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

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

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

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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

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A list of the institutions and affiliations for the authors of this report may be found in the Acknowledgments at the end of this article.

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panel comprising 16 540 individuals from 10 independent studies. We found 18 genome-wide significant loci, and we provided evidence of replication for 8 of them. Our results confirm 7 previously known loci and introduce 11 novel loci that are implicated in pathways related to the metabolic syndrome (*APOC1*, *HNF1A*, *LEPR*, *GCKR*, *HNF4A*, and *PTPN2*) or the immune system (*CRP*, *IL6R*, *NLRP3*, *IL1F10*, and *IRF1*) or that reside in regions previously not known to play a role in chronic inflammation (*PPP1R3B*, *SALL1*, *PABPC4*, *ASCL1*, *RORA*, and *BCL7B*). We found a significant interaction of body mass index with *LEPR* ($P < 2.9 \times 10^{-6}$). A weighted genetic risk score that was developed to summarize the effect of risk alleles was strongly associated with CRP levels and explained $\approx 5\%$ of the trait variance; however, there was no evidence for these genetic variants explaining the association of CRP with coronary heart disease.

Conclusions—We identified 18 loci that were associated with CRP levels. Our study highlights immune response and metabolic regulatory pathways involved in the regulation of chronic inflammation. (*Circulation*. 2011;123:731-738.)

Key Words: genetics ■ inflammation ■ meta-analysis ■ myocardial infarction ■ genome-wide association 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 lme4 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 (λ_{gc}), by comparing each study's median χ^2 value to 0.4549, the median χ^2 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 ($P < 5 \times 10^{-8}$) loci and sought additional evidence for suggestive loci ($5 \times 10^{-8} < P < 10^{-5}$) 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 al⁹ 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

Table 1. Association of 17 Genome-Wide Significant Loci With CRP Levels in the Discovery Panel

SNP	Band	Significant SNPs	Coded Allele	Allele Frequency	β^* (SE)	<i>P</i>	Gene
rs2794520	1q23.2	121	C	0.66	0.193 (0.007)	9.5×10^{-189}	<i>CRP</i>
rs4420638	19q13.32	16	A	0.80	0.240 (0.010)	2.1×10^{-129}	<i>APOC1</i>
rs1183910	12q24.31	186	G	0.67	0.152 (0.007)	3.3×10^{-113}	<i>HNF1A</i>
rs4420065	1p31.3	291	C	0.61	0.111 (0.007)	3.2×10^{-64}	<i>LEPR</i>
rs4129267	1q21.3	90	C	0.60	0.094 (0.007)	1.1×10^{-47}	<i>IL6R</i>
rs1260326	2q13	54	T	0.41	0.089 (0.007)	5.4×10^{-43}	<i>GCKR</i>
rs12239046	1q44	13	C	0.61	0.048 (0.007)	1.6×10^{-13}	<i>NLRP3</i>
rs6734238	2p23.3	92	G	0.42	0.047 (0.007)	3.4×10^{-13}	<i>IL1F10</i>
rs9987289	8p23.1	15	G	0.90	0.079 (0.011)	2.3×10^{-12}	<i>PPP1R3B</i>
rs10745954	12q23.2	22	A	0.50	0.043 (0.006)	1.6×10^{-11}	<i>ASCL1</i>
rs1800961	20q13.12	1	C	0.95	0.120 (0.018)	2.3×10^{-11}	<i>HNF4A</i>
rs340029	15q22.2	25	T	0.62	0.044 (0.007)	2.6×10^{-11}	<i>RORA</i>
rs10521222	16q12.1	6	C	0.94	0.110 (0.017)	1.3×10^{-10}	<i>SALL1</i>
rs12037222	1p32.4	11	A	0.24	0.047 (0.008)	4.5×10^{-10}	<i>PABPC4</i>
rs13233571	7q11.23	7	C	0.86	0.054 (0.010)	2.8×10^{-8}	<i>BCL7B</i>
rs2836878	21q22.2	2	G	0.72	0.040 (0.007)	4.0×10^{-8}	<i>PSMG1</i>
rs4903031	14q24.2	1	G	0.21	0.046 (0.008)	4.6×10^{-8}	<i>RGS6</i>

* β coefficient represents 1-unit change in the natural log-transformed CRP (mg/L) per copy increment in the coded allele.

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 β 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 6.9×10^{-4} .

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

SNP	Coded Allele	Replication		Discovery+Replication		<i>R</i> ² †	<i>P</i> for Heterogeneity	Closest Gene
		β^* (SE)	<i>P</i>	Beta*(SE)	<i>P</i>			
rs2794520	C	0.086 (0.010)	9.9×10^{-19}	0.160 (0.006)	2.0×10^{-186}	1.38	7.4×10^{-26}	<i>CRP</i>
rs4420638	A	0.200 (0.032)	3.0×10^{-10}	0.236 (0.009)	8.8×10^{-139}	0.93	0.03	<i>APOC1</i>
rs1183910	G	0.122 (0.021)	8.3×10^{-14}	0.149 (0.006)	2.1×10^{-124}	0.76	0.08	<i>HNF1A</i>
rs4420065	C	0.045 (0.009)	1.5×10^{-6}	0.090 (0.005)	3.5×10^{-62}	0.39	1.1×10^{-9}	<i>LEPR</i>
rs4129267	C	0.045 (0.010)	7.3×10^{-6}	0.079 (0.005)	2.1×10^{-48}	0.31	2.4×10^{-4}	<i>IL6R</i>
rs1260326	T	0.031 (0.010)	1.9×10^{-3}	0.072 (0.005)	4.6×10^{-40}	0.24	2.6×10^{-6}	<i>GCKR</i>
rs12239046	C	0.042 (0.018)	1.8×10^{-3}	0.047 (0.006)	1.2×10^{-15}	0.09	0.77	<i>NLRP3</i>
rs6734238	G	0.072 (0.017)	4.9×10^{-6}	0.050 (0.006)	1.8×10^{-17}	0.14	0.95	<i>IL1F10</i>
rs9987289	A	0.003 (0.031)	3.5×10^{-2}	0.069 (0.011)	3.4×10^{-13}	0.08	0.04	<i>PPP1R3B</i>
rs10745954	A	0.018 (0.015)	1.3×10^{-1}	0.039 (0.006)	1.6×10^{-11}	0.06	1.1×10^{-3}	<i>ASCL1</i>
rs1800961	C	0.023 (0.026)	3.7×10^{-1}	0.088 (0.015)	2.2×10^{-9}	0.06	0.07	<i>HNF4A</i>
rs340029	T	0.004 (0.010)	5.2×10^{-1}	0.032 (0.006)	4.1×10^{-9}	0.08	0.05	<i>RORA</i>
rs10521222	C	0.089 (0.028)	1.4×10^{-3}	0.104 (0.015)	8.5×10^{-13}	0.09	0.34	<i>SALL1</i>
rs12037222	A	0.035 (0.017)	3.9×10^{-2}	0.045 (0.007)	6.4×10^{-11}	0.06	0.40	<i>PABPC4</i>
rs13233571	C	0.049 (0.025)	4.5×10^{-2}	0.054 (0.009)	3.6×10^{-9}	0.08	0.13	<i>BCL7B</i>
rs2836878	G	0.013 (0.011)	2.3×10^{-1}	0.032 (0.006)	1.7×10^{-7}	0.05	0.18	<i>PSMG1</i>
rs4903031	G	0.001 (0.012)	9.1×10^{-1}	0.032 (0.007)	5.1×10^{-6}	0.04	0.21	<i>RGS6</i>

* 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.

