Published in final edited form as:

Hum Genet. 2014 August ; 133(8): 985–995. doi:10.1007/s00439-014-1439-z.

Large Multiethnic Candidate Gene Study for C-Reactive Protein Levels: Identification of a Novel Association at *CD36* in African Americans

Jaclyn Ellis,

Department of Genetics, University of North Carolina, Chapel Hill, NC, 27599

Ethan M. Lange,

Department of Genetics, University of North Carolina, Chapel Hill, NC, 27599

Department of Biostatistics, University of North Carolina, Chapel Hill, NC, United States of America, 27599

Jin Li,

Department of Genetics, University of North Carolina, Chapel Hill, NC, 27599

Josee Dupuis,

National Heart, Lung, and Blood Institute's and Boston University's Framingham Heart Study, Framingham, MA, United States of America, 01702

Department of Biostatistics, Boston University School of Public Health, Boston, MA, United States of America, 02118;

Jens Baumert,

Institute of Epidemiology II, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany, 23538

Jeremy D. Walston,

Division of Geriatric Medicine and Gerontology, Johns Hopkins University School of Medicine, Baltimore, MD, United States of America, 21205

Brendan J. Keating,

Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA, United States of America, 19104

Peter Durda,

Departments of Pathology and Biochemistry, University of Vermont College of Medicine, Burlington, VT, United States of America, 05405

Ervin R. Fox,

Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, MS, United States of America, 39216

Address correspondence and reprint requests to: Leslie A. Lange, Ph.D., Department of Genetics, University of North Carolina, 5112 Genetic Medicine Bldg., Chapel Hill, NC 27599-7264, Phone: (919)966-9562, Fax: (919)843-4682, leslie_lange@med.unc.edu.

Conflict of Interest

The authors have no conflicts of interest to disclose.

Cameron D. Palmer,

Program in Medical and Population Genetics, Broad Institute, Cambridge, MA, United States of America, United States of America, 02142

Divisions of Genetics and Endocrinology and Program in Genomics, Children's Hospital Boston, Boston, MA, United States of America, 02115;

Yan A. Meng,

Program in Medical and Population Genetics, Broad Institute, Cambridge, MA, United States of America, United States of America, 02142

Taylor Young,

Program in Medical and Population Genetics, Broad Institute, Cambridge, MA, United States of America, United States of America, 02142

Deborah N. Farlow,

Program in Medical and Population Genetics, Broad Institute, Cambridge, MA, United States of America, United States of America, 02142

Renate B. Schnabel,

Department of General and Interventional Cardiology, University Heart Center Hamburg, Hamburg, Germany, 21046

Carola S. Marzi,

Institute of Epidemiology II, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany, 23538

Emma Larkin,

Department of Medicine, Division of Allergy, Pulmonary and Critical Care, Vanderbilt University Medical Center, Nashville, TN, United States of America, 37232

Lisa W. Martin,

Division of Cardiology, George Washington School of Medicine, Washington, DC, United States of America, 20037

Joshua C. Bis,

Cardiovascular Health Research Unit and Department of Medicine, University of Washington, Seattle, WA, United States of America, 98101

Paul Auer,

Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA, United States of America, 98109

Vasan S. Ramachandran,

Department of Medicine, Boston University School of Medicine, Boston, MA, United States of America, 02118

Stacey B. Gabriel,

Program in Medical and Population Genetics, Broad Institute, Cambridge, MA, United States of America, United States of America, 02142

Monte S. Willis,

Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC, 27599

James S. Pankow,

Division of Epidemiology and Community Health, University of Minnesota School of Public Health, Minneapolis, MN, United States of America, 55455

George J. Papanicolaou,

Division of Cardiovascular Sciences, National Heart, Lung and Blood Institute, Bethesda, MD, United States of America, 20892

Jerome I. Rotter,

Departments of Pediatrics, Medicine, and Human Genetics, University of California, Los Angeles, CA, United States of America, 90095

Institute for Translational Genomics and Population Sciences, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Los Angeles, CA, United States of America

Division of Genomic Outcomes, Department of Pediatrics, Harbor-UCLA Medical Center, Los Angeles, CA, United States of America

Christie M. Ballantyne,

Section of Cardiovascular Research, Baylor College of Medicine, Center for Cardiovascular Disease Prevention, Methodist DeBakey Heart Center, Houston, TX, United States of America, 77030

Myron D. Gross,

Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN, United States of America, 55455

Guillaume Lettre,

Montréal Heart Institute, Montréal, Canada, H3A 2T5

Département de Médecine, Université de Montréal, Montréal, Canada, H3A 2T5

James G. Wilson,

Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, MS, United States of America, 39216

Ulrike Peters,

Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington, United States of America, 98109

Wolfgang Koenig,

Department of Internal Medicine II–Cardiology, University of Ulm Medical Center, Ulm, Germany, 07304

Russell P. Tracy,

Departments of Pathology and Biochemistry, University of Vermont College of Medicine, Burlington, VT, United States of America, 05405

Susan Redline,

Department of Medicine, Brigham and Women's Hospital and Beth Israel Deaconess Medical Center, Boston, MA, United States of America

Alex P. Reiner,

Department of Epidemiology, University of Washington, Seattle, WA, United States of America, 98195

Emelia J. Benjamin, and

National Heart, Lung, and Blood Institute's and Boston University's Framingham Heart Study, Framingham, MA, United States of America, 01702

Department of Epidemiology, Boston University School of Public Health, Boston, MA, United States of America, 02118

Leslie A. Lange

Department of Genetics, University of North Carolina, Chapel Hill, NC, 27599

Abstract

C-reactive protein (CRP) is a heritable biomarker of systemic inflammation and a predictor of cardiovascular disease (CVD). Large-scale genetic association studies for CRP have largely focused on individuals of European descent. We sought to uncover novel genetic variants for CRP in a multi-ethnic sample using the ITMAT Broad-CARE (IBC) array, a custom 50,000 SNP gene-centric array having dense coverage of over 2,000 candidate CVD genes. We performed analyses on 7570 African Americans (AA) from the Candidate gene Association Resource (CARE) study and race-combined meta-analyses that included 29,939 additional individuals of European descent from CARE, the Women's Health Initiative (WHI) and KORA studies. We observed array-wide significance ($p < 2.2 \times 10^{-6}$) for four loci in AA, three of which have been reported previously in individuals of European descent (*IL6R*, $p = 2.0 \times 10^{-6}$; *CRP*, $p = 4.2 \times 10^{-71}$; *APOE*, $p = 1.6 \times 10^{-6}$). The fourth significant locus, *CD36* ($p = 1.6 \times 10^{-6}$), was observed at a functional variant (rs3211938) that is extremely rare in individuals of European descent. We replicated the *CD36* finding ($p = 1.8 \times 10^{-5}$) in an independent sample of 8041 AA women from WHI; a meta-analysis combining the CARE and WHI AA results at rs3211938 reached genome-wide significance ($p = 1.5 \times 10^{-10}$). In the race-combined meta-analyses, 13 loci reached significance, including ten (*CRP*, *TOMM40/APOE/APOC1*, *HNF1A*, *LEPR*, *GCKR*, *IL6R*, *IL1RN*, *NLRP3*, *HNF4A* and *BAZ1B/BCL7B*) previously associated with CRP, and one (*ARNTL*) previously reported to be nominally associated with CRP. Two novel loci were also detected (*RPS6KBI*, $p = 2.0 \times 10^{-6}$; *CD36*, $p = 1.4 \times 10^{-6}$). These results highlight both shared and unique genetic risk factors for CRP in AA compared to populations of European descent.

