Meta-Analysis of Genome-Wide Association Studies in >80 000 Subjects Identifies Multiple Loci for C-Reactive Protein Levels

Abbas Dehghan, MD, PhD*; Josée Dupuis, PhD*; Maja Barbalic, PhD*; Joshua C. Bis, PhD*; Gudny Eiriksdottir, MSc; Chen Lu, MA; Niina Pellikka, BEng; Henri Wallaschowski, MD; Johannes Kettunen, MSc; Peter Henneman, MSc; Jens Baumert, PhD; David P. Strachan, MD; Christian Fuchsberger, PhD; Veronique Vitart, PhD; James F. Wilson, BSc, DPhil; Guillaume Paré, MD, MSc; Silvia Naïtza, PhD; Megan E. Rudock, PhD; Ida Surakka, BSc; Eco J.C. de Geus, PhD; Behrooz Z. Alizadeh, PhD; Jack Guralnik, MD, PhD; Alan Shuldiner, MD; Toshiko Tanaka, PhD; Robert Y.L. Zee, PhD; Renate B. Schnabel, MD, MSc; Vijay Nambi, MD; Maryam Kavousi, MD, MSc; Samuli Ripatti, PhD; Matthias Nauck, MD; Nicholas L. Smith, PhD; Albert V. Smith, PhD; Jouko Sundvall, PhD; Paul Scheet, PhD; Yongmei Liu, MD, PhD; Aimo Ruokonen, MD, PhD; Lynda M. Rose, MSc; Martin G. Larson, ScD; Ron C. Hoogeveen, PhD; Nelson B. Freimer, MD; Alexander Teumer, Dipl-Math; Russell P. Tracy, PhD; Lenore J. Launer, PhD; Julie E. Buring, DSc; Jennifer F. Yamamoto, MA; Aaron R. Folsom, MD, MPH; Eric J.G. Sijbrands, PhD, MD; James Pankow, PhD; Paul Elliott, MBBS, PhD, FMedSci; John F. Keany, MD; Wei Sun, MD, PhD; Antti-Pekka Sarin, BSc; João D. Fontes, MD; Sunita Badola, MSc; Brad C. Astor, PhD, MPH; Albert Hofman, MD, PhD; Amneli Pouta, MD, PhD; Karl Werdan, MD; Karin H. Greiser, MD; Oliver Kuss, PhD; Henriette E. Meyer zu Schwabedissen, MD; Joachim Thiery, MD; Yalda Jamshidi, PhD; Ilja M. Nolte, PhD; Nicole Soranzo, PhD; Timothy D. Spector, MD, MSc, FRCP; Henry Völzke, MD; Alexander N. Parker, PhD; Thor Aspelund, PhD; David Bates, MD, MSc; Lauren Young; Kim Tsui; David S. Siscovick, MD, MPH; Xiuling Guo, PhD; Jerome I. Rotter, MD; Jerome I. Rotter, MD; Manuela Uda, PhD; David Schlessinger, PhD; Igor Rudan, MD; Andrew A. Hicks, PhD; Brenda W. Penninx, PhD; Barbara Thorand, PhD, MPH; Christian Gieger, PhD, MS; Joe Coresh, MD, MPH; Gonneke Willemsen, PhD; Tamara B. Harris, MD, MSc; Andre G. Uitterlinden, PhD; Marjo-Riitta Järvelin, MD, MSc, PhD; Kenneth Rice, PhD; Dörte Radke; Veikko Salomaa, MD, PhD; Ko Willems van Dijk, PhD; Eric Boerwinkle, PhD; Ramachandran S. Vasan, MD; Luigi Ferrucci, MD, PhD; Quince D. Gibson, MBA; Stefania Bandinelli, MD; Harold Snieder, PhD; Dorret I. Boomsma, PhD; Xiangjun Xiao; Harry Campbell, MBChB, MD; Caroline Hayward, PhD; Peter P. Pramstaller, MD; Cornelia M. van Duijn, PhD; Leena Peltonen, MD, PhD; Bruce M. Psaty, MD, PhD; Vilmundur Gudnason, MD, PhD; Paul M. Ridker, MD, MPH; George Homuth, PhD; Wolfgang Koening, MD, PhD*; Christie M. Ballantyne, MD*; Jacqueline C. M. Witteman, PhD*; Emelie J. Benjamin, MD, ScM*; Markus Perola, MD, PhD*; Daniel I. Chasman, PhD*

**Background**—C-reactive protein (CRP) is a heritable marker of chronic inflammation that is strongly associated with cardiovascular disease. We sought to identify genetic variants that are associated with CRP levels.

