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ORIGINAL INVESTIGATION

Large multiethnic Candidate Gene Study for C-reactive protein levels: identification of a novel association at *CD36* in African Americans

Jaclyn Ellis · Ethan M. Lange · Jin Li · Josee Dupuis · Jens Baumert · Jeremy D. Walston · Brendan J. Keating · Peter Durda · Ervin R. Fox · Cameron D. Palmer · Yan A. Meng · Taylor Young · Deborah N. Farlow · Renate B. Schnabel · Carola S. Marzi · Emma Larkin · Lisa W. Martin · Joshua C. Bis · Paul Auer · Vasan S. Ramachandran · Stacey B. Gabriel · Monte S. Willis · James S. Pankow · George J. Papanicolaou · Jerome I. Rotter · Christie M. Ballantyne · Myron D. Gross · Guillaume Lettre · James G. Wilson · Ulrike Peters · Wolfgang Koenig · Russell P. Tracy · Susan Redline · Alex P. Reiner · Emelia J. Benjamin · Leslie A. Lange

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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 multiethnic 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 7,570 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 8,041 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

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J. Ellis · E. M. Lange · J. Li · L. A. Lange (✉)
Department of Genetics, University of North Carolina, 5112 Genetic Medicine Bldg., Chapel Hill, NC 27599-7264, USA
e-mail: leslie_lange@med.unc.edu

E. M. Lange
Department of Biostatistics, University of North Carolina, Chapel Hill, NC 27599, USA

J. Dupuis · E. J. Benjamin
National Heart, Lung, and Blood Institute's and Boston University's Framingham Heart Study, Framingham, MA 01702, USA

J. Dupuis
Department of Biostatistics, Boston University School of Public Health, Boston, MA 02118, USA

J. Baumert · C. S. Marzi
Institute of Epidemiology II, Helmholtz Zentrum München, German Research Center for Environmental Health, 23538 Neuherberg, Germany

J. D. Walston
Division of Geriatric Medicine and Gerontology, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

B. J. Keating
Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA

P. Durda · R. P. Tracy
Departments of Pathology and Biochemistry, University of Vermont College of Medicine, Burlington, VT 05405, USA

loci were also detected (*RPS6KB1*, $p = 2.0 \times 10^{-6}$; *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.

Introduction

C-reactive protein (CRP) is a pentameric acute-phase protein that is a hallmark of low-grade systemic inflammation (Ridker 2010). 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 (Robbie and Libby 2001). Associations between CRP and CVD outcomes have been remarkably consistent despite varying study designs, target populations, and case classification methods (Pankow et al. 2001). Observational studies have shown increased levels of CRP to be present in individuals with factors such as older age (Koenig et al. 1999), female sex (Slade et al. 2000), smoking (Harris et al. 1999), obesity (Nappo et al. 2013), diabetes, atherosclerotic CVD (Mendall et al. 1996; Tracy et al. 1997), sleep curtailment (van Leeuwen et al. 2009) and sleep apnea (Larkin et al. 2005). Heritability estimates for CRP concentration range from 35 to 40 % (Schnabel et al. 2009), supporting that genetic factors are likely to influence variation in CRP levels (Pankow et al. 2001).

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*) (Reiner et al. 2008). 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* (Dehghan et al. 2011). To our knowledge, only two published GWASs for CRP in individuals of African ancestry have been conducted (Doumatey et al. 2012; Reiner et al. 2012), and the first was based on a relatively small cohort of individuals (Dehghan et al. 2011). This earlier study identified several variants in the *CRP* gene that were associated with CRP, but no other loci were statistically significant (Doumatey et al. 2012). The latter study, which included 8,280 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* (Reiner et al. 2012).

We sought to extend what is known regarding the genetic underpinnings of CRP by performing multiethnic meta-analyses, including individuals of both African and European ancestry genotyped across a densely covered

E. R. Fox · J. G. Wilson
Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, MS 39216, USA

C. D. Palmer · Y. A. Meng · T. Young · D. N. Farlow · S. B. Gabriel
Program in Medical and Population Genetics, Broad Institute, Cambridge, MA 02142, USA

C. D. Palmer
Divisions of Genetics and Endocrinology and Program in Genomics, Children's Hospital Boston, Boston, MA 02115, USA

R. B. Schnabel
Department of General and Interventional Cardiology, University Heart Center Hamburg, 21046 Hamburg, Germany

E. Larkin
Division of Allergy, Pulmonary and Critical Care, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN 37232, USA

L. W. Martin
Division of Cardiology, George Washington School of Medicine, Washington, DC 20037, USA

J. C. Bis
Cardiovascular Health Research Unit and Department of Medicine, University of Washington, Seattle, WA 98101, USA