Keywords

C-reactive protein; Inflammation; Multi-ethnic; Candidate gene

INTRODUCTION

C-reactive protein (CRP) is a pentameric acute-phase protein that is a hallmark of low-grade systemic inflammation [1]. Vascular inflammation is thought to play a role in the

development and progression of atherosclerosis, ultimately leading to plaque rupture and cardiovascular disease (CVD) events such as myocardial infarction [2]. Associations between CRP and CVD outcomes have been remarkably consistent despite varying study designs, target populations, and case classification methods [3]. Observational studies have shown increased levels of CRP to be present in individuals with factors such as older age [4], female sex [5], smoking [6], obesity [7], diabetes, atherosclerotic CVD [8,9], sleep curtailment [10] and sleep apnea[11]. Heritability estimates for CRP concentration range from 35–40% [12], supporting that genetic factors are likely to influence variation in CRP levels [3].

Multiple candidate gene and genome-wide association studies (GWAS) have been performed for CRP resulting in several reported associated loci. These associations include genes known to be involved in the regulation of inflammatory and metabolic pathways, some of which were not previously known to directly influence CRP levels. The Women's Genome Health Study identified single nucleotide polymorphisms (SNPs) associated with CRP in the leptin-receptor gene (*LEPR*), glucokinase regulatory protein (*GCKR*), and hepatic transcription factor gene (*HNF1A*) [13]. Recently, a GWAS meta-analysis of participants of European ancestry confirmed association of previously identified loci with CRP and introduced 11 novel loci, including *NLRP3*, *HNF4A*, *RORA*, *IRF1*, and *IL1F10* [14]. To our knowledge, only two published GWASs for CRP in individuals of African ancestry have been conducted [15,16], and the first was based on a relatively small cohort of individuals[14]. This earlier study identified several variants in the *CRP* gene that were associated with CRP, but no other loci were statistically significant [15]. The latter study, which included 8280 African American (AA) women from the Women's Health Initiative (WHI) study, also identified a number of variants associated with CRP in the *CRP* gene as well as significant evidence for associations in or near *IL1F10/IL1RN*, *TREM2*, *HNF1A* and *TOMM40/APOE* [16].

We sought to extend what is known regarding the genetic underpinnings of CRP by performing multi-ethnic meta-analyses, including individuals of both African and European ancestry genotyped across a densely covered gene-based array. Participants for the primary analyses came from eight community-based cohorts from the Candidate Gene and Association Resource (CARE) consortium (AAs and European Americans [EAs]), WHI (EAs) and the Cooperative Health Research in the Region of Augsburg (KORA) study (Europeans). All participants had available genotype data from the ITMAT Broad-CARE (IBC) Chip, a custom 50,000 SNP gene-centric array having dense coverage of over 2000 candidate genes within CVD related pathways. An independent sample of AA participants from the WHI study with IBC chip data were used as a follow-up sample for interesting findings.

MATERIALS AND METHODS

Each study was reviewed by a local ethics board and all participants consented to genetic research. Genotype and phenotype data for all study participants, with the exception of KORA participants, are available through the NCBI dbGaP resource (www.ncbi.nlm.nih.gov/gap).

Study samples

CARe—The CARe (Candidate Gene Association Resource) consortium consists of nine studies. The purpose of the consortium was to bring together deeply-phenotyped prospective cohort studies to increase power for genetic association scans of CVD and other disorders [17]. Cohorts included in these analyses of CRP levels are: Atherosclerosis Risk in Communities (ARIC) (n=7572 EA; n=1983 AA), Coronary Artery Risk in Young Adults (CARDIA) (n=1318 EA; n=1118 AA), Cleveland Family Study (CFS) (n=281 EA; n=369 AA), the Cardiovascular Health Study (CHS) (n=3919 EA; n=736 AA), Framingham Heart Study (FHS) (n=7543 EA), Jackson Heart Study (JHS) (n=2026 AA), and Multi-Ethnic Study of Atherosclerosis (MESA) (n=2051 EA; n=1338 AA).

WHI—The Women's Health Initiative (WHI) is one of the largest (n=161,808) studies of women's health ever undertaken in the U.S. [18]. A diverse population was recruited from 1993–1998 at 40 clinical centers across the U.S. A total of n=4389 EA WHI subjects with CRP measures were included in the current study.

KORA—The MONItoring of trends and determinants in Cardiovascular disease/Cooperative Health Research in the Region of Augsburg (MONICA/KORA) study is a series of population-based surveys conducted in the region of Augsburg in Southern Germany [19]. The sample used in the current study consisted of n=2866 EA subjects with CRP measures selected from 1075 participants for KORA S12 and 1800 participants for KORA F3.

Further details of the participating CARe, WHI and KORA studies are reported in the Supplemental Materials.

IBC genotype array

The IBC SNP array is described in detail in Keating et al. [20]. The IBC SNP array includes 49,320 SNPs selected across ~2000 candidate loci for CVD. The array includes SNPs that capture patterns of genetic variation in both European- and African-descent populations. Genotyping for the CARe cohorts was performed at the Broad Institute (Cambridge, MA).

Quality control of genetic data

Criteria for DNA sample exclusion based on genotype data included sex mismatch, discordance among duplicate samples, or sample call rate <90%. Approximately 2.5% of CARe subjects initially included in genotyping efforts did not pass the required call rate. For each set of duplicates or monozygotic twins, data from the sample with the highest genotyping call rate were retained. SNPs were excluded when monomorphic, the call rate was <95%, or when significant departures from expected Hardy-Weinberg equilibrium (HWE) genotype proportions were observed ($p < 10^{-5}$ in EAs). Given the genetic admixture in AAs, there was no HWE filter used for these samples. After these exclusions were applied, data remained on 47,539 SNPs.

Data analysis

Participants with CRP measurement over 100 mg/L were excluded from analysis as these observations would be highly influential and potentially a result of acute infection. We natural log-transformed CRP level in order to generate an approximately normal distribution of model residuals, conditional on the covariates, to meet linear model assumptions. We assumed an additive genetic model in all tests of association. We used the linear regression model implemented in PLINK [21] for studies with unrelated individuals and the linear mixed effects model implemented in the program GWAF for cohorts with related individuals [22] to test for association between log-CRP and genotype at each SNP, adjusting for covariates. All models were stratified by cohort and race. Covariate adjustment was applied for age, sex, current smoking, body mass index and the first 10 principal components calculated using the program EIGENSTRAT [23] to control for potential population substructure. After obtaining cohort- and race-specific results, we performed a fixed-effects, inverse-variance-weighted meta-analysis using the METAL software [24]. Meta-analysis was performed separately by race and race-combined. Heterogeneity of effects across studies was tested in METAL using Cochran's Q statistic.