Table 3. Association of 3 Suggestive Loci With CRP Levels That Reached Genome-Wide Significance After Combining Discovery and Replication Panel

SNP	Coded Allele	Discovery		Replication		Discovery+Replication			<i>P</i> for Heterogeneity	Closest Gene
		β^* (SE)	<i>P</i>	β^* (SE)	<i>P</i>	β^* (SE)	<i>P</i>	<i>R</i> ² †		
rs2847281	A	0.034 (0.007)	1.7×10^{-7}	0.018 (0.016)	4.2×10^{-2}	0.031 (0.006)	2.2×10^{-8}	0.04	0.97	<i>PTPN2</i>
rs6901250	A	0.034 (0.007)	1.2×10^{-6}	0.038 (0.015)	1.2×10^{-2}	0.035 (0.006)	4.8×10^{-8}	0.02	0.89	<i>GPRC6A</i>
rs4705952	G	0.038 (0.008)	4.1×10^{-6}	0.065 (0.018)	3.0×10^{-4}	0.042 (0.007)	1.3×10^{-8}	0.05	0.47	<i>IRF1</i>

* 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 I in the online-only Data Supplement shows the QQ plot ($\lambda=1.09$), 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 ($P < 5 \times 10^{-8}$) (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 ($0.05/64 = 7.8 \times 10^{-4}$) 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 (*PPP1R3B*), 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,⁹ *CRP*, *APOE* (*APOC1*), *HNF1A*, *LEPR*, *IL6R*, *GCKR*, and *ASCL1*, with CRP levels (Bonferroni significance level: $0.05/7 = 7.1 \times 10^{-3}$).

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 ($P < 2.8 \times 10^{-5}$) (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

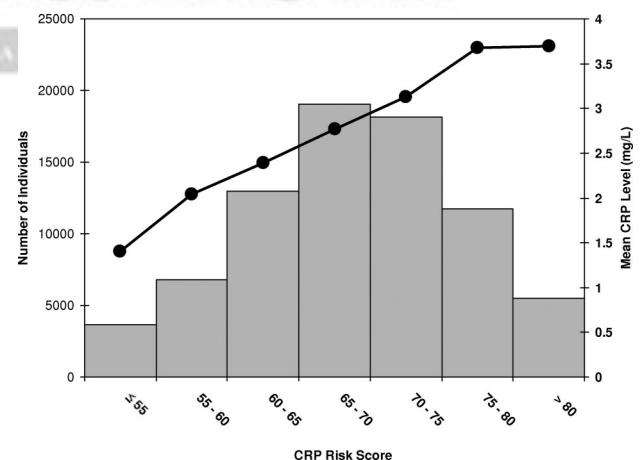


Figure 1. Mean CRP level (right vertical axis) shown as solid black dots connected by solid lines for categories of the genetic risk score. The shaded bars show the distribution of the genetic risk score in the whole population (left vertical axis). The CARdiovascular disease, Living and Aging in Halle (CARLA) Study was not included because of missing values for some of the selected SNPs.

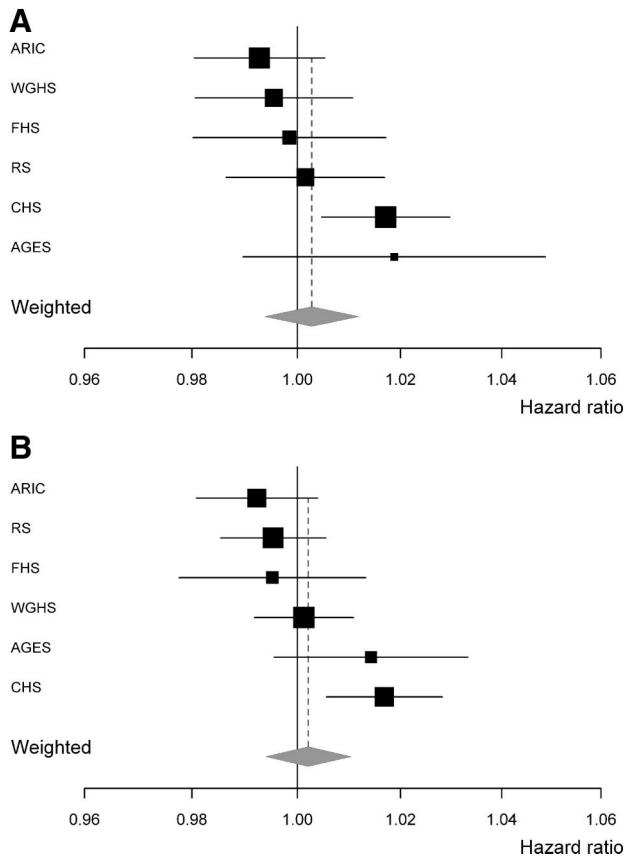


Figure 2. The forest plots show the meta-analysis of the association of the CRP genetic risk score with MI (A) and CHD (B). The horizontal axis indicates the hazard ratio for MI or CHD per unit increase in the rescaled genetic risk score. ARIC indicates Atherosclerosis Risk in Communities; RS, Rotterdam Study; FHS, Framingham Heart Study; WGHS, Women's Genome Health Study; AGES, Age, Gene/Environment Susceptibility-Reykjavik; and CHS, Cardiovascular Health Study.

with age, sex, BMI, and smoking (Table IV in the online-only Data Supplement). After adjustment for the number of tests, we found a significant interaction between BMI and the *LEPR* SNP rs4420065 ($P < 2.9 \times 10^{-6}$).

We examined the association of the SNPs related to CRP with risk of MI and CHD. These studies comprised 1845 cases of MI and 2947 cases of CHD. Neither the individual SNPs nor the combined genetic risk score showed consistent or genome-wide significant associations with risk of clinical events (Figure 2).

Discussion

Through a meta-analysis of GWA scans from 15 cohort studies comprising 66 185 subjects and a replication sample of 16 540 subjects, we identified 18 loci associated with circulating CRP levels and provided evidence of replication for 8 of them. Our results confirm 7 gene-annotated loci reported by Ridker et al.⁹ Furthermore, we introduce 11 novel loci associated with CRP levels, annotating *NLRP3*, *IL1F10*, *PPP1R3B*, *HNF4A*, *RORA*, *SALL1*, *PAPBC4*, *BCL7B*, *PTPN2*, *GPRC6A*, and *IRF1*.

A number of these genes including *APOC1*, *HNF1A*, *LEPR*, *GCKR*, *HNF4A*, and *PTPN2* 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 3.¹⁹ *HNF4A* is part of a complex regulatory network in the liver and pancreas for glucose homeostasis.²⁰ Mutations in the *HNF4A* gene cause MODY type 1.²¹ *HNF4A* is a transcription factor involved in the expression of several liver-specific genes including *HNF1A*.²¹ Defects in the expression of *GCKR* result in deficient insulin secretion.²² *PTPN2*, which modulates interferon gamma signal transduction at the β cell level,²³ was recently identified as a novel susceptibility gene for type 1 diabetes mellitus.²⁴ *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.²⁵

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.²⁶ The NALP3 inflammasome triggers an innate immune response and can be activated by endogenous "danger signals," as well as compounds associated with pathogens.^{27,28} Activated NALP3 inflammasome functions as an activator of nuclear factor- κ B signaling. Nuclear factor- κ B is a transcription factor that affects CRP expression in Hep3B cells.²⁹

Our genetic risk score explained $\approx 5\%$ of the variation in CRP levels, showing that genetic factors are of importance in determining CRP levels. In comparison, BMI as the main nongenetic determinant of CRP was reported to explain 5% to 7% of the variation in CRP levels in AGES³⁰ and up to 15% in FHS.³¹ 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.⁹ However, without adjustment for these covariates, $<5\%$ of the variation in CRP levels was explained (D. Chasman, PhD, personal written communication, May 2009).