**Methods and Results**—We performed a genome-wide association analysis of CRP in 66 185 participants from 15 population-based studies. We sought replication for the genome-wide significant and suggestive loci in a replication study.
C-reactive protein (CRP) is a general marker of systemic inflammation. High CRP levels are associated with increased risks of mortality1 and major diseases including diabetes mellitus,2 hypertension,3 coronary heart disease (CHD),4 and stroke.5 The heritability of CRP levels is estimated to be 25% to 40%,6–8 suggesting that genetic variation is a major determinant of CRP levels. A genome-wide association (GWA) study in 6345 women found 7 loci associated with CRP levels.9 These loci were in or close to genes encoding CRP (CRP), leptin receptor (LEPR), interleukin-6 receptor (IL6R), glucokinase regulator (GCKR), hepatic nuclear factor 1-α (HNF1A), apolipoprotein E (APOE), and achaete-scute complex homolog 1 (ASCL1). Findings from other GWA studies did not extend the number of loci related to CRP.10,11

Clinical Perspective on p 738

In this study, we sought to discover additional genes related to CRP levels using GWA scans in 66 185 participants from 15 population-based cohort studies and replicate our findings in 16 540 participants from 10 independent studies. To investigate whether the genetic variants identified interact with nongenetic determinants of CRP such as age, sex, smoking, and body mass index (BMI), we examined gene-environment interactions. Finally, the extent to which the genes associated with circulating CRP levels, individually or jointly, affect the risk of cardiovascular diseases is still unknown. To address this question, we examined the association of genetic variants with myocardial infarction (MI) and CHD.

Methods

Subjects and Measurements

Participants were of European ancestry. All studies had protocols approved by local institutional review boards. Participants provided written informed consent and gave permission to use their DNA for research purposes. Baseline characteristics for all participating studies are presented in Table I in the online-only Data Supplement. Baseline measures of clinical and demographic characteristics were obtained at the time of cohort entry except for British 1958 Birth Cohort (B58C), the Framingham Heart Study (FHS), Northern Finland Birth Cohort 66 (NFBC66), and the Atherosclerosis Risk in Communities (ARIC) study, in which measures were obtained at the time of phenotype measurement.

GWA Analysis

Genome-wide scans were performed independently in each cohort with the use of various genotyping technologies (Table VII in the online-only Data Supplement). Investigators in each study performed association analysis using the genotype-phenotype data within their cohort. Each study imputed single-nucleotide polymorphisms (SNPs) with reference to HapMap release 22 CEU and provided results for a common set of SNPs for meta-analysis. Except for the FHS, all studies conducted a linear regression analysis adjusted for age (except for NFBC66 and B58C), sex (except for the Women’s Genome Health Study [WGHS]), and site of recruitment (if necessary) for all SNPs based on an additive genetic model. In the Erasmus Rucphen Family (ERF) study, adjustments for the family structure in the GWA analysis were based on the model residuals in the score test, which accounted for pedigree structure as implemented in GenABEL software12 function “mmscore.”13 In the FHS, a linear mixed effects model was employed with the use of the lmekin function of the kinship package in R with a fixed additive effect for the SNP genotype, fixed covariate effects, and random family-specific additive residual polygenic effects.14 In each study, we estimated the genomic inflation rate, stated as lambda (λgc), by comparing each study’s median χ² value to 0.4549, the median χ² for the null distribution15 (Table I in the online-only Data Supplement). P values for each cohort were adjusted for underlying population structure with the genomic inflation coefficient.

Discovery Panel and Replication Panel

The 15-study discovery panel included 5 studies from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium,16 4 studies from the European Special Population Network (EUROSPAN), and 6 additional independent studies comprising 66 185 participants. The replication studies included 10 independent studies and 16 540 participants.

Meta-Analysis

To calculate the combined P values and β coefficients, we used an inverse-variance weighted fixed-effects meta-analysis. We used METAL, a software package designed to perform meta-analysis on GWA data sets.17 We applied an a priori threshold 5.0 × 10⁻⁸ for genome-wide significance.18 When >1 genome-wide significant SNP clustered at a locus, we took the SNP with the smallest P value as the lead SNP. To investigate the validity of our findings, we sought replication of the lead SNP in genome-wide significant (P ≤ 5.0 × 10⁻⁸) loci and sought additional evidence for suggestive loci (5.0 × 10⁻⁵ < P < 10⁻⁴) in our replication panel. We ran a fixed-effect meta-analysis to combine the results of the discovery and replication panels. The first GWA study on serum CRP published by Ridker et al19 was based on part of the WGHS population. To confirm that our findings were not entirely influenced by these previously published results, we performed a meta-analysis excluding the WGHS population.

