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Multi-Ancestral Analysis of Inflammation-Related Genetic Variants and C-Reactive Protein in the Population Architecture using Genomics and Epidemiology (PAGE) Study

Jonathan M. Kocarnik, PhD, MPH[1], **Sarah A. Pendergrass, PhD, MS**[2], **Cara L. Carty, PhD, MS**[1], **James S. Pankow, PhD, MPH**[3], **Fredrick R. Schumacher, PhD**[4], **Iona Cheng, PhD, MPH**[5], **Peter Durda, BS**[6], **JoséLuis Ambite, PhD**[7], **Ewa Deelman, PhD**[7], **Nancy R. Cook, ScD, MS**[8], **Simin Liu, MD, ScD, MPH, MS**[9], **Jean Wactawski-Wende, PhD**[10], **Carolyn Hutter, PhD, MS**[11], **Kristin Brown-Gentry, MS**[12], **Sarah Wilson, MS**[12], **Lyle G. Best, MD**[13], **Nathan Pankratz, PhD**[14], **Ching-Ping Hong, MS**[3], **Shelley A. Cole, PhD, MS**[15], **V. Saroja Voruganti, PhD**[15], **Petra Bůžková, PhD, MS**[16], **Neal W. Jorgensen, MS**[16], **Nancy S. Jenny, PhD**[6], **Lynne R. Wilkens, DrPH, MS**[17], **Christopher A. Haiman, ScD**[4], **Laurence N. Kolonel, MD, PhD**[17], **Andrea LaCroix, PhD, MPH**[1], **Kari North, PhD, MA**[18], **Rebecca Jackson, MD**[19], **Loic Le Marchand, MD, PhD, MPH**[17], **Lucia A. Hindorff, PhD, MPH**[20], **Dana C. Crawford, PhD**[12], **Myron Gross, PhD, MS**[21], and **Ulrike Peters, PhD, MPH**[1]

[1]Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA

[2]Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA

[3]Division of Epidemiology and Community Health, University of Minnesota, Minneapolis, MN

[4]Keck School of Medicine, University of Southern California, Los Angeles, CA

[5]Cancer Prevention Institute of California, Fremont, CA

[6]Department of Pathology, University of Vermont, Burlington, VT

[7]Information Sciences Institute, University of Southern California, Marina del Rey, CA

[8]Department of Medicine, Brigham & Women's Hospital, Boston, MA

[9]Department of Epidemiology, University of California Los Angeles, Los Angeles, CA

[10]Department of Social and Preventive Medicine, University at Buffalo, Buffalo, NY

[11]Epidemiology and Genomics Research Program, DCCPS, NCI, Bethesda, MD

[12]Center for Human Genetics Research, Vanderbilt University, Nashville, TN

[13]Missouri Breaks Industries Research Inc., Timber Lake, SD

[14]Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN

[15]Department of Genetics, Texas Biomedical Research Institute, San Antonio, TX

Correspondence: Ulrike Peters, 1100 Fairview Ave N, M4-B402, PO Box 19024, Seattle, WA 98109, Tel: (206)667-2879, Fax: (206)667-7850, upeters@fhcrc.org.

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[16]Department of Biostatistics, University of Washington, Seattle, WA

[17]Cancer Research Center, University of Hawaii, Honolulu, HI

[18]Department of Epidemiology, University of North Carolina, Chapel Hill, NC

[19]Department of Internal Medicine, The Ohio State University, Columbus, OH

[20]Division of Genomic Medicine, NHGRI, NIH, Bethesda, MD

[21]Masonic Cancer Center, University of Minnesota, Minneapolis, MN

Abstract

Background—C-reactive protein (CRP) is a biomarker of inflammation. Genome-wide association studies (GWAS) have identified single nucleotide polymorphisms (SNPs) associated with CRP concentrations and inflammation-related traits such as cardiovascular disease, type 2 diabetes, and obesity. We aimed to replicate previous CRP-SNP associations, assess whether these associations generalize to additional race/ethnicity groups, and evaluate inflammation-related SNPs for a potentially pleiotropic association with CRP.

Methods and Results—We selected and analyzed 16 CRP-associated and 250 inflammation-related GWAS SNPs among 40,473 African American, American Indian, Asian/Pacific Islander, European American, and Hispanic participants from 7 studies collaborating in the Population Architecture using Genomics and Epidemiology (PAGE) study. Fixed-effect meta-analyses combined study-specific race/ethnicity-stratified linear regression estimates to evaluate the association between each SNP and high-sensitivity CRP. Overall, 18 SNPs in 8 loci were significantly associated with CRP (Bonferroni-corrected $p<3.1\times10^{-3}$ for replication, $p<2.0\times10^{-4}$ for pleiotropy): Seven of these were specific to European Americans, while 9 additionally generalized to African Americans (1), Hispanics (5), or both (3); 1 SNP was seen only in African Americans and Hispanics. Two SNPs in the *CELSR2/PSRC1/SORT1* locus showed a potentially novel association with CRP: rs599839 ($p=2.0\times10^{-6}$) and rs646776 ($p=3.1\times10^{-5}$).

Conclusions—We replicated 16 SNP-CRP associations, 10 of which generalized to African Americans and/or Hispanics. We also identified potentially novel pleiotropic associations with CRP for two SNPs previously associated with coronary artery disease and LDL cholesterol. These findings demonstrate the benefit of evaluating genotype-phenotype associations in multiple race/ethnicity groups, and of looking for pleiotropic relationships among SNPs previously associated with related phenotypes.

Keywords

genetic epidemiology; inflammation; C-reactive protein; race and ethnicity; single nucleotide polymorphism; pleiotropy

Introduction

C-reactive protein (CRP) is an acute-phase reactant protein produced by the liver. Circulating levels rise sharply following inflammatory stimulation from infection or injury, then fall rapidly following stimulus resolution. In situations of chronic underlying disease,

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however, CRP remains slightly raised over time, serving as a biomarker to characterize systemic inflammation[1, 2]. Elevated CRP levels have been associated with a large number of outcomes and traits, such as cardiovascular events[1, 2], atherosclerosis[3], stroke[4], type 2 diabetes (T2D)[5], fitness level and body composition[6], and cancer[7, 8]. As such, serum CRP is an important biomarker for the presence or development of disease among apparently healthy individuals[1, 2].

Heritability estimates for CRP are between 25 and 56%[9–11]. Genome-wide association studies (GWAS) have identified single nucleotide polymorphisms (SNPs) in 21 loci associated with CRP concentrations[12–14]. Environmental factors associated with increased CRP levels include female sex[9, 15], hormone replacement therapy (HRT) use[16], smoking[2], and obesity[1, 2], while factors associated with decreased CRP levels include exercise[17] and the use of aspirin, NSAIDs, and statins[1].