Adipose tissue can induce chronic low-grade inflammation by producing proinflammatory cytokines such as interleukin-6.³² 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.³³

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 al¹⁰ 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-C1-C2* 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 hitherto has been less

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.

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See the online-only Data Supplement.

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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.



Circulation
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SUPPLEMENTAL MATERIAL

**Meta-analysis of genome-wide association studies in over 80,000
subjects identifies 11 novel loci for C-reactive protein levels**

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Study-specific method sections

1. Method section for the discovery panel

The Age, Gene/Environment Susceptibility study (AGES)

The AGES-Reykjavik Study cohort originally comprised a random sample of 30,795 men and women born in 1907-1935 and living in Reykjavik in 1967¹. A total of 19,381 people attended, resulting in a 71% recruitment rate. The study sample was divided into six groups by birth year and birth date within one month. One group was designated for longitudinal follow-up and was examined in all stages. One group was designated a control group and was not included in the examinations until 1991. Other groups were invited to participate in specific stages of the study. Between 2002 and 2006, the AGES-Reykjavik study re-examined 5,764 survivors of the original cohort who had participated before in the Reykjavik Study. Participants came in a fasting state to the clinic. The AGES Reykjavik Study GWAS was approved by the National Bioethics Committee (VSN: 00-063) and the Data Protection Authority.

Genotyping was performed using the Illumina 370CNV BeadChip array on 3,664 participants. Sample exclusion criteria included sample failure, genotype mismatch with reference panel, and sex mismatch, resulting in clean genotype data on 3,218 individuals with CRP level measured. Standard protocols for working with Illumina data were followed with clustering score greater than 0.4. From a total of 353,202 SNPs, 325,094 were used for imputation after exclusion of SNPs with call rate <97%, HWE deviation <1 × 10⁻⁶, mishap (PLINK haplotype-based test for non-random missing genotype data²) p <1 × 10⁻⁹, and mismatched positions between Illumina, dbSNP and/or HapMap.

High sensitivity CRP was measured in serum on a Hitachi 912, using reagents from Roche Diagnostics and following the manufacturer's instructions. Both within- and between-assay quality control procedures were used and the coefficient of variation of the method was 1.3% to 3.4%, respectively, through the period of data collection. The assay could detect a minimal CRP concentration of 0.1 mg/L and values below this level were classified as undetectable. All participants in this study had detectable CRP levels.

The Atherosclerosis Risk in Communities (ARIC)

The ARIC study is a longitudinal cohort study of atherosclerosis and its clinical sequelae. It recruited a population-based sample of 15,792 men and women aged 45-64 years from four US communities in 1987-89³. For this study, the analysis was restricted to subjects of European descent. Affymetrix 6.0 array genotypes were obtained in 8,861 self-identified whites: 734 individuals were excluded for the following reasons: 1) discordant with previous genotype data, 2) genotypic sex did not match phenotypic sex, 3) suspected first-degree relative of an included individual based on genome-wide genotype data, 4) genetic outlier (as assessed by average Identity by State (IBS) using PLINK and >8 standard deviations along any of first 10 principal components in EIGENSTRAT after 5 iterations. SNPs without chromosomal location, monomorphic SNPs, SNPs whose genotype frequencies between 2 freezes differed by $p < 10^{-6}$, SNPs with HWE $p < 10^{-6}$ or call rate <90% were excluded from analysis. Imputation of ~2.5 million autosomal SNPs in HapMap with reference to release 22 of the CEU sample was conducted using the algorithm implemented in MACH.

CRP was assessed using the immunoturbidimetric CRP-Latex (II) high-sensitivity assay from Denka Seiken (Tokyo, Japan). This assay, which has been validated against the Dade Behring method (Deerfield, Ill)⁴, was performed according to the manufacturer's protocol and using a BN2 analyzer (Dade Behring (Deerfield, Il). To assess repeatability of measurements, 421 blinded replicates were measured on different dates. The reliability coefficient was 0.99.

British 1958 birth cohort (B58C)

The B58C is a national population sample followed periodically from birth. At age 44-45 years, 9,377 cohort members were examined by a research nurse in the home as described previously⁵ and non-fasting blood samples were collected with permission for DNA extraction and creation of immortalised cell cultures (<http://www.b58cgene.sgul.ac.uk/collection.php>). A total of 1,480 cell-line-derived DNA samples from unrelated subjects of white ethnicity, with nationwide geographic coverage, were used as controls by the Wellcome Trust Case Control Consortium (WTCCC)⁶.

Genotyping was performed using the Affymetrix 500K Mapping Array Set using the call algorithm CHIAMO as implemented by the WTCCC⁶. Genotypes at other loci were imputed by the program IMPUTE version 0.1.2⁷, using 490,032 autosomal SNPs with CHIAMO calls and the linkage disequilibrium patterns in the HapMap CEU panel. Analysis of imputed genotypes used Marchini's SNPTEST⁷ version 1.1.3 with the “-proper” option and supplementary regression modeling used STATA version 10.0.

Details of the blood collection, CRP measurement and covariate adjustment have been described elsewhere⁸. In brief, CRP antigen levels were measured by high-

sensitivity nephelometric assay using latex particles coated with monoclonal antibodies to human CRP in the BN Prospec protein analyzer (Dade Behring, Marburg, Germany). Fuller details of the laboratory protocols are provided on-line (<http://www.b58cgene.sgul.ac.uk/crp1958sop.pdf>). Levels were adjusted for sex, laboratory batch, time of day, month of examination, and postal delay. Adjustment for age was not required as all subjects were aged 44-45 years. Valid CRP measurements were available for 1,461 (98.7%) of the 1,480 subjects genotyped in the WTCCC.

Ethical approvals for the 2002-2004 fieldwork, including consent procedures, and for this within-cohort genotype-phenotype analysis were obtained from the Southeast England Multi-centre Research Ethics Committee.

Cardiovascular Health Study (CHS)

The CHS is a population-based cohort study of risk factors for CHD and stroke in adults ≥ 65 years conducted across four field centers⁹. The original predominantly European ancestry cohort of 5,201 persons was recruited in 1989-1990 from random samples of the Medicare eligibility lists and an additional 687 African-Americans were enrolled subsequently for a total sample of 5,888. DNA was extracted from blood samples drawn on all participants at their baseline examination in 1989-90.

In 2007-2008, genotyping was performed at the General Clinical Research Center's Phenotyping/Genotyping Laboratory at Cedars-Sinai using the Illumina 370CNV BeadChip system on 3,980 CHS participants who were free of cardiovascular disease (CVD) at baseline, consented to genetic testing, and had DNA available for genotyping. Because the other populations in this meta-analysis were primarily of European ancestry,

the self-described CHS African-Americans were excluded from this analysis to reduce the possibility of confounding due to population stratification. Genotyping has been attempted to date in 3,397 white participants, and was successful in 3,291 persons with a sample call rate >95%. In CHS, the following exclusions were applied to identify a final set of 306,655 autosomal SNPs: call rate <97%, HWE $P < 10^{-5}$, >1 duplicate error or Mendelian inconsistency (for reference CEPH trios), heterozygote frequency = 0, SNP not found in HapMap's CEPH panel. After limiting the sample to those with successful genotypes and CRP measurements, the final dataset for this analysis comprised data on 306,655 SNPs in 3,265 CHS participants. Imputation was performed using BAMB v0.99 (0.91 for QT) with reference to HapMap CEU using release 22, build 36 using one round of imputations and the default expectation-maximization warm-ups and runs. SNPs were excluded for variance on the allele dosage ≤ 0.01 .

Blood was drawn in the morning after an overnight fast. Samples were promptly centrifuged at 3000g for 10 minutes at 4°C. Aliquots of plasma were stored in a central laboratory at -70°C. CRP was measured in all stored baseline plasma samples by a high-sensitivity immunoassay, with an inter-assay coefficient of variation of 6.25%¹⁰.