Based on a simulation analysis performed, the effective number of independent tests was estimated to be 26,482 for the AAs and 20,544 for samples of primarily European ancestry after accounting for the linkage disequilibrium between markers on the IBC array [25]. To maintain an approximate overall type 1 error rate of 5%, a uniform statistical threshold of $\alpha = 2.2 \times 10^{-6}$ (0.05/25,000) was recommended to declare array-wide (experiment-wide) significance [25].

Genotyping and imputation in WHI AA follow-up sample

Genome-wide genotyping for WHI AA participants was performed at Affymetrix using the Affymetrix 6.0 array. A total of 8421 AAs had genotype data that passed quality control. A reference sample of 761 AA NHLBI Exome Sequencing Project (ESP) participants was used for imputation of *CD36* rs3211938 into 8041 AA WHI individuals with GWAS data using the programs MaCH 1.0.18 [26] and minimac [27]. Additional details on the genotype imputation are given in Auer et al. [28].

RESULTS

Characteristics of the 7570 AA and 29,939 European-ancestry study participants from CARE, WHI and KORA are reported in Table 1.

African Americans

Four loci reached IBC array-wide significance ($p < 2.2 \times 10^{-6}$) in AAs (Table 2), including three loci reported in previous GWAS for CRP in individuals of European descent (*CRP*, *IL6R*, *APOE*). The fourth significant result, at rs3211938 in the gene encoding the cluster differentiation 36-membrane protein (*CD36*; $p = 1.4 \times 10^{-6}$), has not been reported previously for association with CRP. A locus zoom plot of the *CD36* region is presented in Supplementary Figure 1, demonstrating the focused association at rs3211938. A Forest plot illustrating cohort specific results is presented in Supplementary Figure 2. We further tested

this SNP using imputed genotype data in an independent sample of 8041 African Americans women from WHI. The association was confirmed, with the minor allele of *CD36* rs3211938 associated with 0.128 (± 0.030) lower CRP levels ($p=1.8\times 10^{-5}$). Together, in a combined meta-analysis of CARE and WHI AA participants, results at *CD36* rs3211938 reached genome-wide significance ($p=1.5\times 10^{-10}$). Of note, *CD36* rs3211938 was not studied in the recent WHI AA GWAS [16]. There was no evidence for any heterogeneity in the results across the AA cohorts for any of the four significant loci (Table 2).

Out of the five loci previously reported to be associated with CRP in AA women [16], we had good proxies for SNPs at *CRP* and *TOMM40* but no available satisfactory proxies for the remaining gene regions (*HNFI1A*, *ILF10/IL1RN*, and *TREM2*). Our top result occurred at *CRP* rs3093058 ($p=4.2\times 10^{-71}$) (Table 2), which is in strong linkage disequilibrium (LD) with, and our best available proxy for (based on 1000 Genomes data in individuals of African ancestry (YRI)), rs16827466 ($r^2=0.89$), the top variant reported by Reiner et al. [16]. Our significant result at *APOE* was for a SNP, rs769450 ($p=2.0\times 10^{-6}$), which is in modest LD ($r^2=0.28$) with the top SNP, rs1160985 in nearby *TOMM40*, reported by Reiner et al. Our best proxy SNP for rs1160985 (rs405509, $r^2=0.66$) demonstrated nominal evidence for association with CRP ($p=1.2\times 10^{-4}$). We had poor proxies available for the top SNPs reported for the other three significant loci in Reiner et al. Our best proxy for *ILF10/IL1RN* rs6734238, rs17042795 ($r^2=0.28$) demonstrated a trend towards association ($p=0.068$), as did our best proxy for *HNFI1A* rs7979473 (rs1169293, $r^2=0.33$, $p=0.052$). We did not find any evidence for association at our best proxy (rs6933067, $r^2=0.39$, $p=0.14$) for the reported novel *TREM2* rs7748513 association. When our analyses were restricted to AA women, rs3093058 was significant ($p=9.4\times 10^{-44}$), while rs769450 ($p=0.0011$), rs1169293 ($p=0.027$) and rs6933067 ($p=0.029$) were nominally significant. No evidence for association was observed for rs405509 ($p=0.24$) or rs17042795 ($p=0.38$).

Combined Race Meta-Analysis

We observed significant signals ($p<2.2\times 10^{-6}$) at 13 loci, including seven loci widely reported to be associated with circulating CRP levels (*CRP*, *TOMM40/APOE/APOC1*, *HNFI1A*, *LEPR*, *GCKR*, *IL6R*, and *IL1RN*) and three (*BAZ1B/BCL7B*, *NLRP3* and *HNFI4A*) recently reported as significant by the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium (Table 3) [14]. We also observed array-wide significance for *ARNTL*, which was reported by CHARGE to be “suggestive” (i.e. nominally associated with $p<1.0\times 10^{-5}$ with CRP levels), and two novel loci, *RPS6KB1* and *CD36*.

The direction and size of the effects were largely consistent between AAs and individuals of European descent for most of these 13 loci (Table 3). In some cases, the frequency of the minor allele was considerably different between the populations, which could largely explain the absence of significant evidence for association in one population or the other. One SNP, rs2075650 in *TOMM40*, had highly discrepant results between AAs and individuals of European descent (see Supplementary Figure 3). There was no evidence of association for the variant in AAs; in fact, there was a near trend for an effect in the opposite direction as was observed in participants of European descent. Both AAs and individuals of European descent had evidence for association at nearby SNP rs769450 (AAs see Table 2;

Europeans, $\beta(\text{SE}) = 0.033(0.009)$, $p = 1.0 \times 10^{-4}$). The two variants are in weak LD (estimated r^2 in YRI = 0.088, r^2 in CEU = 0.13) in populations of African and European ancestry.

Analyses of European Americans not included in CHARGE consortium

A subset of the current study samples was also included in the CHARGE report, which included ~66,000 participants of European descent with CRP and genotype measurements [14]. Genotype data used in the CHARGE report were obtained across a variety of genome-wide marker platforms; genotype imputation was used to probabilistically infer missing genotype data for SNPs in the HapMap database. The current study, which includes 29,939 samples of European descent, has 21,900 overlapping European-ancestry samples with the CHARGE report (subjects of European descent from ARIC, CHS, FHS and KORA). Notably, no AA participants were included in the CHARGE report.