CRP levels have previously been shown to vary according to race/ethnicity, and tend to be higher in populations with high rates of diabetes and obesity. Compared to those of European ancestry, those of African[15, 18] and American Indian[19] ancestry tend to have elevated CRP levels and those of Asian ancestry tend to have lower CRP levels[20, 21]. Many initial genetic studies of CRP were performed primarily on populations of European descent, and studies examining CRP-associated variants in other race/ethnicity populations have had moderate success in generalizing some, but not all, of these loci[18, 22–24]. Generalization and fine-mapping of loci in non-European ancestry populations is thus an important step in further elucidating genetic contributions to CRP concentrations in multiple race/ethnicity groups.

Many of the genetic variants associated with serum CRP levels demonstrate pleiotropic effects, and have also been associated with various other outcomes related to inflammation. SNP rs1205 in the gene encoding CRP (*CRP*), for example, has been associated not only with CRP[12, 22], but also with heart rate variability[25] and colon cancer[7]. Given the multiple phenotypes associated with CRP, we hypothesize that genetic variants associated with inflammation-related phenotypes, such as cardiovascular disease, T2D, or obesity, might also be associated with CRP. Identifying additional pleiotropic associations could aid in elucidating shared biological pathways and relationships.

We seek to replicate previously reported associations between SNPs and serum CRP, as well as generalize these associations to four additional race/ethnicity groups: African Americans, Hispanics, Asian/Pacific Islanders, and American Indians. We also evaluate several SNPs previously associated with inflammation-related phenotypes for an association with CRP, both overall and stratified by race/ethnicity groups. The size and diversity of our study population provides an opportunity to replicate previous findings, generalize existing associations to additional race/ethnicity groups, and discover novel pleiotropic associations with this inflammatory biomarker.

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Methods

Study description

Data for this analysis were generated as part of the Population Architecture using Genomics and Epidemiology study (PAGE), previously described in detail[26]. Briefly, PAGE is a consortium of large, well-characterized population-based studies, investigating the epidemiologic architecture of genetic variants associated with complex diseases across various race/ethnicity groups.

Study populations

We included 40,473 participants: 7,228 from Epidemiologic Architecture for Genes Linked to Environment (EAGLE), based on data from three National Health and Nutrition Examination Surveys (NHANES); 1,593 from the Multiethnic Cohort Study (MEC); 9,901 from the Women's Health Initiative (WHI); and 21,751 from Causal Variants Across the Life Course (CALiCo), itself a consortium of five cohort studies. Participants from CALiCo included: 11,303 from Atherosclerosis Risk in Communities (ARIC); 3,249 from Coronary Artery Risk Development in Young Adults (CARDIA); 4,070 from the Cardiovascular Health Study (CHS); and 3,129 from the Strong Heart Study (SHS).

Studies included one or more of five race/ethnicity groups, totaling 24,958 European American, 8,471 African American, 2,935 Hispanic, 980 Asian/Pacific Islander, and 3,129 American Indian participants. Several of these studies have previously been utilized to evaluate genetic associations with CRP, including participants in ARIC[13], CHS[13, 27, 28], and CARDIA[29]. Within EAGLE, we included NHANES 1999–2002 data and excluded NHANES III data (due to non-high-sensitivity CRP measurements). Each study was approved by a local institutional review board, and all participants gave informed consent.

Measurements

Baseline high-sensitivity C-reactive protein (hsCRP) measurements were available in each study as part of previous investigations, with assay method and instrument used to measure CRP varying by study (Supplemental Table 1). Because the distribution of CRP is skewed, the values were natural-log transformed for analyses. Demographic and other epidemiologic information was obtained according to the enrollment protocols of each study (Table 1, Supplemental Table 2).

SNP Selection and Genotyping

In 2008, PAGE investigators identified 266 SNPs previously associated with various phenotypes of interest, focusing on variants related to cardiovascular disease traits, lipids, body mass index (BMI), and T2D. These included: 16 SNPs associated with CRP/inflammation, 21 with cardiovascular disease or myocardial infarction, 26 with BMI/obesity, 51 with T2D or glucose levels, 82 with HDL/LDL/total cholesterol, 29 with triglycerides, 9 with stroke, and 33 with other related phenotypes (Supplemental Table 3; previous trait association refers to the first reported association at the time of SNP selection). Genotyping methods for PAGE have been previously described[26]. For each SNP, quality control

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thresholds included SNP and sample call rates >90%, concordance of blinded replicates >98%, and no clear evidence of Hardy-Weinberg disequilibrium (p>0.001).

Due to funding constraints, each PAGE study chose a custom subset of SNPs to genotype depending on the phenotypes they had available. As such, the number of SNPs available varied in each study, with 30 SNPs genotyped in ARIC, 7 in CARDIA, 113 in CHS, 196 in EAGLE, 103 in MEC, 19 in SHS, and 94 in WHI (Table 2, Supplemental Table 3). While this meant that not all SNPs were genotyped in every study, an effort was made to ensure overlap (56% of SNPs were genotyped in more than one study). Since race/ethnicity group availability differed by study, this also meant that not all SNPs were available for analyses in each race/ethnicity group. Nearly all SNPs were available for the European American (n=265), African American (n=266), and Hispanic groups (n=261), while fewer SNPs were available for the Asian/Pacific Islander (n=150) and American Indian groups (n=19). These overlap issues reduced the overall sample size available for any given SNP- and race/ethnicity-specific association, though numbers were still large for most analyses (Table 2, Supplemental Table 4). To avoid small sample size issues in the analysis, for each SNP only study-specific race/ethnicity-specific results with at least 100 genotyped subjects were included in meta-analyses. This restriction meant the exclusion of the WHI American Indian results (≤86 participants for each SNP), which reduced the overall number of SNPs available for analysis in the American Indian group from 96 to 19.

Statistical analysis

Each study fit linear regression models to test the association between natural log-transformed CRP and each SNP, coded additively (0/1/2 copies of the coded allele). Separate models were fit for five race/ethnicity groups: European American, African American, Hispanic, Asian/Pacific Islander, and American Indian. MEC performed analyses separately for Hawaiian and Japanese ancestry participants. Race/ethnicity-specific models were adjusted for age, sex, center, and/or principal components, as appropriate for each study. Sampling weights were used in the WHI regression analyses to account for sample selection criteria. Additional models were stratified by sex.