The Erasmus Rucphen Family study (ERF)

The ERF study is a young genetically isolated population from the southwest of the Netherlands which was founded in the middle of 18th century. Minimal immigration and/or marriages occurred between surrounding settlements due to social and religious reasons. While the estimated number of founders is <400, the population experienced a fast expansion and at the moment this region counts roughly 20,000 inhabitants. The ERF

population includes 3,000 individuals, who were not selected based on health information, but rather comprise of living descendants of 22 couples. These couples had at least 6 children baptized in the community church around 1850-1900. Details about the genealogy of the population have been described elsewhere¹¹⁻¹³.

The Illumina HumanHap300 BeadChip array was used to conduct genotyping of the individuals. SNPs with call rate <95% and HWE $p < 10^{-6}$ were excluded. Data on CRP plasma levels and SNP genotypes was available for 1,792 ERF participants. Imputation was conducted using the algorithm implemented in MACH. To get imputed data, more restrictive SNP filters including a minor allele frequency >0.01, SNP call rate >0.98, and HWE p-value $> 1 \times 10^{-6}$ were applied and 491,875 passed the filters. In total 2,586,725 SNPs were imputed using phased haplotypes of HapMap CEU trios.

Fasting plasma samples were collected. Plasma CRP was measured in 2,256 individuals using the US CRP ELISA (cat.# DSL-10-42100) of Diagnostic Systems Laboratories, Inc.(expected range within the 90th percentile is 254-16104 ng/ml). The measurements were performed conform the manufactures protocol.

Framingham Heart Study (FHS)

The FHS is a collection of three cohorts recruited to investigate cardiovascular disease and its risk factors¹⁴. Serum CRP measurements were available from the seventh exam (1998-2001) of the Framingham Offspring cohort (children of the original cohort and their spouses enrolled in 1971)^{15,16}, and the Third Generation cohort (Offspring adult children)¹⁷ first examination, recruited from 2002-2005.

Genotyping was performed using Affymetrix 500K SNP arrays, supplemented with the MIPS 50K array. Genotypes of 8,481 individuals passed our QC criteria that included call rate $\geq 97\%$, no excess Mendelian errors (< 1000) and average heterozygosity within 5 SD of mean (between 25.758% and 29.958%). SNPs analyzed had minor allele frequency $\geq 1\%$, call rate $\geq 95\%$ and HWE $p \geq 10^{-6}$. The analysis reported included data on 425,593 SNPs in 6,899 Framingham participants with measured CRP levels. The MACH software was used to perform imputation based on the haplotypes of the HapMap CEU trios, and 2,046,740 SNPs with good imputation quality, as measured by the ratio of observed to expected variance ≥ 0.3 , were analyzed for association with CRP levels. The SNPs used for imputation passed more stringent filters including a minor allele frequency ≥ 0.01 , SNP call rate ≥ 0.97 , HWE p-value $\geq 1 \times 10^{-6}$, differential missingness p-value $\geq 1 \times 10^{-9}$ and < 100 Mendelian errors; 378,163 SNPs passed these quality control criteria.

CRP was measured in fasting serum samples using a high-sensitivity assay (Dade Behring BN100). The minimum detectable dose of this assay is 0.16 mg/L, with a standard curve range of 0.16-1000 mg/L. The intra-assay coefficient of variation was 3.2%, while the inter-assay coefficient of variation was 5.3%. The final population for this analysis included 6,899 individuals (Offspring $n=3,852$, Third Generation $n=3,047$).

The Health 2000 Study

Health 2000 is a large Finnish cross-sectional health examination survey carried out in 2000–2001¹⁸. The overall study cohort was a two-stage stratified cluster sample (8,028 persons) representing the entire Finnish population aged 30 years and above. A subcohort of 2,080 participants was selected for a GWA study for metabolic syndrome. Cases were

selected according to the IDF Worldwide Definition of the Metabolic Syndrome (<http://www.idf.org/home/index.cfm?node=1429>). Controls were selected for not carrying the trait and were matched for age, sex and residence.

Genotyping for the Health 2000 Study was completed using Illumina's 610-Quad BeadChips for 598,203 SNPs. We excluded 4,295 markers from the analysis because HWE was not met at a level $p < 0.0001$, and 5,842 because of low call rate ($< 95\%$), leaving 588,066 SNPs for the association analysis. Imputation was conducted using the algorithm implemented in MACH.

Venous blood samples were drawn from the antecubital vein after an overnight fast. High sensitivity CRP (hs-CRP) concentrations were determined using a chemiluminescent immunometric assay (Immulite, Diagnostic Products Corporation, Los Angeles, CA, USA).

The MONICA/KORA Augsburg Study (KORA)

The presented data were derived from the third population-based Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) / Cooperative Health Research in the Region of Augsburg (KORA) survey S3¹⁹. This cross-sectional survey covering the city of Augsburg (Germany) and two adjacent counties was conducted in 1994/95 to estimate the prevalence and distribution of cardiovascular risk factors among individuals aged 25 to 74 years as part of the WHO MONICA study. The MONICA/KORA S3 study comprises 4,856 subjects. Among them, 3,006 subjects participated in a follow-up examination of S3 in 2004/05 (MONICA/KORA F3). All participants underwent standardized examinations including blood withdrawals for plasma and DNA. For the

KORA genome-wide association study, 1,644 subjects, aged 45 to 69 years were selected from the KORA S3/F3 samples.

Genotyping for KORA F3 was performed using Affymetrix 500K Array Set consisting of two chips (Sty I and Nsp I). Hybridisation of genomic DNA was done in accordance with the manufacturer's standard recommendations. Genotypes were determined using BRLMM clustering algorithm (Affymetrix 500K Array Set). For quality control purposes, we applied a positive control and a negative control DNA every 48 samples. On chip level, only subjects with overall genotyping efficiencies of at least 93% were included. In addition, the called gender had to agree with the gender in the KORA study database. Imputation of genotypes was performed using maximum likelihood method with the software MACH v1.0.9. After exclusion of subjects with missing information of CRP concentrations, the final population available for this analysis included 1,587 individuals.

CRP was measured in EDTA plasma by a high sensitivity in-house immunoradiometric assay (IRMA) in MONICA/KORA S3, using a five-point calibration with WHO International Reference Standard 85/506. The assay range was 0.05-10 mg/L. Samples with concentrations >10 mg/L were remeasured at higher dilutions. CRP concentrations were determined in triplicate, and the mean was used for analysis. The inter-assay CV for CRP over all ranges was 12%²⁰.

The MICROS Study

The GenNOVA study is an ongoing, comprehensive genetic and health care survey conducted in South Tyrol (Italy). It includes the MICROS study, from which the present

proband was sampled. They were volunteers from three Alpine, isolated, German-speaking villages located in Val Venosta, a region enclosed within the Austria and Switzerland borders²¹.

Genotyping was performed on 1,335 participants, using the Illumina HumanHap300v2 SNP Chip. We excluded from imputation SNPs with a call rate below 98%, HWE $P < 10^{-6}$ and minor allele frequency < 0.01 . The remaining 292,917 SNPs were used for MACH v1.0.16 imputation based on HapMap CEU release 22 build 36.

High sensitivity CRP was measured from fasting serum using the CRP-Dynamic AD-Assay (Invicon diagnostic concepts GmbH, Munich, Germany) on an ADVIA1800 Clinical Chemistry Analyzer (Siemens Healthcare Diagnostics GmbH, Eschborn, Germany). Concentrations from 0.01 to 32 mg/l, with a within-run precision $< 5.0\%$, and a total precision of $< 7.0\%$ can be measured with this system. A total of 1,215 individuals with genotypes and CRP measured were included in this study.

