

Genome Analyses of >200,000 Individuals Identify 58 Loci for Chronic Inflammation and Highlight Pathways that Link Inflammation and Complex Disorders

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C-reactive protein (CRP) is a sensitive biomarker of chronic low-grade inflammation and is associated with multiple complex diseases. The genetic determinants of chronic inflammation remain largely unknown, and the causal role of CRP in several clinical outcomes is debated. We performed two genome-wide association studies (GWASs), on HapMap and 1000 Genomes imputed data, of circulating amounts of CRP by using data from 88 studies comprising 204,402 European individuals. Additionally, we performed *in silico* functional analyses and Mendelian randomization analyses with several clinical outcomes. The GWAS meta-analyses of CRP revealed 58 distinct genetic loci ($p < 5 \times 10^{-8}$). After adjustment for body mass index in the regression analysis, the associations at all except three loci remained. The lead variants at the distinct loci explained up to 7.0% of the variance in circulating amounts of CRP. We identified 66 gene sets that were organized in two substantially correlated clusters, one mainly composed of immune pathways and the other characterized by metabolic pathways in the liver. Mendelian randomization analyses revealed a causal protective effect of CRP on schizophrenia and a risk-increasing effect on bipolar disorder. Our findings provide further insights into the biology of inflammation and could lead to interventions for treating inflammation and its clinical consequences.

Introduction

Inflammation plays a key role in the development of complex diseases, such as cardiovascular disease,¹ type 2 dia-

betes,² Alzheimer disease,³ and schizophrenia.⁴ C-reactive protein (CRP) is a sensitive marker of chronic low-grade inflammation,⁵ and elevated serum amounts of CRP have been associated with a wide range of diseases.^{6–8}

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Unraveling the genetics of inflammation could provide further insights into the underlying biology of inflammation and could identify therapeutic targets for attenuating inflammation.

The genetic determinants of CRP have only been partly characterized. In 2011, our group published a HapMap-based genome-wide association study (GWAS) meta-analysis including a discovery panel of up to 65,000 individuals and found 18 loci that were associated with amounts of CRP.⁹ Increasing GWAS sample size and denser mapping of the genome with further advanced imputation panels could help to identify further genes associated with the phenotypes of interest.^{10,11} Furthermore, by using genetic instrumental variables (i.e., a genetic score), Mendelian randomization (MR) allows investigation of the potential causal effect of an exposure on clinical outcomes and could help to elucidate the causal pathways that link the exposure with the outcome.¹² The causal role of CRP in the development of diseases is still controversial,¹³ and the causal pathways that link inflammation to complex disorders are only partly understood.

We applied two large-scale GWASs on circulatory amounts of CRP by using HapMap and 1000 Genomes (1KG) imputed data to identify genetic determinants of chronic inflammation. Because body mass index (BMI) is a major determinant of CRP amounts, we additionally conducted a GWAS adjusted for BMI to identify associated loci independent of BMI. To identify any sex differences in genetic determinants of chronic inflammation, we further conducted GWASs in men and women separately. We applied *in silico* functional analyses on the identified loci to obtain better insights into the biological processes potentially regulating chronic inflammation. Finally, we conducted MR analyses to provide an improved understanding of the causal relation between CRP and several related clinical outcomes.

Material and Methods

GWAS for Circulating Amounts of CRP

We conducted a meta-analysis of GWASs including individuals of European ancestry within the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Inflammation Working Group (CIWG).¹⁴ All participating studies were approved by an institutional review board (see details in the [Supplemental Data](#)). The CIWG invited cohorts for participation in the HapMap GWAS meta-analysis of CRP amounts in 2012. In 2014, in light of our assessment that showed complementary values of HapMap and

1KG imputed GWASs,¹⁰ we invited studies to participate in the 1KG GWAS meta-analysis. The 1KG GWAS could help to identify loci that were not covered in the HapMap GWAS and fine-map loci found in the HapMap GWAS. Cohorts were allowed to participate in either the HapMap or 1KG GWAS or both. Here, we present a meta-analysis of both HapMap (204,402 individuals from 78 studies) and 1KG (148,164 individuals from 49 studies) imputed-genotype GWASs. All participating cohorts implemented a pre-specified study plan comprising study design, data quality check, data analysis, and data sharing. We measured serum CRP in mg/L by using standard laboratory techniques ([Supplemental Data](#)) and transformed the values by natural log. Individuals with auto-immune diseases, individuals taking immune-modulating agents (if this information was available), and individuals with CRP amounts 4 SD or more away from the mean were excluded from all analyses. The characteristics of the participants are presented in [Table S1](#). We filtered individuals and genetic variants on the basis of study-specific quality-control criteria ([Table S2](#)). At each individual study site, we tested genetic variants for association with amounts of CRP by using an additive linear regression model adjusted for age, sex, and population substructure and accounting for relatedness, if relevant. Before meta-analysis, we filtered variants on the basis of imputation quality at an R^2 index > 0.4 . To avoid type I error inflation, we corrected study-specific GWASs for genomic inflation. For the HapMap study, we conducted fixed-effect meta-analyses for each genetic variant by using the inverse-variance-weighted (IVW) method implemented in GWAMA¹⁵ and performed a second genomic control on the meta-analysis level. For the 1KG imputed GWAS, we used METAL¹⁶ to perform a fixed-effect meta-analysis. We removed variants that were available in only $< 50\%$ of the samples. The HapMap meta-analysis included 2,437,193 variants, and the 1KG GWAS included 10,019,203 variants. We considered associations with $p < 5 \times 10^{-8}$ to be genome-wide significant. We used a stringent distance criterion—a minimum of 500 kb between two significant variants—to identify distinct loci. In each locus, the variant with the smallest p value was called the lead variant. Additionally, we performed sex-stratified analyses among HapMap imputed studies, and we tested for heterogeneity between sex-specific effect estimates as described previously¹⁷ by using the false-discovery rate (FDR) of Benjamini-Hochberg to assess significance of the p value for sex difference (< 0.05). We conducted BMI-adjusted analyses in the 1KG meta-analysis to determine the role of BMI in mediating the genetic associations with CRP and to increase power to detect associations not mediated by BMI.

LD Score Regression

Because population stratification is a major concern in GWASs and can lead to false-positive associations, we applied linkage disequilibrium (LD) score regression (LDSC) to distinguish whether the inflation of test statistics observed in the CRP GWAS was due to the polygenic architecture of CRP or reflected confounding bias

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due to cryptic relatedness or population stratification. The LD score measures collective genetic variation acquired from all genetic variants in LD with the index tagging (causal) variant.¹⁸ A higher LD score of an index variant implicates more nearby genetic variants in high LD with the index variant, which makes it more likely that the index variant tags causal variant(s). More genetic variants in LD with the index genetic variant (i.e., a higher LD score due to polygenicity) could yield higher (i.e., inflated) test statistics. In contrast, higher test statistics caused by cryptic population stratification will not be related to the LD score. LDSC analysis performs regression of the summary statistics from the GWAS meta-analysis (χ^2 statistics from the GWAS) on the LD scores across the genome. An LDSC intercept that equals 1 suggests no confounding bias, whereas an inflated intercept (larger than 1) suggests contribution of confounding due to relatedness to the test statistics. We used the LDHub web interface to perform LDSC.¹⁹ We filtered variants to the subset of HapMap 3 variants and excluded variants with duplicated rs numbers, ambiguous variants, minor allele frequency (MAF) < 0.01, and reported sample size < 66.7% of the total sample size. We used the default European LD score file based on the European 1KG reference panel.