Inverse-variance-weighted fixed effect meta-analyses were used to estimate both overall and race/ethnicity stratified associations for each SNP. Because studies differed in SNPs genotyped and participant race/ethnicity, the overall number of observations available for each SNP-specific meta-analysis varied. Overall combined-race/ethnicity meta-analysis numbers were large for most SNPs (mean number of observations 12,361, range 802–36,299). In the race/ethnicity-stratified meta-analyses, the average number of observations across available SNPs was 7,722 for European Americans, 2,367 for African Americans, 1,766 for Hispanics, 617 for Asian/Pacific Islanders, and 3,129 for American Indians (Table 2, Supplemental Table 4). Additional meta-analyses were stratified by sex. For each SNP, heterogeneity between studies and within race/ethnicity groups was tested using Q and I^2 statistics. Meta-regression was used to evaluate the heterogeneity between race/ethnicity groups and sexes. Meta-analyses were performed using Stata version 12[30]. We used a conservative Bonferroni-corrected p-value to adjust for multiple comparisons, accounting for the maximum number of tested SNPs to determine the statistical significance threshold

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($0.05/16=3.1\times10^{-3}$ for replication and $0.05/250=2.0\times10^{-4}$ for pleiotropy discovery). Since not all SNPs were available in each race/ethnicity group, this correction may have been overly stringent for some groups.

Results

European American and African American were the largest race/ethnicity groups in our analysis (Table 1, Supplemental Table 2). Median CRP concentration varied by study and race/ethnicity: the lowest levels were observed in MEC and Asian/Pacific Islanders, and the highest levels were observed in WHI and American Indians. American Indian participants were on average younger than participants in other groups. Overall there were more women than men (65.8%) due to the focus on women in WHI. Mean BMI values varied by race/ethnicity, with the highest values in the African American and American Indian groups and the lowest values in the Asian/Pacific Islander group. Extensive efforts were made to harmonize variables across PAGE studies, though some study heterogeneity may remain.

In the race/ethnicity-combined analyses, we identified 18 SNPs in 8 loci associated with CRP: 10 of 16 CRP-associated SNPs replicated and 8 of 250 inflammation-related SNPs demonstrated a pleiotropic association with CRP (Table 3). These included: 5 SNPs in the *CRP* locus; 4 in *APOE/APOC1/TOMM40;* 2 each in *GCKR*, *HNF4A*, and *CELSR2/PSRC1/SORT1*; and 1 each in *HNF1A, IL6R*, and *LEPR*. Several of these SNPs were correlated with each other, with the amount of correlation varying by race/ethnicity (Supplemental Table 5). Of the 18 SNPs (in 8 loci) reaching statistical significance, 15 SNPs (in 7 loci) had previously been associated with CRP, while rs599839 (near *PSRC1*) and rs646776 (near *CELSR2*) have not. The SNP rs6857 (in *PVRL2*) has not itself been previously associated with CRP, though it is in close proximity to the known *APOE/APOC1/TOMM40* locus. Since many of these SNPs have previously been associated with other phenotypic traits, the observed associations with CRP suggest that many of these SNPs may have potentially pleiotropic effects.

In race/ethnicity-stratified analyses, 16 SNPs reached statistical significance in European Americans, along with 5 SNPs in African Americans and 9 SNPs in Hispanics. No SNPs reached statistical significance among the Asian/Pacific Islander or American Indian groups, though not all SNPs were evaluated and power was reduced in these smaller groups. Notably, for the 18 SNPs that were significant in the race/ethnicity-combined analysis, only 9 were available in the Asian/Pacific Islander group and none were available in the American Indian group. Most SNPs showed no evidence of heterogeneity, either within or between race/ethnicity groups. For two SNPs showing some evidence of heterogeneity between race/ethnicity groups (rs2075650 and rs4420638, Figure 1), sensitivity analyses using random-effects meta-analyses provided similar results (Supplemental Figure 1).

For generalizability, 7 SNPs were associated only in European Americans, while 10 SNPs in 5 loci demonstrated statistically significant associations with CRP in more than one race/ethnicity group: 3 SNPs in the *APOE/APOC1/TOMM40* locus, 4 SNPs in *CRP*, and 1 each in *HNF1A, IL6R*, and *LEPR* (Table 3). For the three SNPs where a statistically significant effect was seen in European Americans, African Americans, and Hispanics, two showed

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similar effect size estimates across race/ethnicity groups: SNP rs2228145 in *IL6R* (β=−0.10, −0.13, −0.18, respectively; p-heterogeneity=0.16), and SNP rs429358 in *APOE* (β=−0.24, −0.23, −0.36, respectively; p-heterogeneity=0.23). Two SNPs also followed this trend in European Americans and Hispanics: SNP rs2075650 in *TOMM40* (β=−0.22 and −0.21, respectively; p-heterogeneity=0.76), and SNP rs6857 in *PVRL2* (β=−0.23 and −0.28, respectively; p-heterogeneity=0.50). Two SNPs in *CRP* showed a potential exception to this trend, where a larger effect was seen for African Americans than for European Americans (rs1800947, β=−0.61 vs. −0.30; p-heterogeneity=0.02) or than for European Americans and Hispanics (rs1205, β=−0.27 vs. −0.17 and −0.22, respectively; p-heterogeneity=0.02). In general, however, race/ethnicity-specific effect estimates for a given SNP were in the same direction and of similar magnitude across race/ethnicity groups regardless of the statistical significance of the association. We did observe several exceptions to this trend, where the effect estimate for one group was null or in the opposite direction compared to all other groups, though these exceptions could be due to chance.

We also evaluated whether any of these associations differed by sex (Table 4, Supplemental Table 6). Most of the SNPs which were statistically significant overall remained so in sex-stratified analyses. Two SNPs in *GCKR*, rs1260326 and rs780094, demonstrated a statistically significant difference in effect between males and females (p-heterogeneity=1.5×10^{-8}), with a stronger effect seen in females than males. Several other SNPs were also suggestive for a potential difference in effect by sex: rs1417938 in *CRP* (p-heterogeneity=0.03), rs4420638 and rs429358 in *APOE/APOC1/TOMM40* (p-heterogeneity=0.03), and rs2650000 in *HNF1A* (p-heterogeneity=0.01). For these SNPs, the direction of the effect was generally similar in both sexes, while effect estimates were slightly larger and p-values were smaller among the females than the males, as expected given the larger sample size. For the two SNPs in *GCKR*, the p- values were actually slightly smaller in the female-stratified results than in the overall combined results, despite almost half the sample size.