Examination of Heterogeneity

We examined between-study heterogeneity with Cochran’s Q test. On the basis of Bonferroni adjustment for 18 tests, heterogeneity was
considered significant at a $P$ value $<2.8 \times 10^{-3}$. We explored the source of heterogeneity for significant SNPs by fitting a covariate (age, gender, BMI, or smoking) in a meta-regression model.

### Gene-Environment Interaction

For all genome-wide significant SNPs, we examined gene-by-age, gene-by-sex, gene-by-BMI, and gene-by-smoking interactions in each study by introducing an interaction term into a linear model with age, sex, and the covariate of interest as the independent variables and natural log–transformed CRP as the outcome. A meta-analysis was performed to combine the reported interaction $\beta$ and $P$ values across studies for each of the top SNPs. On the basis of Bonferroni adjustment for 72 tests (18 SNPs for 4 environmental factors), we used a significance threshold at $6.9 \times 10^{-4}$.

### Table 2. Association of 17 Genome-Wide Significant Loci With CRP Levels in the Replication Panel and Combined With the Discovery Results

<table>
<thead>
<tr>
<th>SNP</th>
<th>Coded Allele</th>
<th>$\beta^*$ (SE)</th>
<th>$P$</th>
<th>$\beta^*$ (SE)</th>
<th>$P$</th>
<th>$R^2$†</th>
<th>$P$ for Heterogeneity</th>
<th>Closest Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2794520</td>
<td>C</td>
<td>0.086 (0.010)</td>
<td>9.9 $\times$ 10$^{-19}$</td>
<td>0.160 (0.006)</td>
<td>2.0 $\times$ 10$^{-186}$</td>
<td>1.38</td>
<td>7.4 $\times$ 10$^{-26}$</td>
<td>CRP</td>
</tr>
<tr>
<td>rs4420638</td>
<td>A</td>
<td>0.200 (0.023)</td>
<td>3.0 $\times$ 10$^{-10}$</td>
<td>0.236 (0.009)</td>
<td>8.8 $\times$ 10$^{-139}$</td>
<td>0.93</td>
<td>0.03</td>
<td>APOC1</td>
</tr>
<tr>
<td>rs1183910</td>
<td>G</td>
<td>0.122 (0.021)</td>
<td>8.3 $\times$ 10$^{-14}$</td>
<td>0.149 (0.006)</td>
<td>2.1 $\times$ 10$^{-124}$</td>
<td>0.76</td>
<td>0.08</td>
<td>HNF1A</td>
</tr>
<tr>
<td>rs4420065</td>
<td>C</td>
<td>0.045 (0.009)</td>
<td>1.5 $\times$ 10$^{-6}$</td>
<td>0.090 (0.005)</td>
<td>3.5 $\times$ 10$^{-42}$</td>
<td>0.39</td>
<td>1.1 $\times$ 10$^{-9}$</td>
<td>LEPR</td>
</tr>
<tr>
<td>rs4129267</td>
<td>C</td>
<td>0.045 (0.010)</td>
<td>7.3 $\times$ 10$^{-6}$</td>
<td>0.079 (0.005)</td>
<td>2.1 $\times$ 10$^{-48}$</td>
<td>0.31</td>
<td>2.4 $\times$ 10$^{-4}$</td>
<td>IL6R</td>
</tr>
<tr>
<td>rs1260326</td>
<td>T</td>
<td>0.031 (0.010)</td>
<td>1.9 $\times$ 10$^{-3}$</td>
<td>0.072 (0.005)</td>
<td>4.6 $\times$ 10$^{-40}$</td>
<td>0.24</td>
<td>2.6 $\times$ 10$^{-6}$</td>