Furthermore, we applied cross-trait LDSC to estimate genetic correlation of chronic inflammation (by using the HapMap GWAS meta-analysis) with other phenotypes by using published GWAS summary statistics.²⁰ In brief, the cross-product of two GWAS test statistics is calculated at each genetic variant, and this cross-product is regressed on the LD score. The slope of the regression is used for estimating the genetic covariance between two phenotypes.

Identification of Additional Distinct Variants in Associated Loci

To identify additional distinct variants in the associated loci, we performed joint approximate conditional analysis with the 1KG meta-analysis summary statistics and the LD matrix derived from the first cohort of the Rotterdam Study (RS-I) ($n = 5,974$). We used the Genome-wide Complex Trait Analysis (GCTA) tool, which performs a genome-wide stepwise procedure to identify variants according to their distinct association with CRP (i.e., conditional p).^{21,22} We only used variants with an imputation quality of $R^2 > 0.8$ in the reference set (RS-I). This approximate conditional analysis could reveal different lead signals in a locus where multiple associated variants were in the final joint association model. We tested the distinct variants identified in *CRP* jointly for an association with CRP by using individual-level data from the second and third cohorts of the Rotterdam Study (RS-II and RS-III, totaling 5,024 subjects) and the Women's Genome Health Study (WGHS) of 16,299 individuals.

Proportion of CRP Variance Explained

We estimated the variance explained in serum amounts of CRP by using the formula $(2 \times \text{MAF}(1 - \text{MAF})\beta^2)/\text{var}(\text{CRP})$, where β is the estimated effect of the individual variants on CRP²³ and $\text{var}(\text{CRP})$ is the estimated variance in natural-log-transformed CRP in the RS-I cohort. We calculated the variance explained for four combinations of associated variants: (1) the lead variant at just the *CRP* locus, (2) the distinct variants derived from the 1KG joint conditional analysis at the *CRP* locus, (3) all lead variants in the distinct loci, and (4) all lead variants in the distinct loci and, when applicable, the distinct variants derived from the approximate joint conditional analysis at associated loci.

Pathway Analysis and Gene Expression

We used Data-Driven Expression-Prioritized Integration for Complex Traits²⁴ (DEPICT v.1 rel173 beta) to systematically prioritize the most likely causal genes, highlight the pathways enriched with these genes, and identify tissues and cell types in which genes from associated loci are highly expressed. DEPICT requires summary statistics from the GWAS meta-analysis. First, we filtered genome-wide-associated variants from both GWAS meta-analyses by MAF > 0.01 and selected variants with a low correlation with other variants according to PLINK (v.1.90) by using a clumping distance of 500 kb between variants and/or index of LD r^2 threshold < 0.1. The settings for the analysis involved the usage of 1KG pilot phase data²⁵ (phase 1 integrated release v.3; unrelated CEU [Utah residents with ancestry from northern and western Europe], GBR [British in England and Scotland], and TSI [Toscani in Italia] individuals; November 23, 2010) with an $r^2 > 0.5$ LD threshold for locus definition, 10,000 permutations for bias correction, and 500 repetitions for FDR calculation. To summarize and visualize the results, we calculated pairwise Pearson correlation coefficients between all gene-specific Z scores for every pair of reconstituted DEPICT gene sets. We used Affinity Propagation Clustering (apcluster command; APCluster R package²⁶) to identify clusters and representative examples of the clusters and used Cytoscape v.3.2.1 to visualize the results. The DEPICT results of the pathway and gene prioritization are summarized as a heatmap (R v.2.3.3; pheatmap v.1.0.8 package²⁷). The gene-specific Z score describes the likelihood that a given gene is part of the corresponding Gene Ontology (GO) term, KEGG pathway, REACTOME pathway, Mouse Phenotype, or protein-protein interaction network.

Also, we performed Multi-marker Analysis of GenoMic Annotation (MAGMA).²⁸ MAGMA performs gene and gene-set analysis and requires the association results of all variants; therefore, we chose the larger HapMap GWAS for MAGMA. We used the Functional Mapping and Annotation (FUMA)²⁹ tool to perform MAGMA and applied standard settings for running MAGMA.

To prioritize the most likely trait-relevant gene for each GWAS locus, we ran colocalization analysis with the "coloc" R package v.3.1³⁰ separately for the HapMap and 1KG GWASs. We used publicly available genome-wide expression quantitative trait locus (eQTL) data from 5,311 whole-blood samples³¹ and from the Genome Tissue Expression (GTEx) V6p portal, which incorporates eQTL data from 44 post-mortem tissues.³² The coloc package uses approximate Bayes factors to estimate the posterior probability that GWAS and eQTL effects share a single causal variant. All significant *cis*-eGenes or *cis*-eProbes ($q < 0.05$ in GTEx; lowest *cis*-eQTL FDR < 0.05 in Westra et al.³¹) were extracted ± 1 Mb from the lead SNP of each locus. The HapMap SNP positions were converted to hg19 positions (UCSC Genome Browser) with the liftOver command from the rtracklayer v.1.38.3 package. We used the SNPs present in both the GWAS and eQTL datasets. For the HapMap GWAS, the 1KG GWAS, and the GTEx eQTL datasets, we performed the test by using association β , standard error of β , and MAF. For the data from Westra et al.,³¹ we used association p value, MAF, and sample size and included only the subset of *cis*-eQTLs that are publicly available (up to a significance FDR < 0.5). We used default priors supplied by the coloc package ($P1 = 1 \times 10^{-4}$, $P2 = 1 \times 10^{-4}$, and $P12 = 1 \times 10^{-5}$; prior probabilities for association in the GWAS datasets, the eQTL datasets, and both). Full MAF data were not available for the eQTL datasets, so we used the GIANT 1KG p1v3 EUR reference panel instead. We visualized the results as a heatmap by using the pheatmap v.1.0.8 R package.²⁷

Mendelian Randomization

To assess the effect of CRP on complex disorders, we performed a two-sample MR study on nine clinical outcomes—Alzheimer disease (AD), bipolar disorder (BD), coronary artery disease (CAD), Crohn disease (CD), inflammatory bowel disease (IBD), rheumatoid arthritis (RA), schizophrenia, and diastolic blood pressure (DBP), and systolic blood pressure (SBP)—to which CRP showed a potentially causal association at a $p < 0.1$ in a previous MR study.¹³ We used the effect estimates of the 48 lead SNPs found to be associated with CRP in the HapMap GWAS and the effect estimates of the four SNPs that were additionally found to be associated with CRP in the 1KG GWAS in a multiple-instrument approach for the MR analyses ($n = 52$ SNPs). Additionally, we separately studied the effect of rs2794520 at the *CRP* locus to minimize the probability of introducing horizontal pleiotropy. We tested the statistical significance of the association between the instrument and CRP with the following formula:

$$F = \frac{R^2(n-1-k)}{(1-R^2) \times k}$$

R^2 is the CRP variance explained by the genetic instrument (0.014 for the rs2794520 SNP and 0.065 for the 52-SNP score), n is the number of individuals included in the CRP GWAS, and k is the number of variants included in the genetic score. The F statistic was 273 for the 52-SNP score and 2,902 for the rs2794520 SNP, indicating that both instruments were strong.

For the clinical outcomes, we used summary statistics from the most recent meta-analysis of GWASs. For DBP and SBP, we used data from the UK Biobank. The details of the outcome studies are summarized in Table S12. We implemented four different methods of MR analyses: the IVW method, MR-Egger, weighted median (WM), and penalized weighted median (PWM). We used the Two-SampleMR package in R for the MR analyses.³³ Further, we applied the Bonferroni method to correct for multiple testing ($0.05/9$ phenotypes = 5.6×10^{-3}). When the Q statistic of the IVW analyses provided evidence for heterogeneity, the WM estimates were used for significance. The MR methods are described briefly below.

