild type for each genotype as the reference group. The results obtained for unadjusted models were nearly identical to the covariate-adjusted results (data not shown). To account for multiple tagSNPs and genes tested in CARDIA, p-values were determined empirically by permutation testing. In addition, we performed permutation tests that provide a global test of significance across all the SNPs each gene, corrected for the number of genes tested. Both the individual SNP and candidate gene experiment-wise significance permutation tests take into account the correlation among tests of SNPs in LD [13]. Candidate gene testing was carried forward from stage 1 in CARDIA to stage 2 in the CLEAR and CHS replication studies if any of the individual SNP p-values were nominally <0.02.

Pooled analysis of CARDIA, CLEAR, and CHS genotype and phenotype data

Summary effect estimates for change in log(CRP) or fibrinogen associated with each additional copy of the minor SNP allele were calculated by including age, gender, and smoking status, BMI, and disease status as covariates using a random-effects meta-analysis model with inverse-variance weighting [14]. Heterogeneity of results between studies was assessed with *Q* statistics, which test for statistically significant differences among the pooled strata estimates. Statistical analyses were carried out by using the STATA software package v.8.2 (Stata Corporation, College Station, Texas).

As an additional method of combining data across studies, we used the Bayesian imputation and regression analysis program of Servin and Stephens [15] implemented in BIMBAM (http://stephenslab.uchicago.edu/software.html) to quantify the strength of evidence for association by computing Bayes Factors (BFs) for each 'typed' and 'untyped' polymorphism in *IL1RN*. Using this approach, patterns of correlation or LD among the markers typed in each phenotypic data set (CARDIA, CLEAR, and CHS) and the more densely-genotyped SeattleSNPs re-sequencing panel were used to impute the genotypes at all *IL1RN* markers in all individuals, allowing the combined data to be used when assessing the strength of the association between genotypes and the phenotype [15].

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Analysis of *IL1RN* genotype, *ILRN* gene expression, and whole blood IL1-RA levels

Healthy, non-smoking individuals between the ages of 18–65 years of age were recruited from the metropolitan Seattle area (n=285). Fasting blood samples were incubated *ex vivo* with the innate immune stimulus peptidoglycan or with media alone. *IL1RN* mRNA and IL1-RA protein levels were measured, as described in detail under Supplemental Methods.

RESULTS

Study participant characteristics

Descriptive characteristics of participants for all 3 population-based studies are shown in Table 1. At the year 7 CARDIA exam, the mean age of the CARDIA study subjects was 33 years, and 53% were women. The CLEAR study participants had a mean age of 67, and 15% were women. The mean age of the CHS participants at study entry was 73 years, and 57% were women. As expected, there was a greater prevalence of CVD risk factors and prevalent CVD among the older CHS and CLEAR participants than among the younger CARDIA cohort.

Association between *IL1RN* variants and CRP phenotype in CARDIA

We screened 121 common SNPs across 16 candidate inflammation genes in 1,627 CARDIA individuals for association with plasma CRP levels. Genotype, allele frequencies, and HWE p-values are shown in Supplemental Table I. Supplemental Table II shows the results for global tests of association for each candidate gene, and Supplemental Table III shows the results of tests of association for each individual SNP. When tested at the level of the candidate gene, the global permutation test for association of *IL1RN* with plasma CRP levels, corrected for multiple testing, was statistically significant (p=0.01). *IL1RN* 13888 (rs2232354) (minor allele frequency 20%), was significantly associated with plasma CRP level ($p = 3 \times 10^{-4}$; experiment-wise corrected p=0.04). When adjusted for age, gender, field center, BMI, and smoking status, each additional copy of the *IL1RN* 13888 (rs2232354) minor allele was associated with 1.19 (1.08 – 1.30) mg/L higher mean CRP level (Table 2), explaining <1% of the variance. One other SNP, *TNF* 4101 (rs1800628), had a nominal p-value below 0.02 (Supplemental Table III).