Discussion

Our multi-ethnic meta-analysis observed 18 SNPs in 8 loci statistically significantly associated with serum CRP concentrations. Ten of these SNPs in 5 loci demonstrated statistically significant associations with CRP in multiple ancestral groups, most of which have not been previously reported in non-European American populations. Among the 18 significant SNPs, 2 SNPs (rs599839 and rs646776, CEU r^2=0.9) in 1 locus (*CELSR2/PSRC1/SORT1*) had not previously been associated with CRP. Three themes emerged from our results: 1) the general consistency of effect at a particular SNP across race/ethnicity groups; 2) variation in association for different SNPs in the same locus can be useful for fine-mapping regions of interest; 3) and the demonstration of pleiotropic effects for specific SNPs.

Generalization

First, for the 10 SNPs which demonstrated a statistically significant association in multiple race/ethnicity groups, the direction and magnitude of the effect was fairly consistent. Several

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SNPs showed a similar statistically significant effect in European Americans, African Americans, and Hispanics (rs2228145 in *IL6R* and rs429358 in *APOE*) or European Americans and Hispanics (rs2075650 in *TOMM40* and rs6857 in *PVRL2*). This trend of similar direction and magnitude across race/ethnicity groups generally held for most SNPs, even where the race/ethnicity-specific associations did not reach statistical significance across all groups (such as rs7310409 in *HNF1A* and rs599839 in *PSRC1*). When combined with previous findings, these results suggest a shared genetic influence between race/ethnicity groups at these SNPs.

However, there were several notable exceptions where large differences were seen between race/ethnicity groups. Two SNPs in *CRP*, for example, showed larger effect sizes in African Americans than in European Americans (rs1800947, rs1205) or Hispanics (rs1205). In other SNPs, an effect was observed in one or two groups but not the others (rs2075650 and rs4420638 in *TOMM40*, p-heterogeneity$<1.6\times10^{-9}$). Such differences could be indicative of population differences in linkage disequilibrium (LD), since tagging variants in European-ancestry populations may not be representative in other groups[18, 31]. Taken together, this may suggest that the functional SNP is likely to be within the LD block shared by those race/ethnicity groups with similar effect estimates for that tagSNP, while for the "outlying" race/ethnicity group the tagSNP may not be well correlated with the functional SNP. Alternatively, differences in generalizability could also be due to a reduced ability to detect an association in some race/ethnicity groups in our study due to smaller sample sizes, differences in allele frequency, smaller effect sizes, or lower correlation with functional variants.

Fine-mapping

A related second theme is that differences in SNP generalizability may be informative of the genetic architecture of loci with multiple SNPs associated with CRP. Previous studies have shown that both average CRP levels and SNP-CRP associations can vary by race/ethnicity group[18, 23]. Additionally, while some polymorphisms have demonstrated an association across race/ethnicity groups (rs1205[22]), others appear specific to particular groups (rs3093058[18, 22]). In our study, some SNPs within the *CRP* and *APOE/APOC1/TOMM40* loci generalized while others did not, providing evidence of potential differences in genetic effect among race/ethnicity groups. Since LD structure also varies by ancestry, we can utilize this information to identify smaller regions that are more likely to be functionally relevant.

We identified three SNPs in the *APOE/APOC1/TOMM40* locus associated with CRP that generalized to other race/ethnicity groups. SNPs rs2075650 and rs6857 generalized only to Hispanics, while rs429358 demonstrated an association in European Americans, African Americans and Hispanics. Located in the third exon of the *APOE* gene, rs429358 is one of two non-synonymous SNPs that define the major ε2, ε3, and ε4 haplotypes of this region[32]. Our results align with previous studies finding this haplotype associated with serum CRP in European Americans[33], African Americans[34], Hispanics[35, 36], and Asian/Pacific Islanders[24]. The magnitude and direction of the effect were fairly consistent across race/ethnicity groups, supporting a jointly consistent association across multiple race/ethnicity groups. Previous

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studies suggest that SNPs at the *APOE/APOC1/TOMM40* locus appear to represent a single signal best represented by rs429358, with other SNPs appearing associated due to correlation[32, 37]. Given the consistency of effect seen in rs429358 across race/ethnicity groups compared to the other SNPs we evaluated in this region, our results appear to support this view, and extend this SNP finding to African Americans and Hispanics.

We also observed race/ethnicity-specific differences in association with CRP among SNPs in the *CRP* locus. Of the five SNPs demonstrated an overall association, four showed differences in race/ethnicity-specific results. While some of these SNPs may not have generalized to other groups due to smaller sample size, ancestry-related differences in genetic contributions to CRP concentrations at this locus are also possible. For example, for many SNPs in this region the allele frequencies in African Americans were different than other race/ethnicity groups, as previous studies have also highlighted[29]. In such a situation, investigating SNPs in multiple populations can be useful for evaluating which SNPs might be associated with CRP in multiple populations (rs1205), and which may be race/ethnicity-specific (rs4131568). Other SNPs may have a larger effect in one race/ethnicity group than another (rs1800947). If confirmed, this type of information could potentially be important for ensuring that genetic information is appropriately interpreted and targeted where there are differences in effect by race/ethnicity.

Pleiotropy

The third theme is that our results show evidence for potentially pleiotropic effects. As previously stated, most of the SNPs evaluated in this study have previously been associated with other inflammation-related phenotypes, such as HDL and LDL cholesterol, triglycerides, and coronary artery disease. Studies have also demonstrated associations between some of these SNPs and phenotypes as diverse as colon and rectal cancer (rs1205[7]), cervical cancer survival (rs1417938[38]), and fasting glucose concentration (rs780094[39]), though only this last trait reached genome-wide significance. Our results support several of these SNPs also being associated with serum CRP concentrations, suggesting that several of these SNPs and loci appear to have pleiotropic effects. This knowledge could be useful for exploring potentially shared disease etiology or mechanisms between phenotypes associated with a given pleiotropic SNP or locus.

We identified a potentially novel pleiotropic association between two correlated SNPs (CEU r^2=0.9) in the *CELSR2/PSRC1/SORT1* locus and serum CRP levels, as these SNPs have previously been associated with several other inflammation-related phenotypes but not CRP. SNP rs599839 has been associated with LDL cholesterol[40–45], coronary artery disease[41–43, 46], myocardial infarction[47], triglyceride metabolism[41], and coronary heart disease[45], while SNP rs646776 has been associated with LDL cholesterol[40] and progranulin levels[48]. Variations at rs646776 have also been strongly associated with transcript concentrations of the *CELSR2*, *PSRC1*, and *SORT1* genes[40]. Several studies have also found a significant association between variation in rs599839 and sortilin mRNA expression in the liver[40, 43, 49]. Sortilin, the gene product of *SORT1*, acts as a multiligand receptor and influences the uptake of LDL particles into cells, with studies suggesting that the G allele of rs599839 offers a protective effect against coronary artery disease mediated through LDL

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cholesterol lowering[43]. Our finding that SNPs in this region are also associated with CRP may provide additional insights on the inflammation pathway, and how these SNPs might impact multiple outcomes. Our potentially novel finding in this region also demonstrates the value of evaluating SNPs previously associated with related phenotypes for additional pleiotropic relationships.