<td>GCKR</td>
</tr>
<tr>
<td>rs12239046</td>
<td>C</td>
<td>0.042 (0.018)</td>
<td>1.8 $\times$ 10$^{-3}$</td>
<td>0.047 (0.006)</td>
<td>1.2 $\times$ 10$^{-15}$</td>
<td>0.09</td>
<td>0.77</td>
<td>NLPR3</td>
</tr>
<tr>
<td>rs6734238</td>
<td>G</td>
<td>0.072 (0.017)</td>
<td>4.9 $\times$ 10$^{-6}$</td>
<td>0.050 (0.006)</td>
<td>1.8 $\times$ 10$^{-17}$</td>
<td>0.14</td>
<td>0.95</td>
<td>IL1F10</td>
</tr>
<tr>
<td>rs9987289</td>
<td>A</td>
<td>0.003 (0.031)</td>
<td>3.5 $\times$ 10$^{-2}$</td>
<td>0.069 (0.011)</td>
<td>3.4 $\times$ 10$^{-13}$</td>
<td>0.08</td>
<td>0.04</td>
<td>PPP1R3B</td>
</tr>
<tr>
<td>rs10745954</td>
<td>A</td>
<td>0.018 (0.015)</td>
<td>1.3 $\times$ 10$^{-1}$</td>
<td>0.039 (0.006)</td>
<td>1.6 $\times$ 10$^{-11}$</td>
<td>0.06</td>
<td>1.1 $\times$ 10$^{-3}$</td>
<td>ASCL1</td>
</tr>
<tr>
<td>rs1800961</td>
<td>C</td>
<td>0.023 (0.026)</td>
<td>3.7 $\times$ 10$^{-1}$</td>
<td>0.088 (0.015)</td>
<td>2.2 $\times$ 10$^{-8}$</td>
<td>0.06</td>
<td>0.07</td>
<td>HNF4A</td>
</tr>
<tr>
<td>rs340029</td>
<td>T</td>
<td>0.004 (0.010)</td>
<td>5.2 $\times$ 10$^{-1}$</td>
<td>0.032 (0.006)</td>
<td>4.1 $\times$ 10$^{-8}$</td>
<td>0.08</td>
<td>0.05</td>
<td>RORA</td>
</tr>
<tr>
<td>rs10521222</td>
<td>C</td>
<td>0.089 (0.028)</td>
<td>1.4 $\times$ 10$^{-3}$</td>
<td>0.104 (0.015)</td>
<td>8.5 $\times$ 10$^{-13}$</td>
<td>0.09</td>
<td>0.34</td>
<td>SALL1</td>
</tr>
<tr>
<td>rs12037222</td>
<td>A</td>
<td>0.035 (0.017)</td>
<td>3.9 $\times$ 10$^{-2}$</td>
<td>0.045 (0.007)</td>
<td>6.4 $\times$ 10$^{-11}$</td>
<td>0.06</td>
<td>0.40</td>
<td>PABPC4</td>
</tr>
<tr>
<td>rs13233571</td>
<td>C</td>
<td>0.049 (0.025)</td>
<td>4.5 $\times$ 10$^{-2}$</td>
<td>0.054 (0.009)</td>
<td>3.6 $\times$ 10$^{-8}$</td>
<td>0.08</td>
<td>0.13</td>
<td>BCL7B</td>
</tr>
<tr>
<td>rs2836878</td>
<td>G</td>
<td>0.013 (0.011)</td>
<td>2.3 $\times$ 10$^{-1}$</td>
<td>0.032 (0.006)</td>
<td>1.7 $\times$ 10$^{-7}$</td>
<td>0.05</td>
<td>0.18</td>
<td>PSMG1</td>
</tr>
<tr>
<td>rs4903031</td>
<td>G</td>
<td>0.001 (0.012)</td>
<td>9.1 $\times$ 10$^{-1}$</td>
<td>0.032 (0.007)</td>
<td>5.1 $\times$ 10$^{-6}$</td>
<td>0.04</td>
<td>0.21</td>
<td>RSS6</td>
</tr>
</tbody>
</table>

*$\beta$ coefficient represents 1-unit change in the natural log–transformed CRP (mg/L) per copy increment in the coded allele.†Median percentage of CRP variance explained by the SNP reported in all participating studies.
Table 3. Association of 3 Suggestive Loci With CRP Levels That Reached Genome-Wide Significance After Combining Discovery and Replication Panel