P. Auer
School of Public Health, University of Wisconsin-Milwaukee, Milwaukee, WI 53206, USA

V. S. Ramachandran
Department of Medicine, Boston University School of Medicine, Boston, MA 02118, USA

M. S. Willis
Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC 27599, USA

J. S. Pankow
Division of Epidemiology and Community Health, University of Minnesota School of Public Health, Minneapolis, MN 55455, USA

G. J. Papanicolaou
Division of Cardiovascular Sciences, National Heart, Lung and Blood Institute, Bethesda, MD 20892, USA

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 2,000 candidate genes within CVD-related pathways. An independent sample of AA participants from the WHI study with IBC chip data was 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 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 (Musunuru et al. 2012). Cohorts included in these analyses of CRP levels are: Atherosclerosis Risk in Communities (ARIC) (n = 7,572 EA; n = 1,983 AA), Coronary Artery Risk in Young Adults (CARDIA) (n = 1,318 EA; n = 1,118 AA), Cleveland Family Study (CFS) (n = 281 EA; n = 369 AA), the Cardiovascular Health Study (CHS) (n = 3,919 EA; n = 736 AA), Framingham Heart Study (FHS) (n = 7,543 EA), Jackson Heart Study (JHS) (n = 2,026 AA), and Multi-Ethnic Study of Atherosclerosis (MESA) (n = 2,051 EA; n = 1,338 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 US (The Women's Health Initiative Study Group 1998). A diverse population was recruited from 1993 to 1998 at 40 clinical centers across the US. A total of n = 4,389 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 (Lowel et al. 2005). The sample used in the current study consisted of n = 2,866 EA subjects with CRP measures selected from 1,071 participants for KORA S12 and 1,795 participants for KORA F3.

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

J. I. Rotter
Departments of Pediatrics, Medicine, and Human Genetics, University of California, Los Angeles, CA 90095, USA

J. I. Rotter
Institute for Translational Genomics and Population Sciences, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Los Angeles, CA, USA

J. I. Rotter
Division of Genomic Outcomes, Department of Pediatrics, Harbor-UCLA Medical Center, Los Angeles, CA, USA

C. M. Ballantyne
Section of Cardiovascular Research, Baylor College of Medicine, Center for Cardiovascular Disease Prevention, Methodist DeBakey Heart Center, Houston, TX 77030, USA

M. D. Gross
Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN 55455, USA

G. Lettre
Montréal Heart Institute, Montréal H3A 2T5, Canada

G. Lettre
Département de Médecine, Université de Montréal, Montréal H3A 2T5, Canada

U. Peters
Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA

W. Koenig
Department of Internal Medicine II-Cardiology, University of Ulm Medical Center, 07304 Ulm, Germany

S. Redline
Department of Medicine, Brigham and Women's Hospital and Beth Israel Deaconess Medical Center, Boston, MA, USA

A. P. Reiner
Department of Epidemiology, University of Washington, Seattle, WA 98195, USA

E. J. Benjamin
Department of Epidemiology, Boston University School of Public Health, Boston, MA 02118, USA

IBC genotype array

The IBC SNP array is described in detail in Keating et al. (Keating et al. 2008). The IBC SNP array includes 49,320 SNPs selected across ~2,000 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 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 (Purcell et al. 2007) for studies with unrelated individuals and the linear mixed-effects model implemented in the program GWAF for cohorts with related individuals (Chen and Yang 2010) 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 ten principal components calculated using the program EIGENSTRAT (Price et al. 2006) to control for potential population substructure. After obtaining cohort- and race-specific results, we performed a fixed-effect, inverse variance-weighted meta-analysis using the METAL software (Willer et al. 2010). Meta-analysis was performed separately by race and race-combined. Heterogeneity of effects across studies was tested in METAL using Cochran's Q statistic.

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	7,572	1,983	1,318	1,118	281	369	3,919	736	7,543	2,026	2,051	1,338	4,389	2,866
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 (years)	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/m^2)	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 ($N = 7{,}570$)

Chr.	No. SNPs $p < 2.2 \times 10^{-6}$	Most significant	Position	A1/A2	Freq.[a]	Beta (SE)[a]	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*

** p value from Cochran's Q test for heterogeneity of effects across cohorts

[a] Allele frequency and beta estimate is presented for second allele (A2)

Based on a simulation analysis, 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 (Lo et al. 2011). 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 (Lo et al. 2011).

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 8,421 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 8,041 AA WHI individuals with GWAS data using the programs MaCH 1.0.18 (Li et al. 2010) and minimac (Howie et al. 2009). Additional details on the genotype imputation are given in Auer et al. (2012a).