Northern Finland Birth Cohort 66 (NFBC-66)

The NFBC-66 is an on-going follow-up study of people whose expected date of birth was in 1966 in the two Northern provinces of Oulu and Lapland²². Primary clinical data collection on parents and the child occurred prenatally and at birth. Data collection on the child continued at several age points; here the samples were drawn at the 31-year examination.

Genotyping for the NFBC-66 was completed using Illumina Infinium for 339,629 SNPs. We excluded 3,345 SNPs from analysis because HWE was not met at a level $p < 0.0001$, 55 because of low call rate ($< 95\%$) and 7,681 because the MAF was $< 1\%$,

leaving 329,091 SNPs for the association analysis. Imputation was conducted using the algorithm implemented in MACH.

Blood samples were drawn after overnight fasting in the morning (between 08.00 and 11.00 h). Serum gel 10ml tubes were left to stand for 30 minutes after drawing the sample at room temperature. After that, within 90 minutes from sample drawing, samples were centrifuged at 1900 G for 10 minutes at +20 C. Serum hs-CRP concentrations were determined by immunoenzymometric assay (Medix Biochemica). A total of 5,125 NFBC-66 participants were included in the analyses.

Orkney Complex Disease Study (ORCADES)

The ORCADES is an ongoing family-based, cross-sectional study in the isolated Scottish archipelago of Orkney. Genetic diversity in this population is decreased compared to Mainland Scotland, consistent with the high levels of endogamy historically. Data for 715 participants aged 18-100 years, from a subgroup of ten islands, were used for this analysis.

We genotyped 318,237 SNPs for each individual using the Illumina HumanHap300 BeadChip. Alleles were called in BeadStudio using Illumina cluster files. Subjects were excluded if they fulfilled any of the following criteria: genotypic call rate <97%, mismatch between reported and genotypic sex, unexpectedly low genomic sharing with first degree relatives, excess autosomal heterozygosity, or outliers identified by IBS clustering analysis. We excluded SNPs on the basis of minor allele frequency (<0.01), HWE ($P < 10^{-5}$), call rate (<97%). MACH v1.0.15 was used to impute over 2 million SNPs from HapMap build 36.

Fasting blood samples were collected and over 200 health-related phenotypes and environmental exposures were measured in each individual. Fasting plasma samples were collected and frozen at -70C within 1 hr of collection. CRP was measured by ELISA. All participants gave informed consent and the study was approved by Research Ethics Committees in Orkney and Aberdeen.

Rotterdam Study (RS)

The RS is a prospective population-based cohort study to investigate the determinants of chronic diseases²³⁻²⁵. In brief, residents of Ommoord, a district of Rotterdam, in the Netherlands, 55 years of age or older, were asked to participate, of whom 7,983 participated. The baseline examination took place in 1990-1993.

The version 3 Illumina Infinium II HumanHap550 SNP chip array was used to conduct genotyping among self-reported Caucasians. Genotyping was successful in 6,240 individuals with a sample call rate >97.5%. SNPs with a call rate <95% and HWE $p < 10^{-6}$ were excluded. The final dataset for this analysis comprised data on 530,683 SNPs in 5,974 RS participants. Imputation was conducted using the algorithm implemented in MACH. To obtain imputed data, more restrictive SNP filters including a minor allele frequency >0.01, SNP call rate >0.98, and HWE p -value $> 1 \times 10^{-6}$ were applied and 491,875 passed the filters. In total 2,586,725 SNPs were imputed using phased haplotypes of HapMap CEU trios.

Non-fasting serum samples were collected. The samples were immediately put on ice and were processed within 30 minutes, after which they were kept frozen at -20 °C until measurement of CRP in 2003-2004. High sensitivity CRP was measured by use of

Rate Near Infrared Particle Immunoassay (Immagine® Immunochemistry System, Beckman Coulter, USA). This system measures concentrations from 0.2 to 1440 mg/l, with a within-run precision <5.0%, a total precision <7.5% and a reliability coefficient of 0.995. A total of 5,567 subjects with both DNA and CRP measurements available are included in the current study.

The Study of Health in Pomerania (SHIP)

The SHIP is a longitudinal population-based cohort study conducted in West Pomerania, the north-east area of Germany²⁶. For the baseline examinations, a sample of 6,267 eligible subjects aged 20 to 79 years was drawn from population registries. Only individuals with German citizenship and main residency in the study area were included. Selected persons received a maximum of three written invitations. In case of non-response, letters were followed by a phone call or by home visits if contact by phone was not possible. The SHIP population finally comprised 4,310 participants (response 68.8%), and 4,103 with CRP measured are included in our analyses. Baseline examinations were conducted between 1997 and 2001.

The SHIP samples were genotyped using the Affymetrix Genome-Wide Human SNP Array 6.0. Hybridisation of genomic DNA was done in accordance with the manufacturer's standard recommendations. The genetic data analysis workflow was created using the Software InforSense. Genetic data were stored using the database Caché (InterSystems). Genotypes were determined using the Birdseed2 clustering algorithm.

For quality control purposes, several control samples were added. On the chip level, only subjects with a genotyping rate on QC probe sets (QC call rate) of at least

86% were included. Genotyping was successful in 4,103 individuals. The overall genotyping efficiency of the GWA was 98.6%. All 869,224 SNPs located on autosomal chromosomes were used for imputation. Imputation of genotypes in SHIP was performed with the software IMPUTE v0.5.0 based on HapMap II.

Non-fasting blood samples were taken in the supine position. Serum levels of hs-CRP were measured on a BN II analyzer (Dade Behring, Marburg, Germany).

Vis Study

The Vis Study includes 1,031 unselected adult participants, aged 18–93 years, who were recruited in a population-based study during 2003 and 2004 in the villages of Vis and Komiza on the Dalmatian island of Vis. The study received appropriate ethical approval, and all participants gave informed consent. All subjects visited the clinical research center in the region where they were examined in person and where fasting blood was drawn. Biochemical and physiological measurements were performed, detailed genealogies reconstructed, questionnaire of lifestyle and environmental exposures collected, and blood samples stored for further analyses. The settlements on Vis island have complex population histories dating back from the Illyrian period and including periods of isolation and large-scale emigration. A subset of 774 participants were genotyped genome-wide and had CRP levels available.

We genotyped 317,503 SNPs for each individual using the Illumina HumanHap300 v1 BeadChip. Alleles were called in BeadStudio using Illumina cluster files. Subjects were excluded if they fulfilled any of the following criteria: genotypic call rate <97%, mismatch between reported and genotypic sex, unexpectedly low genomic

sharing with first degree relatives, excess autosomal heterozygosity, or outliers identified by IBS clustering analysis. We excluded SNPs on the basis of minor allele frequency (<0.01), HWE ($P < 10^{-5}$), call rate ($<97\%$). MACH v1.0.15 was used to impute over 2 million SNPs from HapMap build 36.

Fasting plasma samples were collected and frozen at -70°C within 1 hr of collection. CRP was measured by ELISA.

Women's Genome Health Study (WGHS)

The WGHS is a prospective cohort of female North American health care professionals representing participants in the Women's Health Study who provided a blood sample at baseline and consent for blood-based analyses²⁷. Participants were 45 or older at enrollment and free of cardiovascular disease, cancer or other major chronic illness. The current data are derived from 22,054 WGHS participants for whom whole genome genotype information was available at the time of analysis and self-reported European ancestry could be confirmed by multidimensional scaling analysis of 1,443 ancestry informative markers in PLINK v. 1.06.14. The study protocol was approved by the institutional review board of the Brigham and Women's Hospital (Boston, MA, USA).