<table>
<thead>
<tr>
<th>SNP</th>
<th>Coded Allele</th>
<th>Discovery β* (SE)</th>
<th>P</th>
<th>Replication β* (SE)</th>
<th>P</th>
<th>Discovery + Replication β* (SE)</th>
<th>P</th>
<th>R²†</th>
<th>Heterogeneity</th>
<th>P for Heterogeneity</th>
<th>Closest Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2847281</td>
<td>A</td>
<td>0.034 (0.007)</td>
<td>1.7×10⁻⁷</td>
<td>0.018 (0.016)</td>
<td>4.2×10⁻²</td>
<td>0.031 (0.006)</td>
<td>2.2×10⁻⁸</td>
<td>0.04</td>
<td>0.97</td>
<td>0.09</td>
<td>PTNP2</td>
</tr>
<tr>
<td>rs6901250</td>
<td>A</td>
<td>0.034 (0.007)</td>
<td>1.2×10⁻⁶</td>
<td>0.038 (0.015)</td>
<td>1.2×10⁻²</td>
<td>0.035 (0.006)</td>
<td>4.8×10⁻⁸</td>
<td>0.02</td>
<td>0.89</td>
<td>0.09</td>
<td>GPRC6A</td>
</tr>
<tr>
<td>rs4705952</td>
<td>G</td>
<td>0.038 (0.008)</td>
<td>4.1×10⁻⁶</td>
<td>0.065 (0.018)</td>
<td>3.0×10⁻⁴</td>
<td>0.042 (0.007)</td>
<td>1.3×10⁻⁸</td>
<td>0.05</td>
<td>0.47</td>
<td>0.09</td>
<td>RF1</td>
</tr>
</tbody>
</table>

*β coefficient represents 1-unit change in the natural log–transformed CRP (mg/L) per copy increment in the coded allele.
†Median percentage of CRP variance explained by the SNP reported in all participating studies.

**Genetic Risk Score**

To model the cumulative effect of the identified loci, we created a genetic risk score comprising information from the genome-wide significant SNPs. The risk score was computed for each subject by multiplying the number of alleles associated with higher CRP by the β coefficient from the combined meta-analysis and taking the sum over the SNPs. To make the genetic risk score easier to interpret, we rescaled to range from zero (low CRP level) to 100 (high CRP level).

**Association With MI and CHD**

The association of the genome-wide significant SNPs and the genetic risk score with clinical events was tested in the ARIC study, the Age, Gene/Environment Susceptibility–Reykjavik (AGES) study, the Cardiovascular Health Study (CHS), the FHS, the Rotterdam Study (RS), and the WGHS with the use of incident cases of MI and CHD (ie, occurring after CRP concentrations were measured). Incident MI included fatal and nonfatal MI. Incident CHD included incident fatal and nonfatal MI, fatal CHD, and sudden death. Each study examined the associations with the use of a Cox proportional hazards model adjusted for age and sex. We subsequently combined these results by performing a meta-analysis.

**Results**

The basic characteristics of the participating studies are shown in Table I in the online-only Data Supplement. Figure 1 in the online-only Data Supplement shows the QQ plot (λ = 1.09), and Figure II in the online-only Data Supplement presents the P values for >2.5 million SNPs across 22 autosomal chromosomes. A total of 953 SNPs in 17 loci exceeded the genome-wide significance threshold (P<5×10⁻⁸) (Table 1). Moreover, we found suggestive signals (P<10⁻⁵) in 47 loci. Sixty-four lead SNPs including 17 SNPs from the genome-wide significant loci and 47 SNPs from the suggestive loci were chosen for the replication stage (Table II in the online-only Data Supplement). Six SNPs close to CRP, APOC1, HNF1A, LEPR, IL6R, and IL1F10 exceeded the Bonferroni significance level (0.05/64 = 7.8×10⁻⁶) in the replication stage. In a fixed-effects meta-analysis of the discovery and replication panels, 18 loci showed a genome-wide significant association: 15 loci of the 17 genome-wide significant loci (Table 2) and 3 loci of the 47 suggestive loci (Table 3). In addition to confirming 7 previously reported associations, the genome-wide significant signals marked 11 novel associations within or close to the NLR family, pyrin domain containing 3 (NLRP3), interleukin-1 family, member 10 (IL1F10), protein phosphatase 1, regulatory (inhibitor) subunit 3B (PPPI3B), hepatocyte nuclear factor 4-α (HNF4A), RAR-related orphan receptor A (RORA), Sal-like 1 (SALL1), poly(A) binding protein, cytoplasmic 4 (inducible form) (PABPC4), B-cell chronic lymphocytic leukemia/lymphoma 7B (BCL7B), proteasome assembly chaperone 1 (PSMG1), protein tyrosine phosphatase, nonreceptor type 2 (PTPN2), G protein–coupled receptor, family C, group 6, member A (GPRC6A), and interferon regulatory factor 1 (IRF1). Furthermore, our meta-analysis excluding the WGHS population (Table III in the online-only Data Supplement) confirmed the association of 7 previously known genes, CRP, APOE (APOC1), HNF1A, LEPR, IL6R, GCKR, and ASCL1, with CRP levels (Bonferroni significance level: 0.05/7 = 7.1×10⁻⁴).