Inverse-Variance Weighted

The IVW method obtains the causal estimate by regressing the SNP associations with the outcome on the SNP associations with the risk factor; the intercept is set to 0, and weights are the inverse variances (IVs) of the SNP associations with the outcome. With a single genetic variant, the estimate is the ratio of coefficients β_Y/β_X , and the standard error is the first term of the Δ method approximation β_{Yse}/β_X . When all CRP SNPs are valid IVs, the IVW estimates converge to the true causal effect. When one or more invalid IVs are present (i.e., one SNP has an effect on an outcome through a different pathway than CRP), the IVW estimate deviates from the true causal effect.

MR-Egger

We used MR-Egger to account for potential unbalanced pleiotropy in the multiple-variant instrument.³⁴ When unbalanced pleiotropy is present, an alternative effect (positive or negative) is present between the SNP and the outcome, and it could bias the estimate of the causal association. The MR-Egger method is similar to the IVW analysis but does not force the intercept to pass through the origin. The slope of the MR-Egger regression provides the estimate of the causal association between CRP and the clinical outcome. An MR-Egger intercept that is significantly different from 0 suggests directional pleiotropic effects that could bias uncorrected estimates of the causal effect. MR-Egger regression depends on the InSIDE (instrument strength independent of direct effect) assumption, which states that the strengths of the effect

of the SNP on the outcome are uncorrelated with the direct pleiotropic effect of the SNP on the outcome.

Weighted Median and Penalized Weighted Median

We applied the median-based method to provide robust estimates of causal association even in the presence of horizontal pleiotropy when up to 50% of the information contributed by the genetic variants is invalid.³⁵ In PWM analysis, the effect of each variant is weighted by a factor that corresponds to the Q statistics (heterogeneity test) of the SNP; this means that most variants will not be affected by this correction, but the causal effect of the outlying variants, which are most likely to be invalid IVs, will be down-weighted.

We displayed the individual SNP estimates of causal effect and corresponding 95% confidence intervals (CIs) in a forest plot. To assess whether one of the variants used in the genetic score had disproportionate effects, we performed “leave-one-out” analyses, where one SNP at a time is removed from the score. We depicted the relationship between the SNP effect on CRP and the SNP effect on the clinical outcomes in a scatterplot and plotted the individual SNP effect against the inverse of their standard error in a funnel plot. When unbalanced pleiotropy is absent, the causal-effect estimates of the individual should center around the meta-analysis estimate in the funnel plot.

We used the proportion of CRP variance explained by the genetic instruments (0.014 for the rs2794520 SNP and 0.065 for the 52-SNP score) to perform power calculations for each outcome by using the online tool mRnd.³⁶ We calculated the power to detect a relative 5%, 10%, 15%, and 20% difference in outcome risk. For example, a 10% difference refers to an odds ratio (OR) of at least 0.90 or 1.10 in outcome risk (Table S13).

Results

HapMap GWAS Meta-analysis for Amounts of CRP

The HapMap meta-analysis identified 3,977 genome-wide-significant variants at $p < 5 \times 10^{-8}$ (quantile-quantile [Q-Q] plot, Figure S1; Manhattan plot, Figure S2), which mapped to 48 distinct loci (Table 1; Table S3). Of the previously reported 18 variants for CRP, 16 remained associated. Compared with the variants in the previous GWAS, the rs6901250 variant at the *GPRC6A* locus ($p = 0.09$) and the rs4705952 variants at the *IRF1* locus ($p = 2.7 \times 10^{-3}$) were not significant. The β estimates for natural-log-transformed CRP for each of the associated loci ranged from 0.020 to 0.229. We observed the strongest association for rs2794520 at *CRP* ($\beta = 0.182$ in the natural-log-transformed CRP [mg/L] per copy increment in the coded allele; $p = 4.17 \times 10^{-523}$), followed by rs4420638 at *APOC1* and *APOE* ($\beta = 0.229$, $p = 1.23 \times 10^{-305}$). As in previous GWAS meta-analysis, the lead variant within interleukin-6 receptor (*IL6R*) was rs4129267 ($\beta = 0.088$; $p = 1.2 \times 10^{-129}$). We identified rs1880241 upstream of *IL6* ($\beta = 0.028$; $p = 8.4 \times 10^{-14}$), related to the interleukin-6 pathway. In addition to the previously described interleukin-1 signaling, the *IL1RN-IL1F10* locus (interleukin-1 receptor antagonist and interleukin-1 family member 10), we found rs9284725 within interleukin-1 receptor 1 (*IL1R1*; $\beta = 0.02$; $p = 7.3 \times 10^{-11}$; Table 1). Compared with the overall meta-analysis including both sexes, the sex-specific meta-analyses did not identify