Linkage disequilibrium and haplotype structure of the *IL1RN* gene

The evolutionary haplotype structure of the *IL1RN* gene derived from SeattleSNPs genomic re-sequencing data (Supplemental Figure I) is shown in Supplemental Figure II. Seven *IL1RN* tagSNPs defined 4 major haplo-groups or clades in the European-American population. Clade A is tagged by *IL1RN* 1018 (rs4251961), and 13888 (rs2232354). The correlation coefficient (r^2) between the minor alleles of *IL1RN* 1018 and 13888 was 0.4. In intron 3 of *IL1RN*, there is a well-known 86-bp variable number of tandem repeat (VNTR) polymorphism [16], which consists of two common variants containing either 4 copies (allele 1) or 2 copies (allele 2). In the SeattleSNPs re-sequencing data, clades A, B, and C were associated with allele 1 of the *IL1RN* VNTR polymorphism. Clade D, tagged by the minor allele of *IL1RN* 15132 (rs432014) was in complete LD with allele 2 of the VNTR ($r^2 = 0.99$).

Meta-analysis of *IL1RN* genotype – CRP and other inflammation phenotype associations

The minor allele of *IL1RN* 13888 (rs2232354) was associated with increased plasma CRP levels in CLEAR (p-value = 0.004), confirming the association observed in CARDIA (Table 2). In CHS, the minor allele of the other clade A-tagging SNP, *IL1RN* 1018 (rs4251961), was associated with higher CRP levels (Table 2). In a pooled, meta-analysis of all 3 studies (Table 2), *IL1RN* 1018 (rs4251961) had the strongest evidence for association with CRP phenotype ($p < 1 \times 10^{-4}$; p for heterogeneity = 0.95). There was weaker evidence that *IL1RN* 13888 (rs2232354) was associated with higher CRP (p =0.06; p for heterogeneity = 0.01), and that

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IL1RN 15132 or rs432014 (p =0.06; p for heterogeneity = 0.46) and 15453 or rs380092 (p =0.008; p for heterogeneity = 0.54) were associated with lower CRP. None of the *IL1RN* genotype-CRP phenotype associations changed upon additional adjustment for *CRP* tagSNPs (data not shown).There was no association between *TNF* 4101 (rs1800628) and CRP in the pooled analysis (p=0.44).

Bayesian imputation and regression analysis of all 156 typed and untyped *IL1RN* polymorphisms (including the 86-bp VNTR) confirmed that *IL1RN* 1018 (rs4251961) and an untyped SNP in strong LD, *IL1RN* 19327 (rs315919) had the strongest evidence for association with CRP [log10(Bayes Factors) = 2.2 and 2.0, respectively; permutation p-values = 0.001], followed by *IL1RN* 13888 or rs22323540 [log10(Bayes Factor)=1.5; permutation p-value=0.003]. Allele 2 of the VNTR polymorphism showed much weaker evidence of association with CRP [log10(Bayes Factor)=0.3; permutation p-value = 0.04].

In pooled analysis of fibrinogen phenotype, the minor allele of *IL1RN* 13888 (rs2232354) was associated with 4.11 (95% CI 1.60 – 6.57; p=0.001) mg/dL higher fibrinogen, (p for heterogeneity = 0.55). In CHS, the minor allele of *IL1RN* 1018 (rs4251961) was associated with 0.04 ± 0.01 higher mean plasma log(IL-6) levels (p=0.004), while the minor allele of *IL1RN* 2765 was associated with 0.03 ± 0.01 lower log(IL-6) levels (p=0.02).

IL-1RA production in whole blood *ex vivo* is associated with *IL1RN* genotype

There was an association between the clade A *IL1RN* 1018 (rs4251961) C allele and lower IL-1RA production (normalized for neutrophil count) in response to the innate immune stimulus peptidoglycan ($p = 4.5 \times 10^{-6}$; Table 3). There was also an association between normalized IL-1RA production in response to peptidoglycan and the clade D 15132 (rs432014) genotype; the minor C allele was associated with higher IL-1RA levels (Table 3). There was no association between *IL1RN* genotype and IL-1RA production in blood stimulated with media alone (not shown). In a subgroup of 57 subjects selected to represent equal numbers of each *IL1RN* 1018 (rs4251961), genotype group, there was a non-significant trend (p=0.2) towards higher levels of neutrophil-normalized *IL1RN* transcript with increasing copies of *IL1RN* 1018T (Supplemental Figure III).