Focusing on the CHARGE non-overlap EA sample (EA subjects from CARDIA, CFS, MESA and WHI; $n=8039$), we identified significant evidence for six loci (*CRP*, *TOMM40/APOE/APOC1*, *HNF1A*, *LEPR*, *GCKR*, *IL6R*) widely recognized to be associated with CRP (Table 4). We observed evidence supporting two genes newly reported to be associated with CRP in the CHARGE report (*HNF4A* rs1800961 $p=5.4 \times 10^{-4}$; *NLRP3* rs12239046 $p=0.011$), providing, to our knowledge, the first reported confirmation for these findings. We also observed indirect evidence, through a proxy SNP, supporting another novel CHARGE finding, rs13233571 in the *BAZ1B/BCL7B* gene cluster. We observed nominal evidence at *BAZ1B* rs714052 ($p=7.6 \times 10^{-3}$), which is in strong LD with the reported CHARGE *BCL7B* SNP ($r^2=0.93$ in CEU HapMap samples). Additionally, we observed indirect evidence, through proxy SNP rs6486121 ($p=0.0025$), supporting an association at rs6486122 in *ARNTL* reported to be nominally significant in CHARGE. Interestingly, in this non-overlap EA sample, there was no evidence of association at *RPS6KBI* rs1292034 ($p=0.41$), which we identified as a novel locus in our combined meta-analysis (see Supplementary Figure 4 for cohort specific results). Similar to AA results, there was no evidence for any heterogeneity in the results across the EA cohorts for any of the four significant loci in this non-overlap EA sample (Table 4).

DISCUSSION

We performed a dense candidate gene-based scan of approximately 50,000 SNPs covering approximately 2,000 gene regions in a bi-racial sample of 37,509 individuals, including 21,900 EA subjects previously studied in the CHARGE GWAS [14]. We observed significant evidence for seven loci widely reported to be associated with CRP, which include: *CRP*, *TOMM40/APOE/APOC1*, *HNF1A*, *LEPR*, *IL6R*, *GCKR*, and *IL1RN*. We also observed IBC array-wide significant evidence for association at *HNF4A*, *NLRP3*, and *BAZ1B/BCL7B*, loci that were reported to be significantly associated with CRP in the CHARGE GWAS [14]. Analyses based on an independent subset of European decent samples not included in the CHARGE report provide supporting evidence for these associations. We also observed significant evidence for association at *ARNTL*, a locus reported as suggestive in the CHARGE report. Finally, we identified two novel loci, *CD36*

and *RPS6KBI*, which have not been previously reported to be significantly associated with CRP. The *CD36* association at rs3211938 is specific to AAs. We replicated this finding in an independent AA sample (n=8041) from WHI. A meta-analysis at rs3211938 including results from the CARE AA cohorts and WHI resulted in a genome-wide significant association. No evidence for the *RPS6KBI* rs1292034 association was observed in the non-overlap CHARGE sample of European descent (beta= -0.013, p=0.41); the evidence for this association was considerably stronger in the subset of CHARGE European descent samples (beta= -0.047; p=1.7×10⁻⁶). The direction and size of effect in AA was similar to the CHARGE EA subset for rs1292034, but the association was not significant (beta= -0.039; p=0.079). Given that rs1292034 was not reported to be significantly associated with CRP in the complete CHARGE study, future studies will be necessary to replicate our association between rs1292034 and CRP.

The *CD36* gene encodes a cellular receptor that facilitates fatty acid (FA) uptake and the utilization of key metabolic tissues [29,30]. Individuals with mutations in *CD36* have a defective FA uptake, which could result in a poor metabolic profile and elevated serum lipid levels [30,31]. Patients with elevated lipid levels (i.e. LDL cholesterol) are more likely to develop atherosclerosis, which most broadly defines cardiovascular disease risk. Various scavenger receptors have been recognized for their role in mediating the uptake of oxidized LDL (ox-LDL) leading to the formation of foam cells that is a precursor step in the development of atherosclerotic lesions [32]. *CD36* is a scavenger receptor involved in the uptake of these oxidized lipids and thus plays a role in the formation of atheroma [32]. Because the uptake of ox-LDL occurs on multiple cells types, (endothelial cells, macrophages, vascular smooth muscle cells, etc), the role of *CD36* in atherogenesis is complex. A study using hyperlipidemic mice showed that the deletion of *CD36* or *SR-A* (a type A scavenger receptor) decreases lesion formation but does not abrogate foam cell formation suggesting that scavenger receptors may not be absolutely necessary for this process [32,33]. Interestingly, an *in vitro* experiment showed that the addition of CRP to ox-LDL in a cell culture system stimulated foam cell formation suggesting that CRP may have an active role in enhancing foam cell formation [34]. Together, these findings suggest that CRP and *CD36* may have a cooperative role in atherogenesis.

The influence of natural selection, linked to malaria susceptibility, has resulted in the high genetic variation of *CD36* in populations of African descent [35,36]; as a result, *CD36* mutations are ten times more frequent in African populations versus individuals of primarily European descent [37]. The link to malaria susceptibility results in the gene's role as a receptor for *Plasmodium falciparum* infected erythrocytes, which is found in malaria patients. Variants in this gene have also been reported be associated with metabolic syndrome, [37] high density lipoprotein (HDL) cholesterol levels [17,38], and abnormal serum FA [39–41]. A study using AA participants from HyperGEN demonstrated that *CD36* variants account for ~3.4% of inter-individual HDL variability in the study population [42]. The variant allele at rs3211938 creates a premature stop codon and thus a truncated form of the CD36 protein. This variant has only been identified in populations of African descent, having thought to arise as a result of positive selective pressure, and thus its predictive impact on CD36 expression would not apply to other populations [43,44]. In a recent study

by Love-Gregory, et al., the minor allele (G) for rs3211938 was associated with increased HDL and reduced CD36 protein expression on monocytes [38]. The same coding allele (G) for rs3211938 was associated with lower CRP levels in our analysis, suggesting that *CD36* SNPs may elucidate a biological link between CRP and HDL levels. A recent GWAS study on platelet count and mean platelet volume using 16,388 AA individuals from the Continental Origins and Genetic Epidemiology Network (COGENT) identified novel associations at two intronic SNPs at the *CD36* gene [45]. Previous evidence has demonstrated that one of the variants (rs17154155) is associated with platelet function as well as *CD36* expression on platelets [46,47]; rs17154155 is in LD with the rs3211938 ($r^2=0.27$). Further analysis on less common *CD36* variants in AAs from the National Heart Lung and Blood Institute Exome Sequence Project found rs3211938 to be associated with lower platelet count in this population [48]. Taken together these results highlight the potential for *CD36* variants to be used as a predictive tool for CVD risk in AAs [38].

A recent study from the CARE consortium, using the same AA participants included in this study, also found strong evidence for an association between HDL levels and *CD36* rs3211938 [17]. HDL and CRP levels are modestly correlated across these cohorts (e.g. Spearman's correlation for ARIC= -0.050 and JHS= -0.037). Inclusion of HDL as a covariate in the linear models for the CARE samples modestly impacted the association between CRP and rs3211938 ($p = 1.4 \times 10^{-6}$ for the model unadjusted for HDL vs. $p = 5.4 \times 10^{-6}$ for the HDL-adjusted model), suggesting that the *CD36* association with CRP levels is largely independent of HDL.