Strengths of this study stem from the inclusion of well-characterized study populations with diverse race/ethnicity groups. We had large sample sizes overall, with large numbers for European Americans, African American, and Hispanics. While we had fewer Asian/Pacific Islander and American Indian participants, we were still able to evaluate several SNPs in these groups. While several SNPs in our study demonstrate potential pleiotropic associations with both CRP and inflammation-related traits, we are unable to evaluate whether these effects are truly independent, or if the association with one trait explains the other. Further work is needed to explore these complex relationships.

One limiting factor was that not all participants were genotyped for all SNPs, since SNP panels and race/ethnicity representation varied between participating studies. This reduced the overall sample size available for any given SNP- and race/ethnicity-specific association, though numbers were still large for most analyses, particularly in the European American, African American, and Hispanic groups. Unfortunately, for many SNPs the combination of smaller numbers or unavailable genotype information reduced or eliminated our ability to determine whether CRP associations generalized to the Asian/Pacific Islander or American Indian groups. Race/ethnicity differences in coded allele frequency may have also reduced our ability to detect an association in various groups. For some SNPs, these differences in allele frequency may at least partly explain why an association with CRP in one group did not generalize to another. For these reasons, a lack of generalization in our study should not be interpreted as proof that these SNPs are not associated with CRP in other groups, particularly for the Asian/Pacific Islander or American Indian groups where our ability to detect an association was reduced.

Another potential limiting factor is that we are unable to assess fully whether these potentially pleiotropic associations with CRP are due to correlation with the inflammation-related traits they have previously been associated with. However, previous studies of SNPs in the *CRP* gene have suggested that their associations with cardiovascular events are only slightly attenuated after adjustment for plasma CRP concentration[28]. Additionally, a sensitivity analysis in WHI for five of the SNPs in this study showed little effect when adjusting for previously associated traits (coronary heart disease, LDL cholesterol, and triglyceride levels). Together, this suggests that these pleiotropic associations may not be due to statistical correlation between CRP and the other inflammation-related traits, although this cannot be ruled out for all SNPs. Further exploration of the relationships between these traits is warranted.

While our analysis benefited from an a priori selection of interesting SNPs from previous GWAS, it is not a comprehensive analysis across all known loci given the rapid progress made since SNP selection. However, this limitation is lessened by our inclusion of the initial GWAS findings, which tend to have strong effect sizes, and hence tend to explain a larger

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fraction of the genetic variation than more recent GWAS findings. We applied a Bonferroni-corrected p-value to determine the statistical significance of a given association. While we adjusted for either 16 or 250 independent tests, a potential limitation is that we did not adjust for testing in multiple race/ethnicity groups. However, the Bonferroni adjustment is conservative in nature, and we tested GWAS findings for CRP or related traits. Many, though not all, of our findings greatly surpassed our corrected p-value and reached more stringent genome-wide significance levels ($p<5\times10^{-8}$).

In conclusion, our results support and extend previous observations of associations between CRP and genetic variation in several loci. We generalized several associations between SNPs and serum CRP levels previously identified in European Americans to African Americans or Hispanics, and we also identified a potentially novel CRP locus, previously associated with coronary artery disease and LDL cholesterol. Our findings demonstrate the benefit of evaluating genotype-phenotype associations in multiple race/ethnicity groups, and of looking for pleiotropic relationships among SNPs previously associated with related phenotypes. Additional follow-up and fine-mapping of these loci may lead to better characterization of the functional variants in these regions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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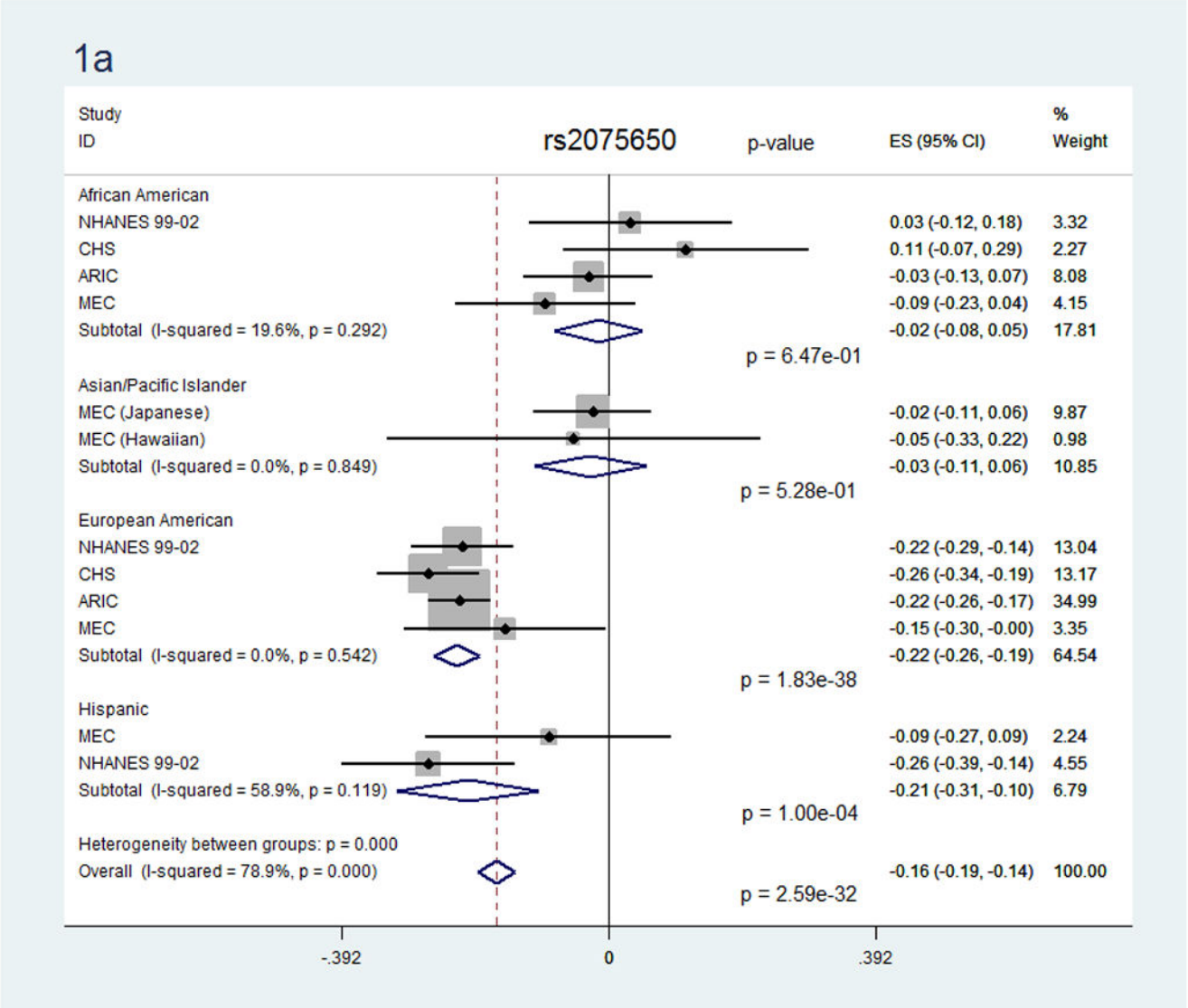