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DISCUSSION

Using data from 3 large studies, we demonstrate that common variants of the IL-1RA gene (*IL1RN*) are associated with multiple systemic inflammation phenotypes. The minor alleles of *IL1RN* 1018 (rs4251961) and 13888 (rs2232354), tagging haplotype clade A, were associated with higher circulating levels of CRP, fibrinogen, and IL-6 levels. The same clade A alleles were associated with reduced cellular IL-1RA production *ex vivo* in response to an inflammatory stimulus.

Our results are supported by several family-based linkage and association studies involving the *IL1RN* gene region in CVD patients. In a recent genome-wide linkage analysis, the region on chromosome 2 containing *IL1RN* was identified as influencing CRP levels in European families with early-onset coronary heart disease (CHD) [17]. In smaller, hospital-based studies of CHD patients, allele 2 of the 86 bp *IL1RN* VNTR polymorphism was associated with lower plasma CRP and fibrinogen concentration [18,19]. Our findings extend the importance of *IL1RN* genotype as a determinant of CRP and fibrinogen levels among the general population of younger and older European American adults. By performing a pooled meta-analysis and imputation analysis to combine genotype and phenotype data across studies, and also to test untyped SNPs identified through a genomic re-sequencing panel, we demonstrated that *IL1RN* 1018 (rs4251961) had the strongest evidence for association with CRP.

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In separate *ex vivo* whole blood analyses, we were able to demonstrate that the amount of IL1-RA produced by leukocytes in response to an inflammatory stimulus is influenced by *IL1RN* genotype. While circulating IL1-RA levels were not measured in our 3 population studies, our cellular IL1-RA results are consistent with a recent report from the Invecchiare in Chianti study [20]. Rafiq et al [20] found the *IL1RN* 1018 (rs4251961), variant (clade A) was strongly associated with lower circulating IL-1RA levels, while another SNP in strong linkage disequilibrium with the VNTR allele 2 haplotype (clade D) was associated with higher soluble IL-1RA levels. Interestingly, Rafiq et al [20] also reported that the *IL1RN* 1018-containing haplotype was associated with increased circulating levels of other inflammatory mediators such as interferon-γ, IL-1β, adiponectin, and α2 macroglobulin, another hepatic acute phase protein. Together with our results, these findings further support the role of common *IL1RN* polymorphisms on circulating levels of multiple biomarkers of systemic inflammation

CRP, fibrinogen, and IL-6 are plasma proteins primarily synthesized in the liver as part of the acute phase response to inflammatory stimuli. The observed *IL1RN* genotype associations are consistent with the known biologic role of IL-1RA, which inhibits overall IL-1 related innate immune responses [21]. IL1-RA is produced in high amounts by peripheral blood neutrophils stimulated with innate immune agonists [22,23]. The soluble form of IL-1RA is also produced by hepatocytes and is itself regulated by pro-inflammatory cytokines and NF-κB and C/EBP, with circulating IL-1RA levels rising during various inflammatory conditions and tissue injury [24]. Therefore the direction of observed IL1RN genotype – inflammatory phenotype associations are consistent with known biology of IL-1RA in inflammation: the minor alleles of *IL1RN* 1018 (rs4251961), and 13888 (rs2232354) are associated with lower IL1-RA production and thus higher inflammation biomarker levels.

Strengths of the current study include the use of 3 large, independent samples to demonstrate replication of *IL1RN* genotype – CRP association, assessment of additional inflammation-related phenotypes, including *ex vivo* cellular IL-1RA production, and the use of meta-analysis methods to combine genotype and phenotype data across studies and to impute untyped SNPs. While the *IL1RN* 1018 variant was strongly associated with cellular IL-1RA production, the precise molecular functional sites remain to be identified. *IL1RN* 1018 (rs4251961) is located 3 bp upstream of a predicted YY1 transcription factor binding site within a region of the intracellular IL-1RA promoter that appears to regulate LPS-induced expression in macrophages [25]. In our Bayesian imputation and regression analysis, *IL1RN* 19327 (rs315919), which is located in the 3’ flanking region and is in strong LD with *IL1RN* 1018 (r^2=0.8), showed nearly as strong a signal of association with CRP. Examination of polymorphism data from the HapMap shows that a*IL1RN* 1018 (rs4251961) is also in strong linkage disequilibrium with 8 other SNPs located in the 3’ untranslated and flanking region of *IL1RN* that form an extended *IL1RN* haplotype. Therefore, additional polymorphic sites located either intrinsic or extrinsic to *IL1RN* may affect athe function of either the secreted or intracellular IL-1RA promoter regions.