Figure 1 presents the average CRP levels across the genetic risk score in the whole population. Individuals in the highest gene score group had a mean CRP level (4.12 mg/L; 95% confidence interval, 4.96 to 5.25) that was more than double the level observed for individuals in the lowest gene score group (1.40 mg/L; 95% confidence interval, 1.31 to 1.49). The percentage of overall variance in CRP that was explained by the genetic risk score ranged from 1.2% to 10.3% across studies in the discovery and replication panels and was more than 5% in half of the studies.

After adjustment for number of tests, significant heterogeneity was found for rs2794520, rs4420065, rs4129267, rs1260326, and rs10745954 (Tables 2 and 3). Meta-regression was used to explore the source of heterogeneity. Sex was associated with heterogeneity for rs10745954 (P<2.8×10⁻⁵) (Table VI in the online-only Data Supplement).

All 18 SNPs that showed genome-wide significant results in the combined meta-analyses were studied for interactions...
A number of these genes including *HNF1A*, *PTPN2*, and *IRF1* are directly or indirectly related to metabolic regulatory pathways involved in diabetes mellitus. Mutations in *HNF1A* are associated with impaired insulin secretion and maturity-onset diabetes mellitus of the young (MODY) type 3. *HNF4A* is part of a complex regulatory network in the liver and pancreas for glucose homeostasis. Mutations in the *HNF4A* gene cause MODY type 1. *HNF4A* is a transcription factor involved in the expression of several liver-specific genes including *HNF1A*. Defects in the expression of *GCKR* result in deficient insulin secretion. *PTPN2*, which modulates interferon gamma signal transduction at the B cell level, was recently identified as a novel susceptibility gene for type 1 diabetes mellitus. *PTPN2* also is linked to the inflammatory pathway. The nuclear isoform of *PTPN2* is a regulator of transcription factor STAT3 in the downstream of interleukin-6 signaling and may affect CRP expression in Hep3B cells.

*CRP*, *IL6R*, *NLRP3*, *ILF10*, and *IRF1* are associated with CRP levels at least partly through pathways related to innate and adapted immune response. *NLRP3* encodes a member of the NALP3 inflammasome complex. The NALP3 inflammasome triggers an innate immune response and can be activated by endogenous “danger signals,” as well as compounds associated with pathogens. Activated NALP3 inflammasome functions as an activator of nuclear factor-κB signaling. Nuclear factor-κB is a transcription factor that affects CRP expression in Hep3B cells.

Our genetic risk score explained ≈5% of the variation in CRP levels, showing that genetic factors are of importance in determining CRP levels. In comparison, BMI as the main non-genetic determinant of CRP was reported to explain 5% to 7% of the variation in CRP levels in AGES and up to 15% in FHS. Ridker et al. reported that 7 SNPs discovered in their study explained 10.1% of the variation in CRP levels after adjustment for age, smoking, BMI, hormone therapy, and menopausal status. However, without adjustment for these covariates, <5% of the variation in CRP levels was explained.

Adipose tissue can induce chronic low-grade inflammation by producing proinflammatory cytokines such as interleukin-6. Therefore, we examined whether adiposity modifies the effect of any of the 18 genes on CRP. We found that BMI modifies the strength of the association between *LEPR* and *CRP*. This interaction was initially found in WGHS.

There is ample evidence that chronic inflammation is involved in atherosclerosis and cardiovascular disease. In this study, we found no association between genetically elevated CRP and risk of CHD. In agreement with our results, Elliott et al. reported in a recent study that variations in the *CRP* gene are not associated with risk of MI and CHD, but they found associations of *LEPR*, *IL6R*, and *APOC1* with CHD. However, the lack of association with clinical events in our study could also be due to lack of power.

Our study has the benefit of a large and homogeneous sample size of 82,725 subjects of European ancestry. This enabled us to find novel genes with small effect on CRP level. Furthermore, this large sample size enabled us to study gene-environment interaction, which to date has been less studied.
feasible. In contrast to most other studies, we used only incident cases of cardiovascular events from well-defined population-based studies to examine the relation between the identified SNPs and clinical disease. The study has several limitations. Although we identified 18 loci associated with CRP levels, other genetic loci associated with CRP concentrations may still be missed by our study. Six of the genome-wide significant loci from the discovery panel were significant after Bonferroni correction in the replication panel. The other identified loci need replication for confirmation in larger samples. We acknowledge that our genetic risk score is based on our own findings and may be less efficient when used in another population. Finally, we did not fine map the identified loci; we therefore acknowledge that the identified SNPs may be in linkage disequilibrium with non-HapMap variants causally related to CRP levels.