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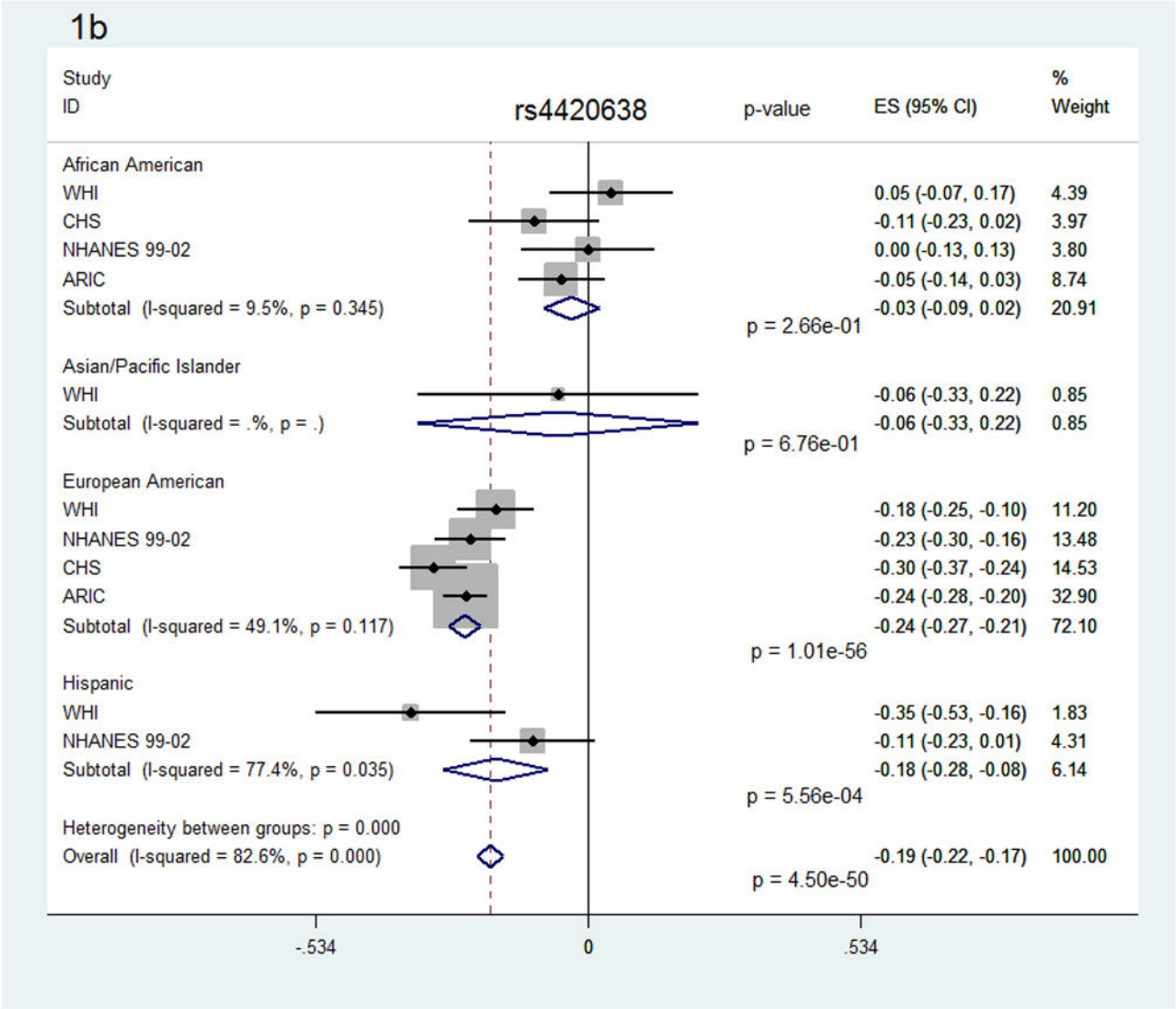

Figure 1.

Forest plots for the association of SNPs rs2075650 (**a**) and rs4420638 (**b**) with C-reactive protein, overall and by race/ethnicity group.

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Table 1

Demographic and epidemiologic characteristics of study populations used in this analysis, by race/ethnicity group and overall (and by study in Supplemental Table 2).

Measure	Unit	European American	African American	Hispanic	Asian/Pacific Islander	American Indian	Overall
Sample size	**n**	**24,958**	**8,471**	**2,935**	**980**	**3,129**	**40,473**
CRP(mg/dL)	Mean of study-specific medians(range)	1.9(1.0–2.9)	2.5(1.3–3.6)	2.3(1.2–3.1)	0.8(0.5–1.0)	3.9(3.9–3.9)	2.3(0.8–3.9)
Age(years)	Mean(SD)	62.1(12.6)	56.8(13.5)	51.3(16.5)	62.3(8.5)	39.3(16.4)	58.5(14.8)
Sex	%Female	66.6%	66.2%	62.5%	67.4%	61.5%	65.8%
BMI(kg/m^2)	Mean(SD)	27.9(5.7)	30.4(6.9)	28.7(5.7)	25.1(4.2)	32.4(7.9)	28.8(6.3)
Smoking	%Current	13.9%	20.8%	16.0%	7.4%	34.0%	16.9%
	%Former	40.0%	29.3%	26.6%	35.5%	23.3%	35.4%
Hormone Replacement Therapy	%Current	31.3%	18.7%	23.4%	39.0%	9.8%	26.6%
	%Former	16.7%	15.8%	15.7%	23.7%	11.4%	16.2%

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Table 2

Number of SNPs and participants available for meta-analysis across studies, overall and by race/ethnicity.