DNA extracted from the baseline blood samples underwent SNP genotyping via the Illumina Infinium II assay for querying of a genome-wide set of SNPs from the Illumina HumanHap300 Duo "+" platform. This panel including the standard content of approximately 318,237 SNPs covering the entire genome from the HumanHap300 panel with an additional focused panel of 45,571 SNPs selected to enhance coverage of cardiovascular candidate genes and SNPs with suspected functional consequences. For

the current analysis, all samples had successful genotype information for >98% of the SNPs, while all SNPs had successful genotype information for >90% of the samples. SNPs with significance $p < 10^{-6}$ for deviations from Hardy-Weinberg equilibrium were excluded from analysis. Self-reported European ancestry was confirmed in 22,054 samples on the basis of a principal component analysis using PLINK² among 1,443 ancestry informative SNPs selected for $F_{st} > 0.4$ in the HapMap²⁸. In total, 339,875 genotyped SNPs passing the criteria for inclusion also had minor allele frequency at least 1 percent. On the basis of linkage disequilibrium relationships in the HapMap (release 21), genotypes for a total of 2,621,896 SNPs were imputed from the 22,054 samples passing the quality criteria using Mach v. 1.0.16. SNP positions were updated in a subsequent step to build 36.1 of the human genome sequence.

All baseline blood samples underwent measurement for hsCRP via a validated immunoturbidimetric method (Denka Seiken, Tokyo, Japan)²⁹. A total of 21,953 participants with genotyped and CRP levels available were included in this study.

2. Method section for the replication panel

The Heredity and Phenotype Intervention (HAPI) Heart Study

The HAPI Heart Study is part of the NHLBI PROGENI Network and was designed to identify genes that interact with environmental exposures to modify risk factors for CVD. From 2003 to 2006, Old Order Amish subjects from Lancaster, PA, aged ≥ 20 years and considered to be relatively healthy were recruited. Details of the study are described elsewhere³⁰.

Genotyping was performed with the Affymetrix GeneChip® Human Mapping 500K Array set, including a total of 500,568 SNPs. The GTYPE-generated chip files were re-analyzed using the BRLMM genotype calling algorithm; the mean genotype call rate in the Stage 1 sample was 98.3%. Uninformative SNPs (n=98,806) with minor allele frequencies (MAF) <2% in the overall sample were removed from further analyses. Finally, 369,241 informative SNPs that passed quality control and Hardy-Weinberg Equilibrium checks (at $p < 0.001$) were retained for analysis. Imputation was performed using MACH. A total of 338,598 autosomal SNPs were used after filtering MAF < 1%, Hardy Weinberg ($p < 1 \times 10^{-6}$) and missingness >5%.

Of the 868 total participants in the HAPI Heart Study, 840 had fasting high sensitivity CRP levels (Cardio CRP; Quest Diagnostics, Horsham, PA; sensitivity 0.2 mg/L) and genome-wide SNP data available for analysis.

The Baltimore longitudinal study on Aging (BLSA) Study

The BLSA study is a population-based study aimed to evaluate contributors of healthy aging in the older population residing predominantly in the Baltimore-Washington DC area³¹. Starting in 1958, participants are examined every one to four years depending on their age. Currently there are approximately 1,100 active participants enrolled in the study. Blood samples were collected for DNA extraction, and genome-wide genotyping was completed for 1,231 subjects using Illumina 550K. The BLSA has continuing approval from the Institutional Review Board of MedStar Research Institute.

The analysis was restricted to subjects of European ancestry and each analysis was further adjusted for the top two principal components derived from an

EIGENSTRAT analysis utilizing ~10,000 randomly selected SNPs from the 550K SNP panel³². Genotyping was completed for 481 participants of European ancestry using a call rate of >98.5% without sex discrepancy based on homozygosity rates and with CRP data. 501,704 autosomal SNPs passed quality control (completeness>99%, MAF >1%, HWE >10⁻⁴) were used to for imputation. HapMap CEU sample (Phase II, release 21a, build 35, January 2007) was used a reference to impute approximately 2.5 million SNPs using MACH. Imputed SNPs with $r^2 < 0.3$ or minor allele frequency of <1% were excluded from the analysis.

Serum CRP was measured using the BNII nephelometer from Dade Behring utilizing a particle enhanced immunonephelometric assay. The assay range is 0.16–1100 ug/mL.

The CARLA Study

The CARLA study is an ongoing cohort study of a representative sample of the inhabitants of the city of Halle, eastern Germany, comprising 1,779 participants aged 45–83 years at baseline (812 women, 967 men)³³. The baseline examination took place between December 2002 and January 2006. A multi-step recruitment strategy aimed to achieve a high response rate. The final response after subtracting exclusions (individuals who were deceased prior to the invitation, had moved away, or were unable to participate due to illness) was 64.1%. The study was approved by the Ethics Committee of Martin-Luther-University Halle-Wittenberg and conforms to the Declaration of Helsinki. A standardized, computer-assisted interview was performed to collect information on socio-demographic and socioeconomic variables, behavioral, biomedical and psychosocial

factors, medical history, and use of medication within the preceding seven days. The medical examination included the recording of sitting blood pressure, waist and hip circumference, weight and height, a venous blood sample, an echocardiogram, and a 20-minute 12-lead resting electrocardiogram.

The determination of CRP was undertaken by the Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics at the Leipzig University Clinics. The laboratory has been accredited according to the accreditation norms ISO 15180 and ISO 17025. Serum high sensitivity CRP levels were measured using a high-sensitivity immunoturbidimetric method (CRP [Latex] HS, Roche, Mannheim, Germany) on a Hitachi autoanalyzer (Roche Diagnostics Mannheim, Germany).

Genotyping of the single nucleotide polymorphisms rs12239046, rs9987289, rs10745954, rs180096, rs340029, rs12037222, rs13233571, rs2836878, rs4903031, rs2847281, and rs1030023 was performed using pre-developed, validated TaqMan®SNP Genotyping Assays (Applied Biosystems, Germany) following the manufacturers instructions. Polymerase chain reaction conditions were as follows: initial denaturation at 95°C for 10 min, followed by 45 cycles of denaturation (92°C for 15 sec) and annealing and extension in 1 step (60°C for 60 s). The post PCR generated fluorescence intensity was quantified using an ABI 7900 Sequence Detector System Software®version 2.3 (Applied Biosystems).

Health, Aging, and Body Composition (Health ABC) Study

The Health ABC study is a prospective cohort study investigating the associations between body composition, weight-related health conditions, and incident functional

limitation in older adults. Health ABC enrolled well-functioning, community-dwelling black (n=1,281) and white (n=1,794) men and women aged 70-79 years between April 1997 and June 1998. Participants were recruited from a random sample of white and all black Medicare eligible residents in the Pittsburgh, PA, and Memphis, TN, metropolitan areas. The current study sample consists of 1,658 white participants with both CRP and genotyping data.

Genotyping was performed by the Center for Inherited Disease Research (CIDR) using the Illumina Human1M-Duo BeadChip system. Genotyping was successful in 1658 Caucasians. Samples were excluded from the dataset for the reasons of sample failure, genotypic sex mismatch, and first-degree relative of an included individual based on genotype data. Analysis was restricted to SNPs with minor allele frequency $\geq 1\%$, call rate $\geq 97\%$ and HWE $p \geq 10^{-6}$. Genotypes were available on 914,263 high quality SNPs for imputation based on the HapMap CEU (release 22, build 36) using the MACH software (version 1.0.16). A total of 2,543,888 imputed SNPs were analyzed for association with CRP levels.

Fasting serum samples were collected at the baseline examination. Serum concentrations of CRP were determined by a commercially available high sensitivity sandwich enzyme immunoassay kit according to the manufacturer's instructions (Biocheck, Burlingame, CA). The intra-assay variability for these assays in serum has been reported as 5.4-7.8% (CV), the inter-assay variability 6.1-9.7 %.