In conclusion, we identified 11 novel loci and confirmed 7 known loci to affect CRP levels. The results highlight immune response and metabolic regulatory pathways involved in the regulation of chronic inflammation, as well as several loci previously unknown to be related to inflammation. Furthermore, LEPR was found to affect CRP differently in the presence of low or high BMI, which may lead to new insights in the mechanisms underlying inflammation.

Acknowledgments

The following is a list of the author affiliations:

From the Department of Epidemiology, Erasmus Medical Center, Rotterdam, Netherlands (A.D., M.K., A.H., C.M.v.D., J.C.M.W.); Member of Netherlands Consortium for Healthy Aging sponsored by Netherlands Genomics Initiative, Leiden, Netherlands (A.D., M.K., A.H., A.G.U., C.M.v.D., J.C.M.W.); Department of Biostatistics, School of Public Health, Boston University, Boston, MA (J.D., C.L.); National Heart, Lung, and Blood Institute and Boston University’s Framingham Heart Study, Framingham, MA (J.D., M.G.L., J.F.Y., J.F.K., J.D.F., R.S.V., E.J.B.); Human Genetics Center and Institute of Molecular Medicine, University of Texas Health Science Center at Houston (M.B., E.B.); Department of Medicine, University of Washington, Seattle (J.C.B.); Icelandic Heart Association, Kópavogur, Iceland (G.E., A.V.S., T.A., V.G.); Unit of Public Health Genomics, Department of Chronic Disease Prevention, National Institute for Health and Welfare, Helsinki, Finland (N.P., M.P.); Institute of Clinical Chemistry and Laboratory Medicine, University of Greifswald, Greifswald, Germany (H.W., M.N.); Department of Human Genetics, Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK (J.K., L.P.); Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands (P.H.); Institute of Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany (J.B., B.T., C.G.); Division of Community Health Sciences, St George’s University of London, London, UK (D.P.S.); Institute of Genetic Medicine, European Academy Bozen/Bolzano (EURAC), Bolzano, Italy (Affiliated Institute of University of Lübeck, Lübeck, Germany) (C.F., A.A.H., P.P.E.); Medical Research Council Human Genetics Unit, Institute of Genetics and Molecular Medicine, Western General Hospital, Edinburgh, UK (V.V., C.H.); Centre for Population Health Sciences, University of Edinburgh, Edinburgh, UK (J.F.W., I.R., H.C.); Center for Cardiovascular Disease Prevention, Harvard Medical School, Boston, MA (G.P., N.B.F.); Istituto di Neurogenetica e Neurofarmacologia, Consiglio Nazionale delle Ricerche, Cagliari, Italy (S.N., M.U.); Department of Epidemiology and Prevention, Wake Forest University School of Medicine, Wake Forest, NC (M.E.R., Y.L.); Institute for Molecular Medicine Finland FIMM, University of Helsinki, Helsinki, Finland (I.S., S.R., A. Sarin); Department of Biological Psychology, VU University, Amsterdam, Netherlands (E.I.C.d.G., G.W., D.I.B.); Unit of Genetic Epidemiology and Bioinformatics, Department of Epidemiology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands (B.Z.A., I.M.N., H.S.); Laboratory of Epidemiology, Demography and Biometry, National Institute on Aging, National Institutes of Health, Bethesda, MD (J.G., L.J.L., T.B.H.); Division of Endocrinology, Diabetes, and Nutrition, University of Maryland School of Medicine, Baltimore (A. Shuldiner; Q.D.G.); Clinical Research Branch, National Institute on Aging, Baltimore, MD (T.T., L.F.); Medstar Research Institute, Baltimore, MD (T.T.); Division of Preventive Medicine, Brigham and Women’s Hospital, Boston, MA (R.Y.L.Z., L.M.R., J.E.B., D.B., P.M.R., D.I.C.); Department of Medicine, Johannes Gutenberg University, Mainz, Germany (R.B.S.); Department of Medicine, Baylor College of Medicine and Center for Cardiovascular Prevention, Methodist DeBakey Heart and Vascular Center, Houston, TX (V.N., R.C.H., B.C.A., J.C., C.M.B.); Department of Epidemiology, University of Washington, Seattle (N.L.S.); Seattle Epidemiologic Research and Information Center of the Department of Veterans Affairs Office of Research and Development, Seattle, WA (N.L.S.); Unit of Disease Risk, Department of Chronic Disease Prevention, National Institute for Health and Welfare, Helsinki, Finland (J.S.); Department of Epidemiology, MD Anderson Cancer Center, University of Texas, Houston (P.S., X.X.); Department of Clinical Chemistry, University of Oulu, Oulu, Finland (A.R.); Interfaculty Institute for Genetics and Functional Genomics, Ernst-Moritz-Arndt University Greifswald, Greifswald, Germany (A.T., G.H.); Departments of Pathology and Biochemistry, Colchester Research Facility, Colchester, Vermont (R.P.T.); Division of Epidemiology and Community Health, University of Minnesota, Minneapolis (A.R.F., J.P.); Department of Internal Medicine, Erasmus Medical Center, Rotterdam, Netherlands (E.J.G.S., A.G.U.); Medical Research Council–Health Protection Agency Centre for Environment and Health, Department of Epidemiology and Biostatistics, School of Public Health, St Mary’s Campus, Imperial College London, London, UK (P.E., M.R.J.); Department of Biostatistics, Department of Genetics, University of North Carolina, Chapel Hill (W.S.); Amgen, Inc, Cambridge, MA (S.B., A.N.P., L.Y., K.T.); Department of Life Course and Services, National Institute for Health and Welfare, Helsinki, Finland (A.P., M.R.J.); Department of Medicine III, Martin Luther University, Halle-Wittenberg, Germany (K.W.); Institute for Medical Epidemiology, Biostatistics, and Informatics, Martin Luther University, Halle-Wittenberg, Germany (K.H.G., O.K.); Division of Cancer Epidemiology, German Cancer Research Centre, Heidelberg, Germany (K.H.G.); Department of Pharmacology, Ernst-Moritz-Arndt University Greifswald, Greifswald, Germany (H.E.M.Z.S.); Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University of Leipzig, Leipzig, Germany (J.T.); Division of Clinical Developmental Sciences, St George’s University of London, London, UK (Y.J.); Department of Twin Research and Genetic Epidemiology Unit, St Thomas’ Campus, King’s College London, St Thomas’ Hospital, London, UK (Y.J.); Wellcome Trust Sanger Institute, Hinxton, UK (N.S.); Department of Twin Research and Genetic Epidemiology Unit, King’s College London, London, UK (T.D.S.); Institute for Community Medicine, Ernst-Moritz-Arndt Universität Greifswald, Greifswald, Germany (H.V., D.R.); University of Iceland, Reykjavik, Iceland (T.A., V.G.); Cardiovascular Health Research Unit, Departments of Medicine, Epidemiology, and Health Services, University of Washington, Seattle (D.S.S., B.M.P.); Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA (X.G., J.I.R.); Laboratory of Genetiic National Institute on Aging, Baltimore, MD (D.S.); Croatian Centre for Global Health, University of Split Medical School, Split, Croatia (I.R.); Department of Psychiatry/EMGO Institute/Neuroscience Campus, VU University Medical Centre, Amsterdam, Netherlands (B.W.P.); Institute of Health Sciences and Biocenter Oulu, Faculty of Medicine, University of Oulu, Oulu, Finland (M.R.J.); Department of Biostatistics, University of Washington, Seattle (K.R.); Unit of Chronic Disease Epidemiology and Prevention, Department of Chronic Disease Prevention, National Institute for Health and Welfare, Helsinki, Finland (V.S.); Departments of Internal Medicine and Human