Table 1. Newly Identified Loci Associated with CRP

Variant ^a	Position ^b	Coded Allele	Frequency of Coded Allele	β^c	Standard Error	p Value	Closest Gene ^d	1KG Lead Variant ^e
Loci Found in the HapMap GWAS								
rs469772	chr1: 91,530,305	T	0.19	-0.031	0.005	5.54×10^{-12}	<i>ZNF644</i>	rs469882
rs12995480	chr2: 629,881	T	0.17	-0.031	0.005	1.24×10^{-10}	<i>TMEM18</i>	rs62105327
rs4246598	chr2: 88,438,050	A	0.46	0.022	0.004	5.11×10^{-10}	<i>FABP1</i>	-
rs9284725	chr2: 102,744,854	C	0.24	0.027	0.004	7.34×10^{-11}	<i>IL1R1</i>	rs1115282
rs1441169	chr2: 214,033,530	G	0.53	-0.025	0.004	2.27×10^{-11}	<i>IKZF2</i>	-
rs2352975	chr3: 49,891,885	C	0.30	0.025	0.004	6.43×10^{-10}	<i>TRAP</i>	rs10049413
rs17658229	chr5: 172,191,052	C	0.05	0.056	0.010	5.50×10^{-9}	<i>DUSP1</i>	rs34471628
rs9271608	chr6: 32,591,588	G	0.22	0.042	0.005	2.33×10^{-17}	<i>HLA-DQA1</i>	rs2647062
rs12202641	chr6: 116,314,634	T	0.39	-0.023	0.004	3.00×10^{-10}	<i>FRK</i>	-
rs1490384	chr6: 126,851,160	T	0.51	-0.025	0.004	2.65×10^{-12}	<i>C6orf173</i>	rs1490384
rs9385532	chr6: 130,371,227	T	0.33	-0.026	0.004	1.90×10^{-11}	<i>L3MBTL3</i>	-
rs1880241	chr7: 22,759,469	G	0.48	-0.028	0.004	8.41×10^{-14}	<i>IL6</i>	rs13241897
rs2710804	chr7: 36,084,529	C	0.37	0.021	0.004	1.30×10^{-8}	<i>KIAA1706</i>	-
rs2064009	chr8: 117,007,850	C	0.42	-0.027	0.004	2.28×10^{-14}	<i>TRPS1</i>	rs6987444
rs2891677	chr8: 126,344,208	C	0.46	-0.020	0.004	1.59×10^{-8}	<i>NSMCE2</i>	rs10956251
rs643434	chr9: 136,142,355	A	0.37	0.023	0.004	1.02×10^{-9}	<i>ABO</i>	9:136146061
rs1051338	chr10: 91,007,360	G	0.31	0.024	0.004	2.27×10^{-9}	<i>LIPA</i>	-
rs10832027	chr11: 13,357,183	G	0.33	-0.026	0.004	4.43×10^{-12}	<i>ARNTL</i>	rs10832027
rs10838687	chr11: 47,312,892	G	0.22	-0.031	0.004	9.12×10^{-13}	<i>MADD</i>	rs7125468
rs1582763	chr11: 60,021,948	A	0.37	-0.022	0.004	2.37×10^{-9}	<i>MS4A4A</i>	rs1582763
rs7121935	chr11: 72,496,148	A	0.38	-0.022	0.004	5.28×10^{-9}	<i>STARD10</i>	-
rs11108056	chr11: 95,855,385	G	0.42	-0.028	0.004	5.42×10^{-14}	<i>METAP2</i>	rs12813389
rs2239222	chr14: 73,011,885	G	0.36	0.035	0.004	9.87×10^{-20}	<i>RGS6</i>	rs2239222
rs4774590	chr15: 51,745,277	A	0.35	-0.022	0.004	2.71×10^{-8}	<i>DMXL2</i>	rs1189402
rs1558902	chr16: 53,803,574	A	0.41	0.034	0.004	5.20×10^{-20}	<i>FTO</i>	rs55872725
rs178810	chr17: 16,097,430	T	0.56	0.020	0.004	2.95×10^{-8}	<i>NCOR1</i>	-
rs10512597	chr17: 72,699,833	T	0.18	-0.037	0.005	4.44×10^{-14}	<i>CD300LF, RAB37</i>	rs2384955
rs4092465	chr18: 55,080,437	A	0.35	-0.027	0.004	3.11×10^{-10}	<i>ONECUT2</i>	-
rs12960928	chr18: 57,897,803	C	0.27	0.024	0.004	1.91×10^{-9}	<i>MC4R</i>	-
rs2315008	chr20: 62,343,956	T	0.31	-0.023	0.004	5.36×10^{-10}	<i>ZGPAT</i>	-
rs2836878	chr21: 40,465,534	G	0.27	0.043	0.004	7.71×10^{-26}	<i>DSCR2</i>	rs4817984
rs6001193	chr22: 39,074,737	G	0.35	-0.028	0.004	6.53×10^{-14}	<i>TOMM22</i>	rs4821816
Additional Loci Found in the 1KG GWAS								
rs75460349	chr1: 27,180,088	A	0.97	0.086	0.014	4.50×10^{-10}	<i>ZDHHC18</i>	-
rs1514895	chr3: 170,705,693	A	0.71	-0.027	0.004	2.70×10^{-9}	<i>EIF5A2</i>	-
rs112635299	chr14: 94,838,142	T	0.02	-0.107	0.017	2.10×10^{-10}	<i>SERPINA1, SERPINA2</i>	-
rs1189402	chr15: 53,728,154	A	0.62	0.025	0.004	3.90×10^{-9}	<i>ONECUT1</i>	-
Additional Loci Found in the BMI-Adjusted 1KG GWAS								
3:47431869	chr3: 47,431,869	D	0.59	0.024	0.004	1.10×10^{-8}	<i>PTPN23</i>	-
rs687339	chr3: 135,932,359	T	0.78	-0.030	0.005	2.80×10^{-10}	<i>MSL2</i>	-

(Continued on next page)

Table 1. Continued

Variant ^a	Position ^b	Coded Allele	Frequency of Coded Allele	β^c	Standard Error	p Value	Closest Gene ^d	1KG Lead Variant ^e
rs7795281	chr7: 74,122,854	A	0.76	0.028	0.005	3.10×10^{-8}	<i>GTF2I</i>	–
rs1736060	chr8: 11,664,738	T	0.60	0.029	0.004	2.60×10^{-13}	<i>FDFT1</i>	–
17:58001690	chr17: 58,001,690	D	0.44	–0.026	0.004	9.50×10^{-10}	<i>RPS6KB1</i>	–
rs9611441	chr22: 41,339,367	C	0.49	–0.022	0.004	1.40×10^{-8}	<i>XPNPEP3</i>	–

^aHapMap variants are presented, except for the 1KG additional findings.

^bPositions are according to UCSC Genome Browser build hg19.

^c β coefficients represent a 1-unit change in the natural-log-transformed CRP (mg/L) per copy increment in allele A1.

^dThe closest gene represents either the gene in which the variant is located or the closest gene.

^eFor HapMap loci, the lead variant from the 1KG GWAS is presented when the loci were also found in the 1KG GWAS.

additional loci for CRP. However, at four genetic variants, we found evidence for heterogeneity in effect estimates between women and men (Table S4), although the directions of associations were consistent.

1KG GWAS Meta-analysis for Amounts of CRP

In the 1KG meta-analysis, 8,002 variants were associated with CRP at $p < 5 \times 10^{-8}$ (Q-Q plot, Figure S3; Manhattan plot, Figure S4). This resulted in 40 distinct loci, of which 36 overlapped the HapMap meta-analysis (Table 1). The lead variant at the *CRP* locus in the 1KG GWAS was rs4287174 ($\beta = -0.185$; $p = 1.95 \times 10^{-398}$), which was in high LD with rs2794520 ($r^2 = 0.98$), the lead variant at the *CRP* locus in the HapMap GWAS. Among eight of the overlapping loci (rs1260326, rs1490384, rs10832027, rs1582763, rs7310409, rs2239222, rs340005, and rs1800961), the lead variant was at the same position in both GWASs. Compared with HapMap variants, the four additional variants identified in 1KG were rs75460349, rs1514895, rs112635299, and rs1189402. The variants rs1514895 and rs1189402 were available in the HapMap GWAS but were not associated at the genome-wide threshold ($p = 1.2 \times 10^{-7}$ and $p = 8.1 \times 10^{-3}$, respectively). The two variants rs75460349 and rs112635299 were not available in the HapMap GWAS or in high LD ($r^2 < 0.8$). rs75460349 is a low-frequency variant with a coded allele frequency of 0.97 ($\beta = 0.086$; $p = 4.5 \times 10^{-10}$). Also, rs112635299 near *SERPINA1* and *SERPINA2* is a low-frequency variant with a MAF of 0.02 ($\beta = 0.107$; $p = 2.1 \times 10^{-10}$). Adjustment for BMI in the 1KG GWAS ($n = 147,827$) revealed six additional loci that were not associated with CRP in the HapMap or 1KG primary analyses (Table 1; Table S5; Q-Q plot, Figure S5; Manhattan plot, Figure S6). The associations at three lead variants were much reduced after adjustment for BMI (rs1558902 [*FTO*], $p_{\text{adjusted}} = 0.40$; rs12995480 [*TMEM18*], $p_{\text{adjusted}} = 0.02$; rs64343 [*ABO*], $p_{\text{adjusted}} = 1.0 \times 10^{-7}$). Both *FTO* and *TMEM18* are well-known obesity-related genes. Except for the *FTO*, *TMEM18*, and *ABO* loci, all distinct loci identified in the primary 1KG analysis were also associated with CRP in the BMI-adjusted 1KG analysis. No genome-wide-significant association was observed on the X chromosome in the 1KG GWAS, which included 102,086 individuals.