InCHIANTI

The InCHIANTI study is a population-based epidemiological study aimed at evaluating the factors that influence mobility in the older population living in the Chianti region in Tuscany, Italy. The details of the study have been previously reported {Ferrucci, 2000 #80. Briefly, 1,616 residents were selected from the population registry of Greve in Chianti (a rural area: 11,709 residents with 19.3% of the population greater than 65 years of age), and Bagno a Ripoli (Antella village near Florence; 4,704 inhabitants, with 20.3% greater than 65 years of age). The participation rate was 90% (n=1,453), and the subjects ranged between 21-102 years of age. Overnight fasted blood samples were taken for genomic DNA extraction and measurement of CRP. Illumina Infinium HumanHap 550K SNP arrays were used for genotyping. The study protocol was approved by the Italian National Institute of Research and Care of Aging Institutional Review and MedStar Research Institute (Baltimore, MD).

The analysis was conducted on 1,202 subjects that passed quality control with a sample call rate >97%, heterozygosity rates >0.3, correct sex specification and with CRP data. 495,343 autosomal SNPs that passed quality control (MAF>1%, completeness >99%, HWE >10⁻⁴) were used for imputation.

Serum CRP was measured using ELISA and colorimetric competitive immunoassay (Roche Diagnostics, GmbH, Mannheim, Germany). Intra-assay and inter-assay CV was 5%.

The Netherlands Study of Depression and Anxiety (NESDA)

The NESDA Study³⁴ is a multi-centre study designed to examine the long-term course and consequences of depressive and anxiety disorders (<http://www.nesda.nl>). NESDA included both individuals with depressive and/or anxiety disorders and controls without psychiatric conditions. Inclusion criteria were age 18-65 years and self-reported western European ancestry, exclusion criteria were not being fluent in Dutch and having a primary diagnosis of another psychiatric condition (psychotic disorder, obsessive compulsive disorder, bipolar disorder, or severe substance use disorder).

For all participants DNA was isolated from the baseline blood sample. Through funding from the fNIH GAIN program (www.fnih.gov/gain), whole genome scan analysis was conducted for 1,859 NESDA (1,702 depressed cases and 157 controls) participants. Perlegen Sciences (Mountain View, CA, USA) performed all genotyping according to strict standard operating procedures. DNA samples were randomly assigned to plates, shipped to Perlegen and identified only by barcode. High-density oligonucleotide arrays (Perlegen 600K) were used yielding 599,164 SNPs. The genotyping calling algorithms are described in prior reports^{35,36}. Eight SNPs with duplicate numbers were deleted and 73 mitochondrial SNPs were removed for later analysis. From the remaining 599,083 SNPs on the Perlegen chip 435,291 passed quality control. A hundred subjects were excluded because of various quality control issues³⁷. Additional exclusions were made based on phenotype leaving 1,706 subjects in the final analysis dataset.

Imputation was then carried out for genotypes for autosomal SNPs that were present in HapMap Phase II but were not present in the genome-wide chip or did not pass direct genotyping QC. Genotypes were imputed using the genotype data from the GWA

chips and data from HapMap (release 22, build 36) genotype data from the 60 CEU HapMap founders as the reference database. The imputation was performed by IMPUTE version 0.3.2 using the default settings and the recommended number 11418 for the effective population size of Caucasians⁷. In this way we extended the genome-wide autosomal SNP dataset from 435,291 to 2,531,819 SNPs.

Blood was drawn after an overnight fast, and was immediately transferred to a local laboratory to start processing within one hour and storage at -85°C for later assaying. CRP was assayed at the Clinical Chemistry department of the VU University Medical Center. High-sensitivity plasma levels of CRP were measured in duplicate by an in-house ELISA based on purified protein and polyclonal anti CRP antibodies (Dako). The CRP assay was standardized against the CRM 470 reference agent. Our analyses included a total of 1,706 participants with CRP assayed and genotypes available. The lower detection limit of CRP is 0.1 mg/l and the sensitivity is 0.05 mg/l. Intra- and inter-assay coefficients of variation were 5% and 10%, respectively.

The Netherlands Twin Register (NTR)

The NTR^{38,39} studies the development of health, lifestyle and personality in children and adult twins and their family members. Between 2004 and 2008, all adult participants in longitudinal studies on health, lifestyle and personality were asked to take part in blood sampling. During a home visit, fasting blood samples were obtained between 7:00 a.m. and 10:00 a.m. for more than 9500 individuals.

As part of the NIH-GAIN Study on Major Depression Disorder 39 a subset of individuals was genotyped by Perlegen, USA using the Affymetrix 600K SNP chip.

SNPs were excluded in case of gross mapping problems, 2 or more genotype disagreements in 40 duplicated samples, 2 or more Mendelian inheritance errors in 38 complete trio samples, minor allele frequency < 0.01 or > 0.05 missing genotypes. More details are published elsewhere³⁶. The final dataset consisted of 435 291 SNPs in 1,777 unrelated individuals. Imputation was conducted using IMPUTE and resulted in a total of 2,562,567 SNPs.

CRP was obtained from heparin plasma and determined using the Immulite CRP assay (Diagnostic Product Corporation, USA). This system has a sensitivity of 0.01 mg/dL, an intra-assay coefficient of variation less than 7.5%, and inter assay coefficient of variation less than 10%. CRP was available in 1596 genotyped individuals.

The SardiNIA study

The SardiNIA study is a longitudinal population-based study to investigate the genetic and epidemiology of aging associated conditions. We recruited and phenotyped 6,148 individuals, male and female, aged 14 and older, from a cluster of four towns in the Lanusei Valley (Sardinia, Italy). During physical examination, a blood sample was collected from each individual and divided into two aliquots. One aliquot was used for DNA extraction and the other to characterize several blood phenotypes, including evaluation of serum levels of high sensitivity (hs)CRP.

During the study, we genotyped 4,305 individuals selected from the whole sample to represent the largest available families with the 500K and the 10K Affymetrix Mapping Array set, with 436 individuals genotyped with both arrays. This genotyping strategy allowed us to examine the majority of our cohort in a cost-effective manner since

genotypes for the SNPs that passed quality control checks could be propagated through the pedigree using imputation. Measurements for hsCRP were available for 4,295 individuals, among the 4,305 genotyped. A total of 362,129 SNPs passed initial quality control checks and we used all the autosomal markers (356,359) to estimate genotypes for all polymorphic SNPs in the CEU HapMap population in all individuals genotyped with the 500K Array. The inference was carried out using the MACH software. We focused on the SNPs for which the imputation procedure predicted $r^2 > 0.30$ between true and imputed genotypes (the average predicted r^2 was 0.86). In total, 2,252,228 markers were successfully imputed or genotyped, with an overall error rate of 2.17% per allele. Taking advantage of the relatedness among individuals in the SardiNIA sample, we carried out a second round of computational analysis to impute genotypes at all SNPs in the individuals who were genotyped only with the Affymetrix Mapping 10K Array, being mostly offspring and siblings of the individuals genotyped at high density. At this second round of imputation, we used a modified version of the Lander-Green algorithm to estimate IBD sharing at the location of the SNPs being tested and identify stretches of haplotype shared with close relatives who were genotyped at higher density and probabilistically infer missing genotypes. Briefly, we first calculated the likelihood of the observed genotype data; then we assigned each missing genotype to a specific value and updated the likelihood pedigree. The ratio of the two likelihoods gave a posterior probability for the proposed genotype conditional on all available data. Furthermore, instead of assigning the most likely genotype, we estimated an expected genotype score, representing the expected number of copies of a reference allele (a fractional number between 0 and 2), which allowed us to partially account for uncertainty in genotype

assignment. The genotype scores were then tested for association with the trait in a simple regression model and a variance component approach was used to account for correlation between different observed phenotypes within each family. With this definition of genotype score, the association test evaluates the additive effect of the marker. The within-family imputation procedure and the association test are implemented in Merlin software^{40,41}. Due to computational constraints, we divided large pedigrees into sub-units with “bit-complexity” of 19 or less (typically, 20-25 individuals) before analysis. In the association model, age and sex were included as covariates in all analyses, and a natural logarithm transformation was applied to the trait values. The analyses included 4195 participants with CRP assayed and genome-wide data.