Race/ethnicity group	European American	African American	Hispanic	Asian/ Pacific Islander	American Indian	Overall
SNPs available in each study						
WHI	94	92	94	91		94
EAGLE	196	196	196			196
MEC	103	103	103	103		103
ARIC	31	31				31
CARDIA	7	7				7
CHS	111	118				118
SHS					19	19
Overall	265	266	261	150	19	266
Observations available per SNP						
Mean	7722	2367	1766	617	3129	12361
Minimum	233	433	243	388	3129	802
Maximum	24319	8158	2908	951	3129	36299

The number of SNPs and observations available are calculated from the number of SNPs genotyped (which varied by study) and the number of participants genotyped in each race/ethnicity group (which varied by study). Each study chose a subset of 266 SNPs to genotype. While these subsets overlapped, not all SNPs were available in all studies. In CHS, seven SNPs were available in African Americans but not in European Americans (rs10010131, rs10938397, rs1501980, rs16890979, rs174547, rs6544713, and rs7679). In WHI, SNPs were excluded from analyses in some race/ethnicity groups with fewer than 100 participants available for that SNP (rs13266634 and rs2383207 for African Americans; rs13266634, rs174547, and rs2383207 for Asian/Pacific Islanders; and all 94 SNPs for American Indians).

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Table 3

Association between serum C-reactive protein and SNPs previously associated with inflammation-related phenotypes

SNP	Allele, position, gene								Within-group	Between- group
Locus	**Previously associated trait**	**Race/ethnicity group**	**n**	**# Studies**	**Coded Allele Frequency**	**Beta**	**SE**	**P-value**	**p-hetero- geneity**	**p-hetero- geneity**
rs1205	T	European American	12400	2	0.33	**−0.17**	**0.01**	**1.03E-31**	0.02	
CRP	3′ UTR, *CRP*	African American	3669	2	0.20	**−0.27**	**0.04**	**8.09E-15**	0.61	
	C-reactive Protein	Hispanic	1842	1	0.35	**−0.22**	**0.04**	**5.37E-09**		
		OVERALL	17911	2		**−0.19**	**0.01**	**1.24E-50**		0.02
rs1800947	G	European American	12404	2	0.06	**−0.30**	**0.03**	**3.10E-25**	0.77	
CRP	Exonic, *CRP*	African American	3676	2	0.01	**−0.61**	**0.13**	**1.28E-06**	0.77	
	C-reactive Protein	Hispanic	1842	1	0.02	−0.36	0.13	6.72E-03		
		OVERALL	17922	2		**−0.32**	**0.03**	**1.13E-30**		0.05
rs1417938	A	European American	3943	1	0.30	**0.14**	**0.03**	**5.57E-07**		
CRP	Intronic, *CRP*	African American	1328	1	0.11	0.20	0.08	1.17E-02		
	C-reactive Protein	Hispanic	1845	1	0.36	**0.14**	**0.04**	**2.71E-04***		
		OVERALL	7116	1		**0.15**	**0.02**	**2.97E-11**		0.79
rs4131568	T	European American	11314	2	0.35	**0.08**	**0.02**	**1.24E-07**	0.31	
CRP	Upstream, *CRP*	African American	3461	2	0.07	−0.08	0.06	1.54E-01	0.96	
	C-reactive protein	Hispanic	1857	1	0.39	0.08	0.04	3.77E-02		
		OVERALL	16632	2		**0.07**	**0.01**	**2.45E-07**		0.02
rs3093058	T	European American	12420	2	0.001	0.32	0.22	1.43E-01	0.53	
CRP	Upstream, *CRP*	African American	3680	2	0.17	**0.48**	**0.04**	**1.40E-40**	0.87	
	C-reactive Protein	Hispanic	1842	1	0.01	**0.67**	**0.20**	**1.02E-03***		
		OVERALL	17942	2		**−0.18**	**0.03**	**1.44E-43**		0.50
rs4420638	G	European American	21378	4	0.18	**−0.24**	**0.02**	**1.01E-56**	0.12	
APOE/APOC/TOMM40	Downstream, *APOC1*	African American	6076	4	0.20	−0.03	0.03	2.66E-01	0.35	
	LDL Cholesterol	Hispanic	2637	2	0.10	−0.18	0.05	5.58E-04	0.04	
		Asian/Pacific Islander	422	1	0.12	−0.06	0.14	6.76E-01		
		OVERALL	30513	5		**−0.19**	**0.01**	**4.50E-50**		**1.64E-09**
rs2075650	G	European American	15926	4	0.14	**−0.22**	**0.02**	**1.83E-38**	0.54	
APOE/APOC/TOMM40	Intronic, *TOMM40*	African American	4729	4	0.14	−0.02	0.03	6.47E-01	0.29	