LD Score Regression

The HapMap GWAS LDSC intercept was 1.03 (standard error = 0.013), and the 1KG intercept was 1.02 (standard error = 0.011). This suggests that a small proportion of the inflation is attributable to confounding bias (~12% for the HapMap GWAS and ~13% for the 1KG GWAS). Hence, the vast majority of inflation is due to the polygenic architecture of circulating amounts of CRP. As depicted in Figure 1, CRP showed strong positive genetic correlations with anthropometric traits (e.g., $R_g = 0.43$ and $p = 5.4 \times 10^{-15}$ for BMI), glycemic phenotypes (e.g., $R_g = 0.33$ and $p = 3.1 \times 10^{-6}$ for type 2 diabetes), lipid phenotypes (e.g., $R_g = 0.29$ and $p = 7.9 \times 10^{-5}$ for triglycerides), and CAD ($R_g = 0.23$ and $p = 2.4 \times 10^{-5}$) (Table S6). By comparison, CRP showed inverse genetic correlations with educational attainment (e.g., $R_g = -0.27$ and $p = 9.2 \times 10^{-7}$ for college completion), lung function (e.g., $R_g = -0.24$ and $p = 4.6 \times 10^{-12}$ for forced vital capacity), and high-density lipoprotein cholesterol ($R_g = -0.30$ and $p = 4.8 \times 10^{-9}$).

Additional Signals at Distinct Loci

Approximate conditional analyses in the 1KG GWAS revealed additional signals at nine loci (Table S7). Five loci showed one secondary signal (*IL6R*, *NLRP3*, *HNF1A*, *CD300LE*, and *APOE* and *APOC1*), the *PPP1R3B* locus had two additional signals, the *LEPR* locus had three additional signals, and the *SALL1* locus had four additional signals, whereas the *CRP* locus showed a total of 13 distinct associated variants. Interestingly, the rs149520992 rare variant (MAF = 0.01) mapping to the *CRP* locus showed an association at $p_{\text{conditional}} = 3.7 \times 10^{-15}$ with $\beta = -0.272$ for the T allele. The GCTA effect estimates for the ten distinct variants identified in the vicinity of *CRP* by the 1KG conditional analysis are highly correlated with these variants' effect estimates obtained from the RS-I and WGHS individual-level data ($r_{\text{RS}} = 0.97$ and $r_{\text{WGHS}} = 0.84$), confirming the reliability of the GCTA estimates.

Explained CRP Variance

The lead variant at the *CRP* locus in both the HapMap (rs2794520) and 1KG (rs4287174) GWASs explained 1.4% of the variance in natural-log-transformed CRP amounts. The distinct *CRP* variants derived from the joint

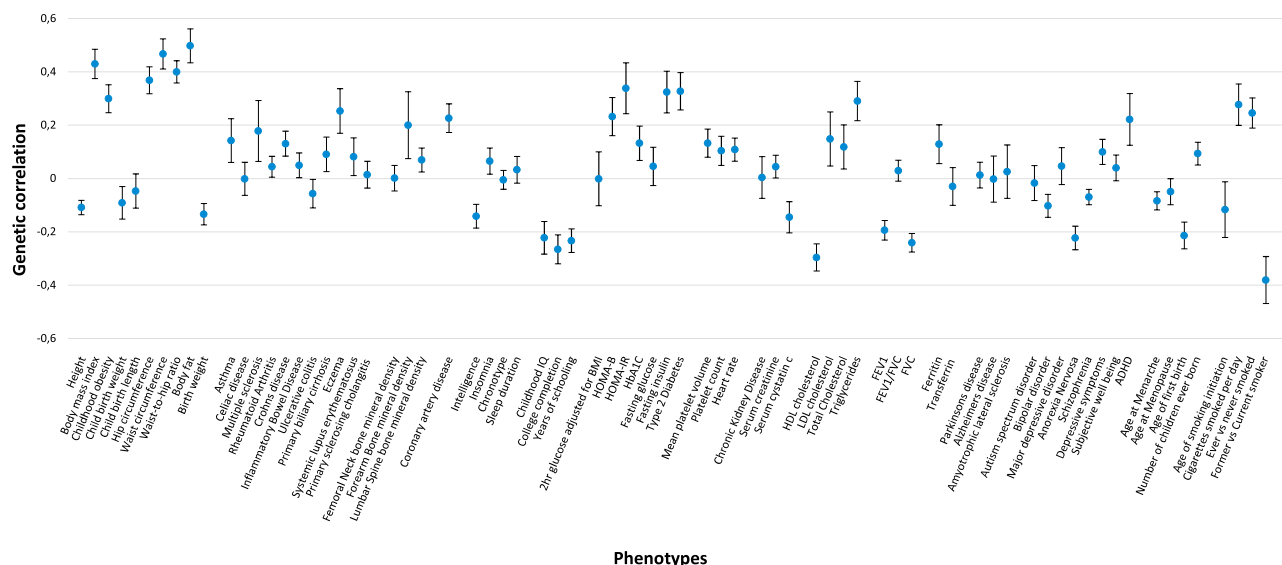


Figure 1. Genome-wide Genetic Correlation between Serum Amounts of CRP and Different Phenotypes and Clinical Diseases
The genetic correlation and its standard error are estimated by LDSC analysis. Abbreviations are as follows: ADHD, attention deficit and hyperactivity disorder; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; HOMA-B, homeostatic model assessment β cell function; HOMA-IR, homeostatic model assessment insulin resistance; and HbA1c, hemoglobin A1c.

conditional analysis in the 1KG GWAS explained 4.3% of the variance. The lead variants at all distinct loci together explained 6.2% of the CRP variance in the HapMap GWAS and 6.5% in the 1KG GWAS. When we added the distinct variants derived from the conditional analysis at associated loci, the variance explained by all associated loci was 11.0% in the 1KG GWAS.

Functional Annotation

We applied DEPICT and MAGMA analyses for functional annotation and biological interpretation of the findings. The DEPICT analysis included 9,497 genome-wide-significant variants covering 283 genes and prioritized 55 candidate genes across 29 regions ($FDR < 0.05$; Table S8). The prioritized genes included *IL6R*, which mapped to the 1q21.3 locus (represented by rs4129267), and *APCS*, which mapped to the 1q32.2 locus. Investigating 10,968 reconstituted gene sets for enrichment, DEPICT highlighted 583 (5.3%) gene sets to be significantly enriched among CRP-associated loci at $FDR < 0.05$ (Table S9). Using further clustering, we identified 66 groups of gene sets that substantially correlated and clustered in two sets, one mainly composed of immune pathways and the other enriched with metabolic pathways (Figure 2). In Figure 3, we present the prioritized genes and the most significant gene sets. We found synovial fluid, liver tissue, and monocytes to be enriched with expression of the prioritized genes ($FDR < 0.05$). We applied MAGMA analysis to the HapMap GWAS, identifying five significantly enriched gene sets (Bonferroni-corrected $p < 0.05$; Table S10). Results included consequences of EGF induction, positive regulation of gene expression, and the interleukin-6 signaling pathway, in line with the most strongly prioritized gene from DEPICT gene prioritization. MAGMA analysis prioritized liver as a sole enriched tissue ($p = 0.048$).