We also found evidence at *ARNTL*, also termed *BMAL1*, which is a core component of the circadian clock and a vital element of the central circadian pacemaker [49]. This locus had suggestive evidence for association in the CHARGE report. Molecular circadian clocks exist in peripheral tissues and coordinate gene transcription involved in a wide range of metabolic processes including gluconeogenesis, lipolysis, adipogenesis, and mitochondrial oxidative phosphorylation to achieve an appropriate internal alignment of metabolic signaling as well as external alignment of cellular processes [50]. It has been well documented that pathologic events display circadian rhythms with an increase in incidences, such as myocardial infarction and ischemia, from dawn to noon [51,52]. Furthermore, experimental circadian misalignment is associated with abnormalities in blood pressure, glucose, and insulin levels [53] while shift work, a real-world model for circadian misalignment, is associated with diabetes and CVD [54,55]. Polymorphisms of *ARNTL* have also been associated with age at menarche [56–58]. Inactivation of *ARNTL* results in altered regulation of blood pressure, lipid metabolism, and glucose homeostasis; these changes have been observed in hypertensive mouse models [59–63]. The association between *ARNTL* and CRP suggests one possible mechanism of correlation between CRP and metabolic dysregulation, thereby increasing CVD risk.

Strengths of this study include the large AA and European-ancestry samples, with measured CRP levels, that have been genotyped on a large commercial candidate-gene-based genotyping panel. The generalizability to other populations remains to be determined. SNPs identified may not be causally linked to variation in CRP, rather they may be in linkage disequilibrium with the causal variants. In addition, the functional mechanisms linking

genetic variants to CRP concentrations remain to be determined. Though we did not observe much evidence for heterogeneity of effect estimates across studies among our top findings, as with any meta-analyses, systemic differences between studies, including different technologies used to measure CRP levels, can lead to reduced power and biased aggregate effect size estimates. Finally, we had limited power to detect low frequency variants and SNPs with low effect size, especially in AAs. Additional genomic loci may be uncovered in larger samples and with broader coverage of genetic variation across the human genome.

Our findings provide increased insight about genetic variants influencing variation in CRP concentrations, including the identification of a newly reported African-specific *CD36* variant associated with CRP level and the confirmation of three CRP loci identified in a recent GWAS. Overall, these findings are consistent with the role of metabolism and inflammatory pathway in the regulation of circulating CRP levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

CARE is supported by contract number HHSN268200625226C from the National Institutes of Health (NIH)/National Heart Lung and Blood Institute (NHLBI). Sources of funding for individual CARE cohorts: **Atherosclerosis Risk in Communities (ARIC)**: NHLBI (N01 HC-55015, N01 HC-55016, N01HC-55017, N01 HC-55018, N01 HC-55019, N01 HC-55020, N01 HC-55021); **Cardiovascular Health Study (CHS)**: NHLBI (N01-HC-85239, N01-HC-85079 through N01-HC-85086, N01-HC-35129, N01 HC-15103, N01 HC-55222, N01-HC-75150, N01-HC-45133, grant HL080295 and contract HHSN268201200036C), with additional support from NINDS and from NIA (AG-023629, AG-15928, AG-20098, and AG-027058); **Coronary Artery Risk Development in Young Adults (CARDIA)**: NHLBI (N01-HC95095 & N01-HC48047, N01-HC48048, N01-HC48049, and N01-HC48050); **Framingham Heart Study (FHS)**: NHLBI (N01-HC-25195 and grant R01 NS17950) with additional support from NIA (AG08122 and AG033193); **Jackson Heart Study (JHS)**: NHLBI and the National Institute on Minority Health and Health Disparities (N01 HC-95170, N01 HC-95171 and N01 HC-95172); **Multi-Ethnic Study of Atherosclerosis (MESA)**: N01 HC-95159, N01-HC-95160, N01-HC-95161, N01-HC-95162, N01-HC-95163, N01-HC-95164, N01-HC-95165, N01-HC-95166, N01-HC-95167, N01-HC-95168, N01-HC-95169 and RR-024156. Funding for CARE genotyping was provided by NHLBI Contract N01-HC-65226. Additional financial support was provided by NHLBI grant R01 HL071862.

The MONICA/KORA Augsburg studies were financed by the Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany and supported by grants from the German Federal Ministry of Education and Research. Part of this work was financed by the German National Genome Research Network (project number 01GS0834), by the German Research Foundation (TH-784/2-1 and TH-784/2-2), by the European Foundation for the Study of Diabetes and through additional funds from the Helmholtz Zentrum München, the German Diabetes Center and the University of Ulm. Furthermore, the research was supported within the Munich Center of Health Sciences as part of the Ludwig Maximilians University innovative.

The authors wish to thank the National Heart, Lung, and Blood Institute (NHLBI) Exome Sequencing Project for providing a reference panel for CD36 rs3211938 imputation. Funding for GO ESP was provided by NHLBI grants RC2 HL-103010 (HeartGO), RC2 HL-102923 (LungGO) and RC2 HL-102924 (WHISP). The exome sequencing was performed through NHLBI grants RC2 HL-102925 (BroadGO) and RC2 HL-102926 (SeattleGO).

Reference List

1. Ridker PM. Inflammation, high-sensitivity C-reactive protein, and vascular protection. *Tex Heart Inst J.* 2010; 37:40–41. [PubMed: 20200625]
2. Robbie L, Libby P. Inflammation and atherothrombosis. *Ann NY Acad Sci.* 2001; 947:167–179. [PubMed: 11795264]