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SNP	Allele, position, gene								Within-group	Between- group
Locus	**Previously associated trait**	**Race/ethnicity group**	**n**	**# Studies**	**Coded Allele Frequency**	**Beta**	**SE**	**P-value**	**p-hetero- geneity**	**p-hetero- geneity**
	LDL Cholesterol	Hispanic	2119	2	0.10	**−0.21**	**0.05**	**1.00E-04**	0.12	
		Asian/Pacific Islander	528	2	0.18	−0.03	0.04	5.28E-01	0.85	
		OVERALL	23302	5		**−0.16**	**0.01**	**2.59E-32**		**1.60E-09**
rs429358	C	European American	6993	2	0.13	**−0.24**	**0.04**	**2.41E-10**	0.61	
APOE/APOC/TOMM40	Exonic, *APOE*	African American	2220	2	0.20	**−0.23**	**0.04**	**5.07E-08**	0.05	
	Cardiovascular Disease	Hispanic	1031	2	0.11	**−0.36**	**0.07**	**5.08E-08**	0.02	
		Asian/Pacific Islander	943	2	0.11	−0.19	0.05	2.25E-04	0.84	
		OVERALL	11187	2		**−0.24**	**0.02**	**1.84E-25**		0.23
rs6857	T	European American	3955	1	0.16	**−0.23**	**0.04**	**2.07E-10**		
APOE/APOC/TOMM40	3′ UTR, *PVRL2*	African American	1331	1	0.06	−0.23	0.11	3.04E-02		
	Cardiovascular Disease	Hispanic	1852	1	0.10	**−0.28**	**0.06**	**6.12E-06**		
		OVERALL	7138	1		**−0.24**	**0.03**	**7.30E-16**		0.79
rs1260326	T	European American	22661	5	0.42	**0.10**	**0.01**	**2.38E-17**	0.16	
GCKR	Exonic, *GCKR*	African American	6506	5	0.15	0.06	0.03	2.06E-02	0.42	
	Triglycerides	Hispanic	2873	3	0.33	0.08	0.03	5.78E-03	0.45	
		Asian/Pacific Islander	951	2	0.54	0.09	0.03	2.04E-03	0.11	
		OVERALL	32991	6		**0.09**	**0.01**	**5.34E-22**		0.72
rs780094	T	European American	17567	5	0.40	**0.10**	**0.01**	**1.53E-16**	0.32	
GCKR	Intronic, *GCKR*	African American	6175	5	0.19	0.03	0.03	2.26E-01	0.23	
	C-reactive Protein	Hispanic	2130	2	0.34	0.07	0.03	2.62E-02	0.26	
		Asian/Pacific Islander	528	1	0.54	0.05	0.03	1.51E-01	0.63	
		OVERALL	26400	6		**0.08**	**0.01**	**5.65E-17**		0.09
rs2650000	A	European American	16506	5	0.35	**−0.12**	**0.01**	**2.62E-23**	0.18	
HNF1A	Upstream, *HNF1A*	African American	5983	5	0.12	−0.09	0.03	5.24E-03	0.49	
	LDL Cholesterol	Hispanic	2125	2	0.36	−0.11	0.03	9.45E-04	0.08	
		Asian/Pacific Islander	500	1	0.45	−0.03	0.03	3.61E-01	0.25	
		OVERALL	25114	6		**−0.11**	**0.01**	**4.34E-26**		0.08
rs7310409	A	European American	3956	1	0.40	**−0.18**	**0.03**	**1.57E-10**		
HNF1A	Intronic, *HNF1A*	African American	1327	1	0.32	−0.15	0.06	7.94E-03		
	C-reactive protein	Hispanic	1855	1	0.41	**−0.13**	**0.04**	**1.13E-03***		

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SNP	Allele, position, gene								Within-group	Between- group
Locus	**Previously associated trait**	**Race/ethnicity group**	**n**	**# Studies**	**Coded Allele Frequency**	**Beta**	**SE**	**P-value**	**p-hetero- geneity**	**p-hetero- geneity**
		OVERALL	7138	1		**−0.16**	**0.02**	**3.33E-14**		0.59
rs2228145	C	European American	17184	4	0.40	**−0.10**	**0.01**	**1.47E-18**	0.09	
IL6R	Exonic, *IL6R*	African American	5942	4	0.14	**−0.13**	**0.03**	**6.72E-05**	0.27	
	C-reactive Protein	Hispanic	1841	1	0.52	**−0.18**	**0.04**	**2.18E-06**		
		OVERALL	24967	5		**−0.11**	**0.01**	**3.66E-26**		0.16
rs1892534	T	European American	11344	2	0.39	**−0.08**	**0.02**	**5.80E-08**	0.13	
LEPR	Downstream, *LEPR*	African American	3471	2	0.46	−0.08	0.03	4.45E-03	0.49	
	C-reactive protein	Hispanic	1851	1	0.50	**−0.12**	**0.04**	**1.99E-03***		
		OVERALL	16666	2		**−0.09**	**0.01**	**1.02E-11**		0.67
rs1800961	T	European American	17660	5	0.031	**−0.15**	**0.03**	**4.75E-06**	0.39	
HNF4A	Exonic, *HNF4A*	African American	6222	5	0.007	0.00	0.10	9.86E-01	0.27	
	HDL Cholesterol	Hispanic	2117	2	0.039	−0.06	0.08	4.30E-01	0.99	
		Asian/Pacific Islander	523	1	0.001	−0.18	0.22	4.05E-01	0.89	
		OVERALL	26522	6		**−0.13**	**0.03**	**9.39E-06**		0.45
rs599839	G	European American	22427	5	0.23	**0.05**	**0.01**	**1.01E-04**	0.68	
CELSR2/PSRC1/SORT1	Downstream, *PSRC1*	African American	6596	5	0.71	0.04	0.02	5.95E-02	0.52	
	Coronary Artery	Hispanic	2811	3	0.22	0.06	0.03	6.96E-02	0.25	
	Disease	Asian/Pacific Islander	946	2	0.08	0.05	0.05	3.24E-01	0.34	
		OVERALL	32780	6		**0.05**	**0.01**	**1.96E-06**		0.96
rs646776	C	European American	22652	5	0.22	0.05	0.01	2.02E-04	0.66	
CELSR2/PSRC1/SORT1	Downstream, *CELSR2*	African American	6479	5	0.35	0.03	0.02	1.97E-01	0.65	
	LDL Cholesterol	Hispanic	2889	3	0.19	0.04	0.03	2.72E-01	0.18	
		Asian/Pacific Islander	951	2	0.07	0.08	0.06	1.82E-01	0.05	
		OVERALL	32971	6		**0.04**	**0.01**	**3.06E-05**		0.78

CAF=Coded Allele Frequency; SE=Standard Error;

*P-values below threshold for replication ($p<3.1\times10^{-3}$) but not pleiotropy discovery ($p<2.0\times10^{-4}$).

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Table 4

SNPs showing a potential sex difference in association with C-reactive protein (p-heterogeneity<0.05).

		Male				**Female**				**Between sex**
Locus	SNP(coded allele)	N	Beta	SE	P-value	N	Beta	SE	P-value	P-heterogeneity
CRP	rs1417938(A)	**3449**	**−0.1**	**0.03**	**1.24E-03***	**3667**	**−0.19**	**0.03**	**2.01E-09**	0.03
APOE/APOC1/TOMM40	rs4420638(G)	**9234**	**−0.23**	**0.02**	**6.99E-27**	**21365**	**−0.17**	**0.02**	**2.01E-26**	0.03
APOE/APOC1/TOMM40	rs429358(C)	**1012**	**−0.16**	**0.04**	**1.18E-04**	**10260**	**−0.27**	**0.03**	**2.96E-23**	0.03
GCKR	rs1260326(T)	10664	0.04	0.01	2.10E-03	**22327**	**0.12**	**0.01**	**8.96E-23**	1.54E-08
GCKR	rs780094(T)	**12095**	**0.04**	**0.01**	**2.85E-03***	**14305**	**0.12**	**0.01**	**4.74E-18**	1.54E-08
HNF1A	rs2650000(A)	**11545**	**−0.13**	**0.01**	**1.48E-18**	**13569**	**−0.09**	**0.01**	**1.16E-10**	0.01

*P-values below threshold for replication ($p<3.1\times10^{-3}$) but not pleiotropy discovery ($p<2.0\times10^{-4}$).