To prioritize the most likely trait-relevant gene for each GWAS locus, we interrogated the GWAS data with *cis*-eQTL data identified from 44 post-mortem tissues and a large whole-blood eQTL meta-analysis by using colocalization analysis (Table S11). Figure S7 presents the GWAS loci that colocalize with *cis*-eQTLs with the corresponding tissue, the colocalizing gene, and the posterior probability that one shared underlying variant drives both associations. Out of the 58 lead gSNPs, 25 SNPs (43%) showed evidence of colocalization with one or more local eQTL effects (posterior probability > 0.9). For example, the rs2293476 locus colocalized with several *cis*-eQTL effects for *PABPC4* and pseudogenes *OXCT2P1*, *RP11-69E11.4*, and *RP11-69E11.8*. The rs10925027 locus colocalized with the *cis*-eQTL effect for *NLRP3* exclusively in the highly powered blood meta-analysis. Out of 25 loci, nine loci had only one colocalizing gene. Altogether, gSNP-associated *cis*-eQTL effects were present in up to 14 different tissues, of which whole blood, esophagus mucosa, skin, and tibial nerve were the most frequent.

Mendelian Randomization Analyses

We observed a protective effect of genetically determined variance in CRP with schizophrenia with an IVW OR of the 52-SNP score of 0.89 (95% CI = 0.81–0.97; $p = 6.6 \times 10^{-3}$; Tables S14 and S15; Figure S8–S11). The MR-Egger intercept was compatible with no unbalanced pleiotropy ($p = 0.48$). The estimate of the rs2794520 variant was comparable to the 52-SNP score estimate (OR = 0.89; 95% CI = 0.84–0.94; $p = 0.046$). The WM and PWM estimates were comparable to the IVW estimate (OR_{WM} = 0.89 and $P_{WM} = 5.1 \times 10^{-3}$; OR_{PWM} = 0.89 and $P_{PWM} = 4.4 \times 10^{-3}$). The “leave-one-out” analysis provided evidence that no single variant was driving the IVW

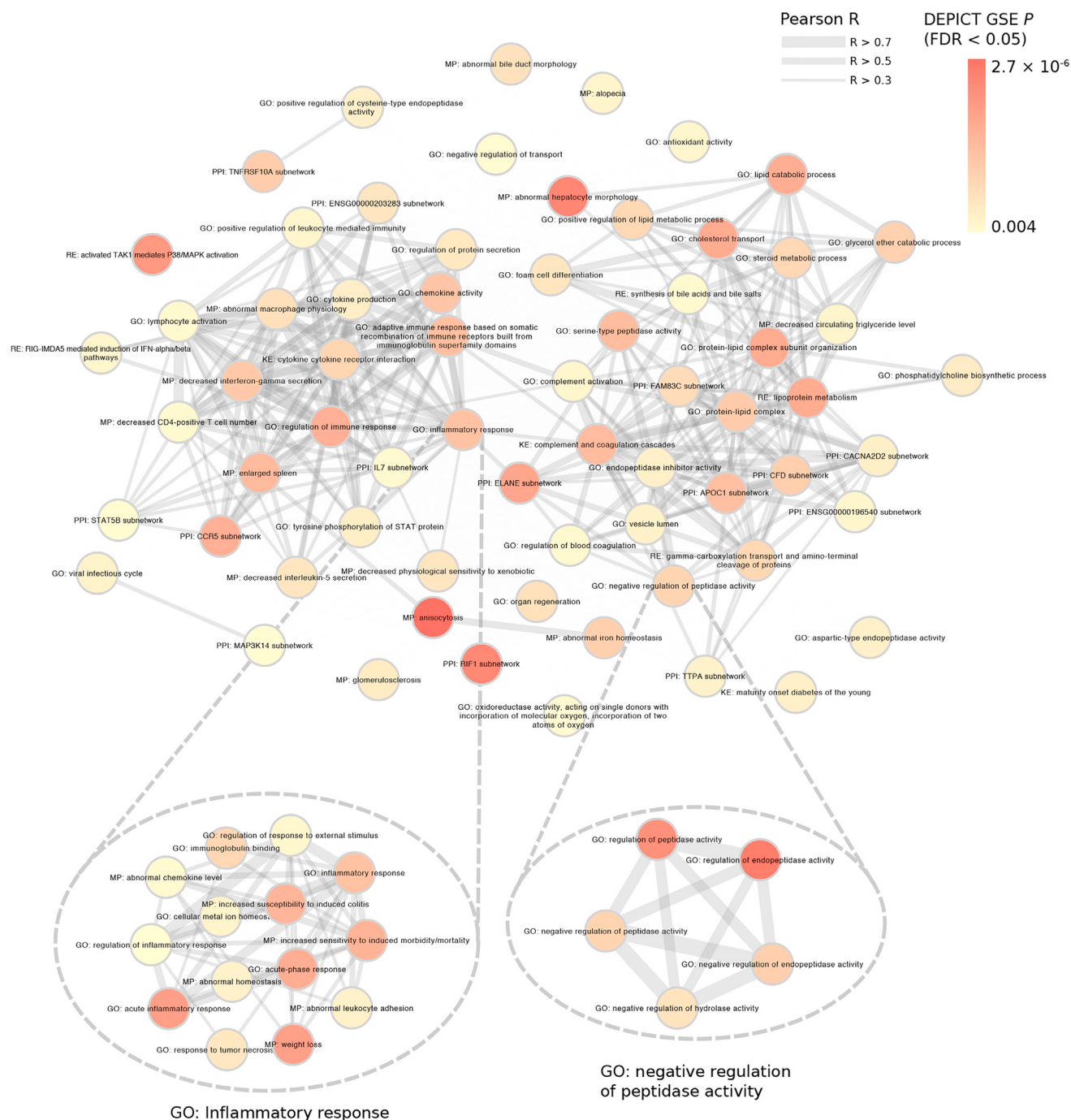


Figure 2. Results of the DEPICT Functional Annotation Analysis

Each node represents an exemplar gene set from affinity-propagation clustering, and links represent corresponding Pearson correlation coefficients between individual enriched gene sets (only the links with $r > 0.3$ are shown). DEPICT GSE P refers to the gene-set enrichment p value for that DEPICT gene-set generated by DEPICT. As an example, outlined are the individual gene sets inside two clusters (“inflammatory response” and “negative regulation of peptidase activity”).

point estimate (Figure S10). The causal OR between the rs2794520 variant and BD was 1.33 (95% CI = 1.03–1.73; $p = 0.032$). For the 52-SNP score, the IVW OR was 1.16 (95% CI = 1.00–1.35; $p = 0.054$). The MR-Egger intercept was compatible with unbalanced pleiotropy ($p = 0.049$). The MR-Egger estimate OR of the 52-SNP score was comparable to the rs2794520 estimate (OR = 1.36; 95% CI = 1.10–1.69; $p = 6.7 \times 10^{-3}$), as were the WM and

PWM estimates (OR_{WM} = 1.33 and $P_{WM} = 3.4 \times 10^{-3}$; OR_{PWM} = 1.32 and $P_{PWM} = 4.3 \times 10^{-3}$).