In summary, we have identified several common variants of the *IL1RN* gene associated with circulating levels of multiple inflammatory biomarkers in European American adults. IL-1RA has beneficial effects in many disease models, and recombinant human IL-1RA is currently approved or under investigation for treatment of diseases such as stroke, diabetes, and arthritis [26–28]. Therefore, the current findings have potential implications for primary risk stratification and targeted drug therapy [29] in a wide range of CVD, metabolic, and other inflammation-related disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Descriptive characteristics of participants from CARDIA, CLEAR, and CHS

Characteristic	CARDIA	CLEAR	CHS
Number	1,627	1,208	4,310
Mean age, years [range]	33 [24 – 39]	67 [31 –92]	73 [65 – 98]
Female sex	1023 (53)	186 (15)	2580 (57)
Current smokers	378 (21)	203 (17)	497 (11)
Body mass index (kg/m^2)	25.5 ± 5.1	28.3 ± 5.2	26.4 ± 4.5
Total cholesterol (mg/dL)	178 ± 35	192 ± 39	212 ± 39
LDL cholesterol (mg/dL)	108 ± 31	112 ± 32	130 ± 36
HDL cholesterol (mg/dL)	51 ± 14	49 ± 16	54 ± 16
Triglycerides (mg/dL)	94 ± 86	148 ± 150	144 ± 79
Diagnosis of hypertension	54 (3)	703 (58)	2530 (56)
Diabetes	22 (1)	195 (16)	657(15)

Data are presented as number (%) or mean ± standard deviation, unless otherwise indicated.

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Table 2

Associations between *IL1RN* tagSNP genotype and plasma log(CRP) levels in CARDIA and in CLEAR and CHS validation populations

IL1RN tag SNP (rs id)	Genotype	CARDIA β ± SE (*p*-value)	CLEAR β ± SE (*p*-value)	CHS β ± SE (*p*-value)	Pooled estimate β [95% CI] (*p*-value)
1018 (rs4251961)	T/T	0	0	0	
	T/C	−0.034 ± 0.056 (0.55)	0.011 ± 0.056 (0.89)	0.092 ± 0.032 (0.004)	
	C/C	0.195 ± 0.084 (0.02)	0.201 ± 0.084 (0.06)	0.109 ± 0.045 (0.017)	
	per C allele	0.062 ± 0.039 (0.11)	0.082 ± 0.051 (0.11)	0.067 ± 0.021 (0.002)	0.068 [0.034 – 0.102]
					P<0.0001
2765 (rs315919)	G/G	0		0	
	G/T	−0.091 ± 0.058 (0.12)		−0.030 ± 0.032 (0.35)	
	T/T	0.072 ± 0.077 (0.35)		−0.119 ± 0.044 (0.008)	
	per T allele	0.014 ± 0.037 (0.70)		−0.053 ± 0.021 (0.01)	−0.026 [−0.074 – 0.023]
					P=0.295
5848 (rs3213448)	G/G	0		0	
	G/A	−0.046 ± 0.064 (0.47)		−0.041 ± 0.036 (0.25)	
	A/A	0.322 ± 0.200 (0.11)		0.003 ± 0.117 (0.98)	
	per A allele	0.011 ± 0.055 (0.84)		−0.030 ± 0.031 (0.33)	−0.013 [-0.065 – 0.038]
					P=0.612
10257 (rs315934)	T/T	0	0	0	
	T/C	−0.102 ± 0.056 (0.07)	−0.083 ± 0.086 (0.33)	0.008 ± 0.032 (0.81)	
	C/C	−0.022 ± 0.156 (0.88)	−0.270 ± 0.253 (0.29)	−0.087 ± 0.077 (0.26)	
	per C allele	−0.072 ± 0.048 (0.14)	−0.098 ± 0.074 (0.19)	−0.012 ± 0.026 (0.64)	−0.033 [−0.078 – 0.012]
					P=0.146
13888 (rs2232354)	T/T	0	0	0	
	T/G	0.112 ± 0.055 (0.04)	0.115 ± 0.070 (0.10)	0.022 ± 0.032 (0.49)	
	G/G	0.565 ± 0.142 (0.0001)	0.356 ± 0.163 (0.03)	0.038 ± 0.072 (0.60)	
	per G allele	0.170 ± 0.050 (0.0001)	0.141 ± 0.057 (0.013)	0.021 ± 0.026 (0.47)	0.103 [−0.002 – 2.08]
					P=0.055
15132 (rs432014)	T/T	0	0	0	
	T/C	−0.061 ± 0.055 (0.27)	−0.070 ± 0.077 (0.36)	−0.019 ± 0.031 (0.36)	
	C/C	0.086 ± 0.094 (0.36)	0.037 ± 0.140 (0.79)	−0.154 ± 0.057 (0.79)	
	C allele	0.002 ± 0.040 (0.97)	−0.021 ± 0.057 (0.72)	−0.054 ± 0.023 (0.03)	−0.038 [−0.075 – −0.001]