3. Pankow JS, Folsom AR, Cushman M, Borecki IB, Hopkins PN, et al. Familial and genetic determinants of systemic markers of inflammation: the NHLBI family heart study. *Atherosclerosis*. 2001; 154:681–689. [PubMed: 11257270]
4. Koenig W, Sund M, Frohlich M, Fischer HG, Lowel H, et al. C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. *Circulation*. 1999; 99:237–242. [PubMed: 9892589]
5. Slade GD, Offenbacher S, Beck JD, Heiss G, Pankow JS. Acute-phase inflammatory response to periodontal disease in the US population. *J Dent Res*. 2000; 79:49–57. [PubMed: 10690660]
6. Harris TB, Ferrucci L, Tracy RP, Corti MC, Wacholder S, et al. Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. *Am J Med*. 1999; 106:506–512. [PubMed: 10335721]
7. Nappo A, Iacoviello L, Fraterman A, Gonzalez-Gil EM, Hadjigeorgiou C, Marild S, Molnar D, Moreno LA, Peplies J, Sioen I, Veidebaum T, Siani A, Russo P. High-sensitivity C-reactive protein is a predictive factor of adiposity in children: results of the identification and prevention of dietary- and lifestyle-induced health effects in children and infants (IDEFICS) study. *J Am Heart Assoc*. 2013; 2(3):e000101. [PubMed: 23744403]
8. Mendall MA, Patel P, Ballam L, Strachan D, Northfield TC. C reactive protein and its relation to cardiovascular risk factors: a population based cross sectional study. *BMJ*. 1996; 312:1061–1065. [PubMed: 8616412]
9. Tracy RP, Psaty BM, Macy E, Bovill EG, Cushman M, et al. Lifetime smoking exposure affects the association of C-reactive protein with cardiovascular disease risk factors and subclinical disease in healthy elderly subjects. *Arterioscler Thromb Vasc Biol*. 1997; 17:2167–2176. [PubMed: 9351386]
10. van Leeuwen WM, Lehto M, Karisola P, Lindholm H, Luukkonen R, et al. Sleep restriction increases the risk of developing cardiovascular diseases by augmenting proinflammatory responses through IL-17 and CRP. *PLoS One*. 2009; 4:e4589. [PubMed: 19240794]
11. Larkin EK, Rosen CL, Kirchner HL, Storfer-Isser A, Emancipator JL, et al. Variation of C-reactive protein levels in adolescents: association with sleep-disordered breathing and sleep duration. *Circulation*. 2005; 111:1978–1984. [PubMed: 15837952]
12. Schnabel RB, Lunetta KL, Larson MG, Dupuis J, Lipinska I, et al. The relation of genetic and environmental factors to systemic inflammatory biomarker concentrations. *Circ Cardiovasc Genet*. 2009; 2:229–237. [PubMed: 20031590]
13. Reiner AP, Barber MJ, Guan Y, Ridker PM, Lange LA, et al. Polymorphisms of the HNF1A gene encoding hepatocyte nuclear factor-1 alpha are associated with C-reactive protein. *Am J Hum Genet*. 2008; 82:1193–1201. [PubMed: 18439552]
14. Dehghan A, Dupuis J, Barbalic M, Bis JC, Eiriksdottir G, et al. Meta-analysis of genome-wide association studies in >80 000 subjects identifies multiple loci for C-reactive protein levels. *Circulation*. 2011; 123:731–738. [PubMed: 21300955]
15. Doumatey AP, Chen G, Tekola AF, Zhou J, Erdos M, et al. C-reactive protein (CRP) promoter polymorphisms influence circulating CRP levels in a genome-wide association study of African Americans. *Hum Mol Genet*. 2012
16. Reiner AP, Beleza S, Franceschini N, Auer PL, Robinson JG, et al. Genome-wide association and population genetic analysis of C-reactive protein in African American and Hispanic American women. *Am J Hum Genet*. 2012; 91:502–512. [PubMed: 22939635]
17. Musunuru K, Romaine SP, Lettre G, Wilson JG, Volcik KA, et al. Multi-ethnic analysis of lipid-associated loci: the NHLBI CARE project. *PLoS One*. 2012; 7:e36473. [PubMed: 22629316]
18. The Women's Health Initiative Study Group. Design of the Women's Health Initiative clinical trial and observational study. *Control Clin Trials*. 1998; 19:61–109. [PubMed: 9492970]
19. Lowel H, Doring A, Schneider A, Heier M, Thorand B, et al. The MONICA Augsburg surveys--basis for prospective cohort studies. *Gesundheitswesen*. 2005; 67(Suppl 1):S13–S18. [PubMed: 16032512]
20. Keating BJ, Tischfield S, Murray SS, Bhangale T, Price TS, et al. Concept, design and implementation of a cardiovascular gene-centric 50 k SNP array for large-scale genomic association studies. *PLoS One*. 2008; 3:e3583. [PubMed: 18974833]

21. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet.* 2007; 81:559–575. [PubMed: 17701901]
22. Chen MH, Yang Q. GWAF: an R package for genome-wide association analyses with family data. *Bioinformatics.* 2010; 26:580–581. [PubMed: 20040588]
23. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, et al. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet.* 2006; 38:904–909. [PubMed: 16862161]
24. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics.* 2010; 26:2190–2191. [PubMed: 20616382]
25. Lo KS, Wilson JG, Lange LA, Folsom AR, Galarneau G, et al. Genetic association analysis highlights new loci that modulate hematological trait variation in Caucasians and African Americans. *Hum Genet.* 2011; 129:307–317. [PubMed: 21153663]
26. Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR. MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. *Genet Epidemiol.* 2010; 34:816–834. [PubMed: 21058334]
27. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet.* 2009; 5:e1000529. [PubMed: 19543373]
28. Auer PL, Srivastava S, Doerge RW. Differential expression--the next generation and beyond. *Brief Funct Genomics.* 2012; 11:57–62. [PubMed: 22210853]
29. Coburn CT, Knapp FF Jr, Febbraio M, Beets AL, Silverstein RL, et al. Defective uptake and utilization of long chain fatty acids in muscle and adipose tissues of CD36 knockout mice. *J Biol Chem.* 2000; 275:32523–32529. [PubMed: 10913136]
30. Tanaka T, Nakata T, Oka T, Ogawa T, Okamoto F, et al. Defect in human myocardial long-chain fatty acid uptake is caused by FAT/CD36 mutations. *J Lipid Res.* 2001; 42:751–759. [PubMed: 11352982]
31. Miyaoka K, Kuwasako T, Hirano K, Nozaki S, Yamashita S, et al. CD36 deficiency associated with insulin resistance. *Lancet.* 2001; 357:686–687. [PubMed: 11247555]
32. Goyal T, Mitra S, Khaidakov M, Wang X, Singla S, et al. Current Concepts of the Role of Oxidized LDL Receptors in Atherosclerosis. *Curr Atheroscler Rep.* 2012
33. Manning-Tobin JJ, Moore KJ, Seimon TA, Bell SA, Sharuk M, et al. Loss of SR-A and CD36 activity reduces atherosclerotic lesion complexity without abrogating foam cell formation in hyperlipidemic mice. *Arterioscler Thromb Vasc Biol.* 2009; 29:19–26. [PubMed: 18948635]
34. Ji SR, Wu Y, Potempa LA, Qiu Q, Zhao J. Interactions of C-reactive protein with low-density lipoproteins: implications for an active role of modified C-reactive protein in atherosclerosis. *Int J Biochem Cell Biol.* 2006; 38:648–661. [PubMed: 16376133]
35. Aitman TJ, Cooper LD, Norsworthy PJ, Wahid FN, Gray JK, et al. Malaria susceptibility and CD36 mutation. *Nature.* 2000; 405:1015–1016. [PubMed: 10890433]
36. Omi K, Ohashi J, Patarapotikul J, Hananantachai H, Naka I, et al. CD36 polymorphism is associated with protection from cerebral malaria. *Am J Hum Genet.* 2003; 72:364–374. [PubMed: 12506336]
37. Love-Gregory L, Sherva R, Sun L, Wasson J, Schappe T, et al. Variants in the CD36 gene associate with the metabolic syndrome and high-density lipoprotein cholesterol. *Hum Mol Genet.* 2008; 17:1695–1704. [PubMed: 18305138]
38. Love-Gregory L, Sherva R, Schappe T, Qi JS, McCreary J, et al. Common CD36 SNPs reduce protein expression and may contribute to a protective atherogenic profile. *Hum Mol Genet.* 2011; 20:193–201. [PubMed: 20935172]
39. Handberg A, Levin K, Hojlund K, Beck-Nielsen H. Identification of the oxidized low-density lipoprotein scavenger receptor CD36 in plasma: a novel marker of insulin resistance. *Circulation.* 2006; 114:1169–1176. [PubMed: 16952981]
40. Silverstein RL. Inflammation, atherosclerosis, and arterial thrombosis: role of the scavenger receptor CD36. *Cleve Clin J Med.* 2009; 76(Suppl 2):S27–S30. [PubMed: 19376978]