We observed evidence against a causal association between either *CRP* rs2794520 (OR = 1.01; 95% CI = 0.91–1.12; $p = 0.88$) or the 52-SNP instrument (OR = 0.96; 95% CI = 0.84–1.09; $p = 0.51$) and CAD. An Egger intercept of 0.014 suggested the presence of unbalanced pleiotropy ($p = 5.8 \times 10^{-3}$), and the MR-Egger causal estimate

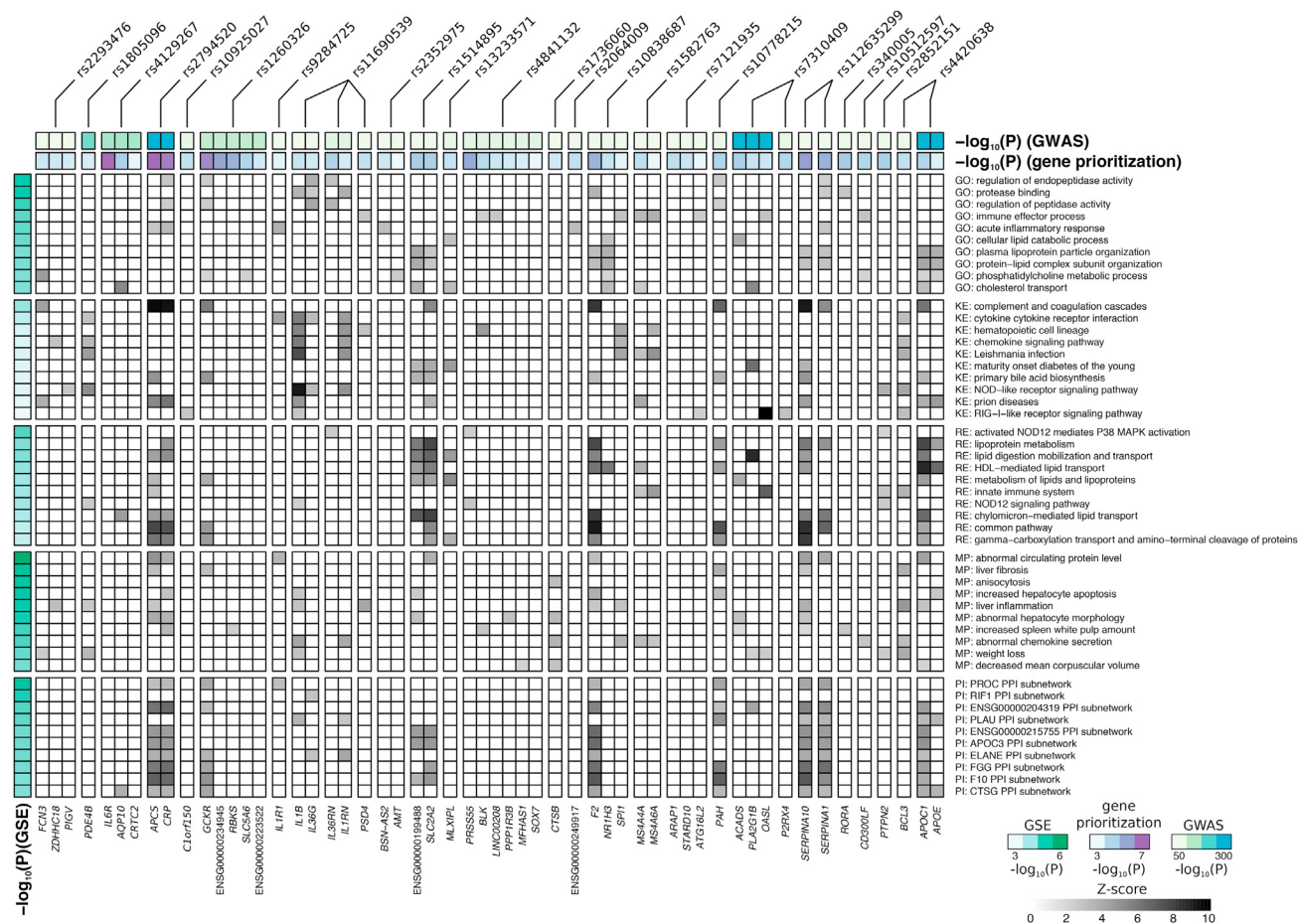


Figure 3. Heatmap Representing the Results of DEPICT Functional Annotation Analysis

Each row represents enriched ($FDR < 0.05$) gene sets, and each column represents prioritized ($FDR < 0.05$) genes. Colors on the heatmap represent each gene's contribution to gene-set enrichment (GSE; depicted as a Z score; only the top ten highest Z scores per gene set are visualized). Sidebars represent p values for GWAS, GSE, and gene prioritization (nominal p value on $\log_{10}$ scale). The top ten gene sets per annotation category are visualized. Abbreviations are as follows: GO, Gene Ontology; KE, Kyoto Encyclopedia of Gene and Genomes; RE, REACTOME pathways; MP, mouse phenotype; and PI, protein-protein interaction.

was $OR = 0.79$ (95% CI = 0.67–0.94; $p = 0.012$). However, the WM and PWM showed no association between CRP and CAD. For AD, there was evidence against an association with rs2794520 ($p = 0.592$), although the IVW OR showed a protective effect ($OR = 0.51$; 95% CI = 0.30–0.88; $p = 0.015$). The Egger intercept of 0.046 suggested unbalanced pleiotropy ($p = 0.042$), and the MR-Egger OR was 0.27 (95% CI = 0.12–0.60). However, the association was null for the WM and PWM analyses ($OR_{WM} = 1.04$ and $P_{WM} = 0.61$; $OR_{PWM} = 1.05$ and $P_{PWM} = 0.53$). We observed evidence against an effect for CD, DBP, IBD, RA, and SBP for the rs2794520 variant and the IVW, MR-Egger, WM, and PWM analyses.

Discussion

Using genomic data from >200,000 individuals, we have identified 58 distinct signals for circulating amounts of CRP, confirming 16 previously identified CRP loci. BMI-adjusted GWASs suggested that the vast majority of

genetic risk variants affect CRP amounts independently of its main determinant (BMI). The genome-wide *in silico* functional annotation analysis highlights 55 genes that are likely to explain the association between 29 signals and amounts of CRP. The data identified gene sets involved in the biology of the immune system and liver as main regulators of serum amounts of CRP. MR analyses supported causal associations between genetically increased CRP and a protective effect on schizophrenia and increased risk of BD.

Obesity is one of the main determinants of chronic low-grade inflammation in the general population.^{37,38} Adjustment for BMI in the CRP GWAS abolished the association at only three lead variants, suggesting that the genetic regulation of chronic low-grade inflammation is largely independent of BMI. Notably, BMI adjustment resulted in the identification of six variants that were not associated with CRP in the BMI-unadjusted GWAS. This supports the notion that adjustment for covariates that explain phenotypic variance could improve the statistical power in linear model analyses of quantitative traits.³⁹ Although adjustment for heritable correlated traits in GWASs could

bias effect estimates (collider bias),⁴⁰ there is consistent evidence in the literature that BMI has a causal direct effect on CRP amounts,⁴¹ and therefore, collider bias in CRP GWASs adjusted for BMI is less likely.

The sex-stratified analyses revealed significant heterogeneity in effect estimates between men and women at only four lead variants, which represent fewer than 10% of all CRP loci. Even among these four loci, the effect directions were similar; thus, the heterogeneity was limited to effect sizes. The data suggest that the difference between men and women in amounts of CRP is less likely to be explained by genetic factors. Furthermore, two signals identified in the former HapMap GWAS of CRP amounts were not significant in the current HapMap GWAS. The effect estimates in the current analyses were too small to identify with our sample size.

The top variant at the *CRP* locus in both the HapMap and 1KG GWASs explained 1.4% of the variance in circulating amounts of CRP. The approximate conditional analysis resulted in 13 variants jointly associated within the *CRP* locus in the 1KG GWAS. With respect to locus definition, we used a more conservative distance criterion than other GWASs, which often use ± 500 kb surrounding the GWAS peak.⁴² Here, we used the criterion that the minimum distance between the boundaries of loci is 500 kb. In order to identify further variants associated with amounts of CRP, we performed approximate conditional analyses, which resulted in multiple putative additional variants also inside and near genes that were not identified in the primary GWAS. As an example, the *CRP* locus spanned >2 Mb according to our criterion. Approximate conditional analysis revealed that two variants, namely rs3027012 near *DARC* and rs56288844 near *FCER1A*, both downstream of *CRP*, were associated with CRP amounts. Furthermore, upstream of *CRP*, we identified a variant near *FCGR2A* (immunoglobulin G Fc receptor II). These results show that for a given lead variant, potentially multiple causal loci—here *DARC*, *FCER1A*, and *FCGR2A* alongside *CRP*—contribute to chronic low-grade inflammation and variation in circulating amounts of CRP.