Sources of Funding
See the online-only Data Supplement.

Disclosures
Dr Ridker has received research grant support from Roche, AstraZeneca, and Amgen and is listed as a coinventor on patents held by the Brigham and Women’s Hospital that relate to the use of inflammatory biomarkers in cardiovascular disease and diabetes mellitus.


**CLINICAL PERSPECTIVE**

C-reactive protein (CRP) is a heritable marker of chronic inflammation that is strongly associated with cardiovascular disease. Although environmental factors such as obesity, smoking, and hormone therapy influence levels of serum CRP, genes play an important role in determining serum CRP levels. The advent of genome-wide association studies has provided an opportunity to identify previously unsuspected genetic loci that influence complex traits. In this study, we collected data on >80,000 subjects from 25 studies and identified 18 genetic loci that are associated with serum CRP levels. These genetic loci provide valuable insights into the pathways that affect serum levels of CRP. Although further investigations are needed to understand the exact mechanisms, our findings highlight immune response and metabolic regulatory pathways involved in the regulation of chronic inflammation, as well as several loci previously unknown to be related to inflammation. However, these single-nucleotide polymorphisms were not associated with incident myocardial infarction or coronary heart disease, either individually or in combination. A better knowledge of the molecular mechanisms that control serum CRP levels may lead to a deeper understanding of the complex interactions underlying the inflammatory response in cardiovascular disease.