41. Sun Y, Scavini M, Orlando RA, Murata GH, Servilla KS, et al. Increased CD36 expression signals monocyte activation among patients with type 2 diabetes. *Diabetes Care*. 2010; 33:2065–2067. [PubMed: 20551015]
42. Coon H, Leppert MF, Eckfeldt JH, Oberman A, Myers RH, et al. Genome-wide linkage analysis of lipids in the Hypertension Genetic Epidemiology Network (HyperGEN) Blood Pressure Study. *Arterioscler Thromb Vasc Biol*. 2001; 21:1969–1976. [PubMed: 11742872]
43. Fry AE, Ghansa A, Small KS, Palma A, Auburn S, et al. Positive selection of a CD36 nonsense variant in sub-Saharan Africa, but no association with severe malaria phenotypes. *Hum Mol Genet*. 2009; 18:2683–2692. [PubMed: 19403559]
44. Sabeti PC, Schaffner SF, Fry B, Lohmueller J, Varilly P, et al. Positive natural selection in the human lineage. *Science*. 2006; 312:1614–1620. [PubMed: 16778047]
45. Qayyum R, Snively BM, Ziv E, Nalls MA, Liu Y, et al. A meta-analysis and genome-wide association study of platelet count and mean platelet volume in african americans. *PLoS Genet*. 2012; 8:e1002491. [PubMed: 22423221]
46. Ghosh A, Murugesan G, Chen K, Zhang L, Wang Q, et al. Platelet CD36 surface expression levels affect functional responses to oxidized LDL and are associated with inheritance of specific genetic polymorphisms. *Blood*. 2011; 117:6355–6366. [PubMed: 21478428]
47. Jones CI, Bray S, Garner SF, Stephens J, de Bono B, et al. A functional genomics approach reveals novel quantitative trait loci associated with platelet signaling pathways. *Blood*. 2009; 114:1405–1416. [PubMed: 19429868]
48. Auer PL, Johnsen JM, Johnson AD, Logsdon BA, Lange LA, et al. Imputation of exome sequence variants into population-based samples and blood-cell-trait-associated loci in African Americans: NHLBI GO Exome Sequencing Project. *Am J Hum Genet*. 2012; 91:794–808. [PubMed: 23103231]
49. Honma S, Ikeda M, Abe H, Tanahashi Y, Namihira M, et al. Circadian oscillation of BMAL1, a partner of a mammalian clock gene Clock, in rat suprachiasmatic nucleus. *Biochem Biophys Res Commun*. 1998; 250:83–87. [PubMed: 9735336]
50. Buijs RM, Scheer FA, Kreier F, Yi C, Bos N, et al. Organization of circadian functions: interaction with the body. *Prog Brain Res*. 2006; 153:341–360. [PubMed: 16876585]
51. Maemura K, Layne MD, Watanabe M, Perrell MA, Nagai R, et al. Molecular mechanisms of morning onset of myocardial infarction. *Ann NY Acad Sci*. 2001; 947:398–402. [PubMed: 11795300]
52. Portman MA. Molecular clock mechanisms and circadian rhythms intrinsic to the heart. *Circ Res*. 2001; 89:1084–1086. [PubMed: 11739270]
53. Scheer FA, Hilton MF, Mantzoros CS, Shea SA. Adverse metabolic and cardiovascular consequences of circadian misalignment. *Proc Natl Acad Sci USA*. 2009; 106:4453–4458. [PubMed: 19255424]
54. Karlsson B, Knutsson A, Lindahl B. Is there an association between shift work and having a metabolic syndrome? Results from a population based study of 27,485 people. *Occup Environ Med*. 2001; 58:747–752. [PubMed: 11600731]
55. Knutsson A, Akerstedt T, Jonsson BG, Orth-Gomer K. Increased risk of ischaemic heart disease in shift workers. *Lancet*. 1986; 2:89–92. [PubMed: 2873389]
56. Anderson CA, Zhu G, Falchi M, van den Berg SM, Treloar SA, et al. A genome-wide linkage scan for age at menarche in three populations of European descent. *J Clin Endocrinol Metab*. 2008; 93:3965–3970. [PubMed: 18647812]
57. Rothenbuhler A, Fradin D, Heath S, Lefevre H, Bouvattier C, et al. Weight-adjusted genome scan analysis for mapping quantitative trait Loci for menarchal age. *J Clin Endocrinol Metab*. 2006; 91:3534–3537. [PubMed: 16804047]
58. Guo Y, Shen H, Xiao P, Xiong DH, Yang TL, et al. Genomewide linkage scan for quantitative trait loci underlying variation in age at menarche. *J Clin Endocrinol Metab*. 2006; 91:1009–1014. [PubMed: 16394082]
59. Bunker MK, Wilsbacher LD, Moran SM, Clendenin C, Radcliffe LA, et al. Mop3 is an essential component of the master circadian pacemaker in mammals. *Cell*. 2000; 103:1009–1017. [PubMed: 11163178]

60. Rudic RD, McNamara P, Curtis AM, Boston RC, Panda S, et al. BMAL1 and CLOCK, two essential components of the circadian clock, are involved in glucose homeostasis. *PLoS Biol.* 2004; 2:e377. [PubMed: 15523558]
61. Shimba S, Ishii N, Ohta Y, Ohno T, Watabe Y, et al. Brain and muscle Arnt-like protein-1 (BMAL1), a component of the molecular clock, regulates adipogenesis. *Proc Natl Acad Sci USA.* 2005; 102:12071–12076. [PubMed: 16093318]
62. Curtis AM, Cheng Y, Kapoor S, Reilly D, Price TS, et al. Circadian variation of blood pressure and the vascular response to asynchronous stress. *Proc Natl Acad Sci USA.* 2007; 104:3450–3455. [PubMed: 17360665]
63. Naito Y, Tsujino T, Kawasaki D, Okumura T, Morimoto S, et al. Circadian gene expression of clock genes and plasminogen activator inhibitor-1 in heart and aorta of spontaneously hypertensive and Wistar-Kyoto rats. *J Hypertens.* 2003; 21:1107–1115. [PubMed: 12777947]

Table 1

Study sample characteristics (% or mean±SD)