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IL1RN tag SNP (rs id)	Genotype	CARDIA β ± SE (*p*-value)	CLEAR β ± SE (*p*-value)	CHS β ± SE (*p*-value)	Pooled estimate β [95% CI] (*p*-value)
					P=0.056
15453 (rs380092)	A/A	0	0	0	
	A/T	−0.090 ± 0.055 (0.10)	−0.105 ± 0.080 (0.19)	−0.008 ± 0.031 (0.81)	
	T/T	−0.057 ± 0.095 (0.55)	−0.226 ± 0.138 (0.10)	−0.152 ± 0.052 (0.02)	
	per T allele	−0.053 ± 0.040 (0.19)	−0.010 ± 0.059 (0.06)	−0.040 ± 0.023 (0.08)	−0.050 [−0.087 – −0.013]
					P=0.008

SE = standard error; CI = confidence interval.

Beta coefficients and S.E.'s were estimated using multiple linear regression models, adjusted for age, sex, clinic, BMI, smoking status (and carotid disease status in CLEAR). For each SNP, the first two estimates represent the change in log(CRP) for heterozygote and rare homozygote genotype groups, compared to common homozygotes. The third (and pooled) estimates correspond to the change in (log)CRP associated with each additional copy of the minor allele (assuming an additive genetic model). *IL1RN* 2765 and 5848 were not typed in the CLEAR subjects.

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Table 3

Whole blood IL-1RA production by *IL1RN* genotypes

	IL1RN SNP	Genotype	Count (frequency)	IL1RA/PMN (Mean RQ)	*p*
rs4251961	1018	TT	107 (0.38)	3.49	
		TC	143 (0.50)	3.45	
		CC	35 (0.12)	3.35	4.50×10^{-6}
rs315919	2765	GG	106 (0.37)	3.43	
		GT	130 (0.46)	3.47	
		TT	49 (0.17)	3.49	0.014
rs3213448	5848	GG	203 (0.71)	3.46	
		GA	77 (0.27)	3.45	
		AA	6 (0.02)	3.46	0.616
rs315934	10257	TT	180 (0.63)	3.45	
		TC	93 (0.33)	3.48	
		CC	12 (0.04)	3.53	0.39

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IL1RN SNP		Genotype	Count (frequency)	IL1RA/PMN (Mean RQ)	p
rs2232354	13888	TT	175 (0.62)	3.47	
		TG	101 (0.36)	3.46	
		GG	8 (0.03)	3.36	0.141
rs432014	15132	TT	157 (0.55)	3.43	
		TC	110 (0.39)	3.49	
		CC	17 (0.06)	3.53	0.0007
rs380092	15453	AA	121 (0.42)	3.45	
		AT	136 (0.48)	3.46	
		TT	28 (0.10)	3.49	0.346

Whole blood IL1RA production in response to peptidoglycan (100 mg/ml) for 6 hours. Mean values are normalized to neturophil (PMN) count and log_{10} transformed. P-values were determined by linear regression, assuming a co-dominant or additive genetic effect.