DEPICT analysis provided further evidence that the genes annotated to the associated CRP variants mainly cluster in the immune and liver biological systems. Notably, the gene set “inflammatory response,” which captures both immune response and liver metabolism, was the main connector network between the two networks. This is in line with the observation that CRP is mainly produced by liver cells in response to inflammatory cytokines during acute and chronic inflammation.⁴³ Interestingly, the analysis highlighted iron homeostasis as an enriched gene set. In agreement, the conditional analysis highlighted a distinct genetic association at the hemochromatosis gene *HFE*, encoding a transmembrane protein of the major histocompatibility complex (MHC) class I family. Previous studies have shown that iron metabolism plays a pivotal role in inflammation.^{44,45} However, genetic pleiotropy could highlight co-regulated pathway-analysis networks

that do not cause inflammation per se. It is also important to note that the results of DEPICT analyses apply to reconstituted gene sets that might sometimes have slightly different overlaying biological themes than the original gene-set annotation.

The MR analyses validate previous evidence that genetically elevated CRP is protective for the risk of schizophrenia,^{13,46} although observational data suggest a positive association between CRP and risk of schizophrenia.⁴⁷ For BD, we observed a positive causal effect, which is in line with previous MR and observational studies.^{13,48} Although the causal underlying mechanisms remain to be elucidated, a hypothesis for the schizophrenia observation might be the immune response to infections early in life. Amounts of acute-phase response proteins in dry blood spots collected at birth are lower for individuals with non-affective psychosis, which includes schizophrenia, than for control individuals, suggesting a weaker immune response at birth.⁴⁹ Also, neonates who have been exposed to a maternal infection and have low amounts of acute-phase response proteins have a higher risk of schizophrenia.⁵⁰ Altogether, the evidence suggests that a deficient immune response could contribute to chronic infection in children and the development of schizophrenia. For AD and CAD, the Egger intercept showed evidence of unbalanced pleiotropy, and the Egger estimate showed a protective effect of CRP on the risk of AD and CAD. However, for both outcomes, the effects of the WM and PWM analyses, as well as analyses using the single rs2794520 variant (which is least likely to be affected by pleiotropy), were null. The MR-Egger estimate relies on the InSIDE assumption, which states that the strength of the association between the genetic variants and CRP is independent of the strength of the direct pleiotropic effects of the genetic variants on the outcome. This assumption can be violated when the genetic variants are associated with a confounder of the CRP-outcome association. Such a scenario can occur when the genetic variants are associated with an exposure that is causally upstream of the exposure under study. In the context of the association between CRP and either AD or CAD, this could be lipids or glycemic phenotypes. Several genetic variants used in the 52-SNP instrument are associated with metabolic phenotypes that might affect amounts of CRP. In agreement, the WM and PWM, in which the InSIDE assumption is relaxed, and the single-variant analysis showed no association. Furthermore, the observation that CRP is not causally related to CAD in the MR analyses is comparable to the findings of previous published studies.⁵¹ Power calculation showed that we had 100% power to detect a 10% difference in CAD risk, so the probability of a false-negative finding is small. Also, CRP is associated with future CAD in observational studies, and randomized trials have shown a beneficial effect of lowering inflammation with the use of statins⁵² and canakinumab⁵³ on CAD risk, but this effect is unlikely to be attributable to CRP.

The strengths of our study are the use of a very large sample size for CRP and the use of both HapMap and 1KG imputed data. Furthermore, we conducted sex-specific and BMI-adjusted analyses to study the effect of sex and body mass on the associations between genetic variants and CRP. To maximize power and to efficiently use the data, we meta-analyzed all available samples in a discovery setting without replication. The consistent association of the variants in >50 studies at a strict Bonferroni-corrected threshold provide confidence that our findings represent true associations. We used both HapMap and 1KG imputed data to identify genetic variants for circulating amounts of CRP. At the start of the project, more studies had HapMap imputed data available. Hence, the sample size and thus power in the HapMap GWAS was higher than that in the 1KG. Also, HapMap could have identified variants that were not identified by the 1KG GWAS.⁵⁴ Nevertheless, 1KG offers better coverage of uncommon variants and includes indels, which are not included in the HapMap reference panel. Including both reference panels, we used all available samples and maximized the possibility of identifying both common and uncommon genetic variants for CRP.

However, we note limitations to our study. GWAS merely identify loci associated with complex phenotypes, and the identification of causal genes remains challenging. We included only individuals of European ancestry; the generalizability of our findings to other races and ethnicities is uncertain. In addition, although our analyses provide support for causal associations, we acknowledge that we might not have identified the causal variants, and we might not have eliminated residual confounding. The colocalization analyses provide evidence for colocalization of CRP GWAS signals and eQTLs; however, they do not provide evidence that the GWAS signal functions on CRP through gene expression. We further note that the method assumes identical LD structure from the GWAS and eQTL datasets. Given that non-European samples make up ~14% of the full dataset, this assumption might be violated for some tissues. Last, we meta-analyzed all available samples in one meta-analysis and did not replicate our findings in an independent sample. Therefore, our findings might need replication.

In conclusion, we performed a large GWAS meta-analysis to identify genetic loci associated with circulating amounts of CRP, a sensitive marker of chronic low-grade inflammation, and found support for a causal relationship between CRP and decreased risk of schizophrenia and increased risk of BD. Given that inflammation is implicated in the pathogenesis of multiple complex diseases, our insights into the biology of inflammation could contribute to future therapies and interventions.

Accession Numbers

All GWAS data reported in this paper are publicly available. Please email s.ligthart@erasmusmc.nl to get more information on how to gain access and download the data.

Supplemental Data

Supplemental Data include 11 figures, 15 tables, and study-specific descriptives and acknowledgments and can be found with this article online at <https://doi.org/10.1016/j.ajhg.2018.09.009>.

Consortia

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Declaration of interests

O.H.F. works at ErasmusAGE, a center for aging research across the life course funded by Nestlé Nutrition (Nestec Ltd.), Metagenics Inc., and AXA. Nestec Ltd., Metagenics Inc., and AXA had no role in the design or conduct of the study; collection, management, analysis, or interpretation of the data; or preparation, review or approval of the manuscript. Bruce M. Psaty serves on the data and safety monitoring board of a clinical trial funded by Zoll LifeCor and on the steering committee of the Yale Open Data Access Project, funded by Johnson & Johnson.

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Web Resources

DEPICT, <https://data.broadinstitute.org/mpg/depict/>
 GCTA, <http://cnsgenomics.com/software/gcta/>
 GIANT 1KG p1v3 EUR reference panel, <http://csg.sph.umich.edu/abecasis/mach/download/1000G.2012-03-14.html>
 GTEx Portal, <https://www.gtexportal.org/>
 GWAMA, <https://www.geenivaramu.ee/en/tools/gwama>
 LD Hub, <http://ldsc.broadinstitute.org/ldhub/>
 METAL, https://genome.sph.umich.edu/wiki/METAL_Documentation
 mRnd power calculations for Mendelian randomization, <http://cnsgenomics.com/shiny/mRnd/>

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