Results

Characteristics of the 7,570 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 Fig. 1, demonstrating the focused association at rs3211938. A forest plot illustrating cohort-specific results is presented in Supplementary Fig. 2. We further tested this SNP using imputed genotype data in an independent sample of 8,041 AA women from WHI. The association was confirmed, with the minor allele of *CD36* rs3211938 associated with 0.128 (±0.030) lower log-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 (Reiner et al. 2012). 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 (Reiner et al. 2012), we had good proxies for SNPs at *CRP* and *TOMM40* but no available satisfactory proxies for the remaining gene regions (*HNF1A*, *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 1,000 Genomes data in individuals of African ancestry (YRI)], rs16827466 ($r^2 = 0.89$), the top variant reported by Reiner et al. (2012). 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 *IL1F10/IL1RN* rs6734238, rs17042795 ($r^2 = 0.28$), demonstrated a trend toward association ($p = 0.068$), as did our best proxy for *HNF1A* 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

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)[a]	Beta (SE) (Eur)[a]	*p* value (Eur)	*p* value Het (Eur)	Freq. (AA)[a]	Beta (SE) (AA)[a]	*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*

[a] Allele frequencies and beta estimates are presented for second allele (A2)

Table 4 Loci associated with CRP in the independent European American ($n = 8{,}039$) replication sample *(CARDIA, CFS, MESA, WHI)*

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

[a] Allele frequency and beta estimate is presented for second allele (A2)

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, HNF1A, LEPR, GCKR, IL6R, and IL1RN*) and three (*BAZ1B/BCL7B, NLRP3* and *HNF4A*) recently reported as significant by the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium (Table 3) (Dehghan et al. 2011). 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 Fig. 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 (SE) = 0.033 (0.009), $p = 1.0\text{x}10^{-4}$]. 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 (Dehghan et al. 2011). 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; $n = 8{,}039$), 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). In addition, 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 *RPS6KB1* rs1292034 ($p = 0.41$), which we identified as a novel locus in our combined meta-analysis (see Supplementary Fig. 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 (Dehghan et al. 2011). 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 (Dehghan et al. 2011). 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 *RPS6KB1,* 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 ($n = 8{,}041$) 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 *RPS6KB1* 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 ($\beta = -0.047$; $p = 1.7 \times 10^{-6}$). 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 (Coburn et al. 2000; Tanaka et al. 2001). Individuals with mutations in *CD36* have a defective FA uptake, which could result in a poor metabolic profile and elevated serum lipid levels (Tanaka et al. 2001; Miyaoka et al. 2001). 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 (Goyal et al. 2012). *CD36* is a scavenger receptor involved in the uptake of these oxidized lipids and thus plays a role in the formation of atheroma (Goyal et al. 2012). 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 (Goyal et al. 2012; Manning-Tobin et al. 2009). 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 (Ji et al. 2006). 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 (Aitman et al. 2000; Omi et al. 2003); as a result, *CD36* mutations are ten times more frequent in African populations vs. individuals of primarily European descent (Love-Gregory et al. 2008). 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, (Love-Gregory et al. 2008) high-density lipoprotein (HDL) cholesterol levels (Musunuru et al. 2012; Love-Gregory et al. 2011), and abnormal serum FA (Handberg et al. 2006; Silverstein 2009; Sun et al. 2010). A study using AA participants from HyperGEN demonstrated that *CD36* variants account for ~3.4 % of inter-individual HDL variability in the study population (Coon et al. 2001). 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 (Fry et al. 2009; Sabeti et al. 2006). 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 (Love-Gregory et al. 2011). 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 (Qayyum et al. 2012). Previous evidence has demonstrated that one of the variants (rs17154155) is associated with platelet function as well as *CD36* expression on platelets (Ghosh et al. 2011; Jones et al. 2009); 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 (Auer et al. 2012b). Taken together these results highlight the potential for *CD36* variants to be used as a predictive tool for CVD risk in AAs (Love-Gregory et al. 2011).

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 (Musunuru et al. 2012). 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 (Honma et al. 1998). 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 (Buijs et al. 2006). 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 (Maemura et al. 2001; Portman 2001). Furthermore, experimental circadian misalignment is associated with abnormalities in blood pressure, glucose, and insulin levels (Scheer et al. 2009) while shift work, a real-world model for circadian misalignment, is associated with diabetes and CVD (Karlsson et al. 2001; Knutsson et al. 1986). Polymorphisms of *ARNTL* have also been associated with age at menarche (Anderson et al. 2008; Rothenbuhler et al. 2006; Guo et al. 2006). Inactivation of *ARNTL* results in altered regulation of blood pressure, lipid metabolism, and glucose homeostasis; these changes have been observed in hypertensive mouse models (Bunger et al. 2000; Rudic et al. 2004; Shimba et al. 2005; Curtis et al. 2007; Naito et al. 2003). 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.

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