	ARIC		CARDIA		CFS		CHS		FHS	JHS	MESA		WHI	KORA
	EA	AA	EA	AA	EA	AA	EA	AA	EA	AA	EA	AA	EA	EU
N	7572	1983	1318	1118	281	369	3919	736	7543	2026	2051	1338	4389	2866
Female, %	53.9	64.1	53.2	59.2	53.0	58.0	56.3	62.5	53.8	60.6	52.0	53.7	100.0	48.9
Current Smoker, %	20.9	26.1	23.0	29.3	24.6	18.3	11.1	16.1	50.0	15.1	10.7	17.9	7.6	21.8
Diabetes, %	7.6	16.5	0.53	0.85	11.1	17.6	14.7	24.6	10.9	15.4	5.8	16.2	7.3	4.5
Age, y	54.1±5.7	52.9±5.7	25.7±3.3	24.4±3.8	44.3±19.3	40.4±18.6	72.8±5.6	73.0±5.7	48.8±13.7	50.0±12.0	62.2±10.1	61.7±9.8	68.2±6.5	52.1±10.7
BMI, kg/m2	26.9±4.7	29.6±5.9	23.7±4.0	25.6±5.7	31.7±9.2	33.1±9.8	26.4±4.5	28.5±5.6	27.4±5.5	32.2±7.8	27.7±5.1	30.1±5.7	27.6±6.8	27.2±4.1
CRP, mg/L	4.0±5.6	5.8±7.1	2.4±3.7	4.3±6.3	3.8±5.2	4.6±6.2	4.3±6.7	6.1±8.2	3.3±6.2	5.1±7.7	3.4±5.1	4.7±6.9	4.2±6.1	2.8±5.2

EA=European American; AA=African American; EU=European

Table 2

Loci associated with CRP in the African American samples (N=7570)

Chr.	No. SNPs P<2.2×10 ⁻⁶	Most Significant	Position	A1/A2	Freq.*	Beta(SE)*	p-value Het**	p-value	Gene
1q23	13	rs3093058	157951939	T/A	0.17	0.39(0.022)	0.69	4.2×10 ⁻⁷¹	CRP
1q21	2	rs8192284	152693594	A/C	0.14	-0.12(0.025)	0.60	2.0×10 ⁻⁶	IL6R
7q21	1	rs3211938	80138385	T/G	0.08	-0.14(0.030)	0.22	1.6×10 ⁻⁶	CD36
19q13	2	rs769450	50102284	G/A	0.37	0.083(0.017)	0.16	1.6×10 ⁻⁶	TOMM40

* Allele frequency and beta estimate is presented for second allele (A2).

** P-value from Cochran's Q-test for heterogeneity of effects across cohorts.

Table 3

Loci associated with CRP in a meta-analysis of African Americans (AA) and participants of European (Eur) descent (n=37,509).

Chr.	Most Significant	A1/A2	Freq. (Eur)*	Beta(SE) (Eur)*	p-value (Eur)	p-value Het (Eur)	Freq. (AA)*	Beta(SE) (AA)*	p-value (AA)	p-value Het(AA)	p-value (Meta)	p-value Het(Meta)	Gene
1p31	rs1805096	G/A	0.38	-0.098(0.009)	3.3×10^{-30}	0.013	0.45	-0.061(0.017)	2.5×10^{-4}	0.98	5.6×10^{-32}	0.044	LEPR
1q21	rs4129267	C/T	0.40	-0.079(0.008)	5.2×10^{-21}	0.32	0.13	-0.12(0.025)	5.7×10^{-7}	0.52	8.0×10^{-26}	0.28	IL6R
1q23	rs3091244	A/G	0.62	-0.17(0.009)	3.5×10^{-91}	0.34	0.45	-0.24(0.017)	5.1×10^{-45}	0.11	7.8×10^{-132}	0.011	CRP
1q44	rs12239046	C/T	0.39	-0.039(0.009)	5.3×10^{-6}	0.93	0.51	-0.064(0.017)	1.4×10^{-4}	0.98	7.3×10^{-9}	0.98	NLRP3
2p23	rs1260326	C/T	0.43	0.094(0.008)	5.3×10^{-29}	0.57	0.14	0.056(0.024)	0.019	0.63	1.0×10^{-29}	0.57	GCKR
2q13	rs4251961	T/C	0.38	0.060(0.009)	1.4×10^{-12}	0.37	0.18	0.081(0.022)	2.0×10^{-4}	0.055	1.8×10^{-15}	0.12	IL1RN
7q11	rs714052	A/G	0.12	-0.065(0.013)	3.9×10^{-7}	0.75	0.042	-0.056(0.044)	0.20	0.80	1.7×10^{-7}	0.91	BAZ1B
7q21	rs3211938	T/G	<0.001	-0.42(0.54)	0.44	0.96	0.085	-0.14(0.030)	1.6×10^{-6}	0.22	1.4×10^{-6}	0.40	CD36
11p15	rs6486121	T/C	0.37	-0.043(0.009)	6.4×10^{-7}	0.91	0.58	-0.037(0.017)	0.026	0.098	1.3×10^{-7}	0.57	ARNTL
12q24	rs2244608	A/G	0.34	-0.11(0.009)	4.6×10^{-39}	0.51	0.14	-0.076(0.024)	0.0016	0.65	9.5×10^{-41}	0.54	HNF1A
17q23	rs1292034	G/A	0.45	-0.037(0.008)	6.0×10^{-6}	0.043	0.82	-0.039(0.02)	0.079	0.53	1.2×10^{-6}	0.13	RPS6KB1
19q13	rs2075650	A/G	0.13	-0.18(0.012)	2.2×10^{-47}	0.59	0.13	0.039(0.02)	0.11	0.75	1.9×10^{-34}	1.7×10^{-9}	TOMM40
20q13	rs1800961	C/T	0.030	-0.13(0.024)	2.0×10^{-7}	0.63	0.0070	-0.14(0.10)	0.18	0.82	7.8×10^{-8}	0.85	HNF4A

* Allele frequencies and beta estimates are presented for second allele (A2).

Table 4
Loci associated with CRP in the independent European American (n=8039) replication sample (*CARDIA*, *CFS*, *MESA*, *WHI*)

Chr.	No. SNPs P<2.2×10 ⁻⁶	Most Significant	Position	A1/A2	Freq.*	Beta(SE)*	het p-value	p-value	Gene
1p31	19	rs1805096	65874845	G/A	0.38	-0.14(0.017)	0.61	2.5×10 ⁻¹⁸	<i>LEPR</i>
1q21	5	rs4129267	152692888	C/T	0.40	-0.10(0.016)	0.69	6.1×10 ⁻¹⁰	<i>IL6R</i>
1q23	9	rs3091244	157951289	G/A	0.38	0.18(0.016)	0.61	2.9×10 ⁻²⁸	<i>CRP</i>
2p23	3	rs1260326	27584444	C/T	0.43	0.085(0.016)	0.35	1.5×10 ⁻⁷	<i>GCKR</i>
12q24	5	rs2244608	119919810	A/G	0.31	-0.12(0.017)	0.38	1.2×10 ⁻¹⁴	<i>HNF1A</i>
19q13	6	rs12721046	50087459	G/A	0.13	0.18(0.023)	0.36	7.3×10 ⁻¹⁵	<i>APOC1</i>

* Allele frequency and beta estimate is presented for second allele (A2).