Serum levels of hsCRP were measured by the high sensitivity Vermont assay (University of Vermont, Burlington), an enzyme-linked immunosorbent assay calibrated with WHO Reference Material¹⁰. The lower detection limit of this assay is 0.007 mg/l, with an inter-assay coefficient of variation of 5.14%.

The TwinsUK cohort

The Twins UK Registry comprises unselected, mostly female volunteers ascertained from the general population through national media campaigns in the UK {Spector, 2006 #96}. Means and ranges of quantitative phenotypes in Twins UK were similar to an age-matched singleton sample from the general population⁴². Zygosity was determined by standardized questionnaire and confirmed by DNA fingerprinting. Written informed consent was obtained from all participants before they entered the studies, which were approved by the local research ethics committee.

Samples from the TwinsUK cohort were genotyped with the Infinium assay (Illumina, San Diego, USA) using four different SNP arrays, the Hap300 Duo, Hap300, Hap550 and Hap610⁴³. SNP calling was performed using the Illuminus software⁴⁴. SNPs were excluded if they violated Hardy–Weinberg equilibrium (HWE) ($p < 1.0 \times 10^{-4}$); had genotype call rates $< 95\%$; or had a minor allele frequency (MAF) of less than 0.01. Individuals were excluded if the sample call rate was less than 95%, autosomal heterozygosity was outside the expected range, genotype concordance was over 97% with another sample and the sample was of lesser call rate, non-Caucasian ancestry either self-identified or identified by cluster analysis in STRUCTURE⁴⁵ or multidimensional scaling by comparison to the three HapMap phase 2 reference populations (CEU, YRI, CHB+JPT; www.hapmap.org), or unexplained relatedness (estimated proportion of allele shared identical by descent > 0.05) to > 120 other samples². This resulted in GWAS data being available for 5,295 twins from the TwinsUK cohort.

CRP concentrations from serum were measured with the Human Cardiovascular Disease (CVD) Panel 2 (acute-phase proteins) LINCOpex Kit (HCVD2-67BK) from Linco (Millipore) and with the Extracellular Protein Buffer Reagent Kit (LHB0001) from Invitrogen. CRP concentrations were expected to be very high and a dilution step was required prior to analysis. The optimal dilution which was not specified in the assay procedure was set at 1:2000. Sample analyses were performed according to the manufacturers' protocol (Sensitivity: CRP 6 pg/mL; Intra-assay: 3.7-13.4%; Inter-assay: 16.9-21%; Accuracy: CRP 121.0%) and assayed in duplicate. Data was collected using the Luminex-100 system (Qiagen LiquiChip). CRP measurements were available for 1,093 of these individuals, of which 552 were DZ twins (i.e. 226 pairs) and 541

singletons. These singletons included 407 MZ twins of which the mean log(CRP) of both twins was used to optimize information.

Non-genotyped autosomal SNPs were imputed using Phase II CEU HapMap data (release 22, build 36) as the reference database using IMPUTE version 0.3.2⁷. Only SNPs with $MAF > 1\%$, $P > 10^{-4}$ for the HWE test calculated using the genotypes inferred after imputation by maximum likelihood expectation and an imputation quality score reflecting the observed by expected variance ratio > 0.5 for TwinsUK (IMPUTE proper_info) were included in the analysis. Using regression analysis, we adjusted log(CRP) for age and batch. No adjustment for sex was made because all individuals were female. Association between the residuals and autosomal SNPs was tested with an F-test in SNPTEST version 1.1.4 using an additive model and the proper option to account for the uncertainty of the genotypes that were imputed. As the TwinsUK cohort data consisted partly of dizygotic twins, the variances of the regression coefficients were corrected for the sibship relations using the Huber-White method for robust variance estimation in R^{46,47}.

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Web Resources

MACH, <http://www.sph.umich.edu/csg/abecasis/MACH/index.html>

SHARE, http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000007.v3.p2

SNPTEST, <http://www.stats.ox.ac.uk/~marchini/software/gwas/snpctest.html>

R-project, <http://www.r-project.org>

MetABEL, <http://mga.bionet.nsc.ru/~yurii/ABEL>

Eigenstrat software, <http://genepath.med.harvard.edu/~reich/EIGENSTRAT.htm>

IMPUTE, <http://www.stats.ox.ac.uk/~marchini/software/gwas/impute.html>

BIMBAM, <http://stephenslab.uchicago.edu/software.html>

EUROSPAN, <http://homepages.ed.ac.uk/s0565445/index.html>

METAL, <http://www.sph.umich.edu/csg/abecasis/Metal/index.html>

Supplementary figures

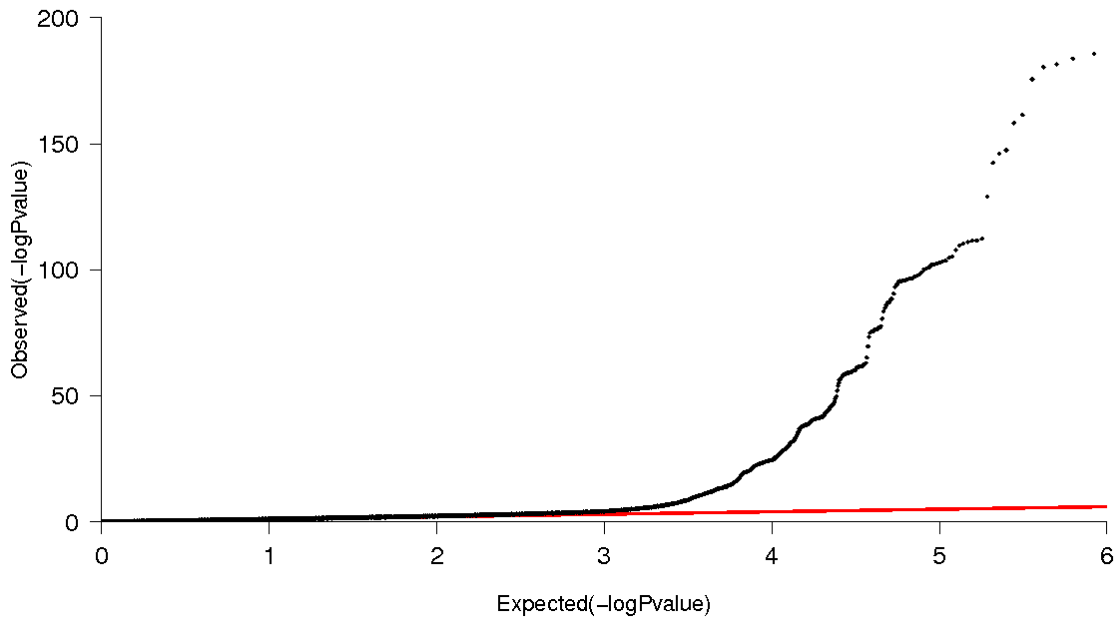
Figure legends

Supplementary Figure I. QQ-plot for the discovery panel

Supplementary Figure II. -Log plot for the discovery panel. Figure **(a)** illustrates the -log p-values for all tests performed for the association of the SNPs with serum CRP levels in the discovery panel. The grey dashed horizontal lines correspond to the p-value threshold of 5×10^{-8} . Figure **(b)** focuses on SNPs with a p-value larger than 1.0×10^{-10}

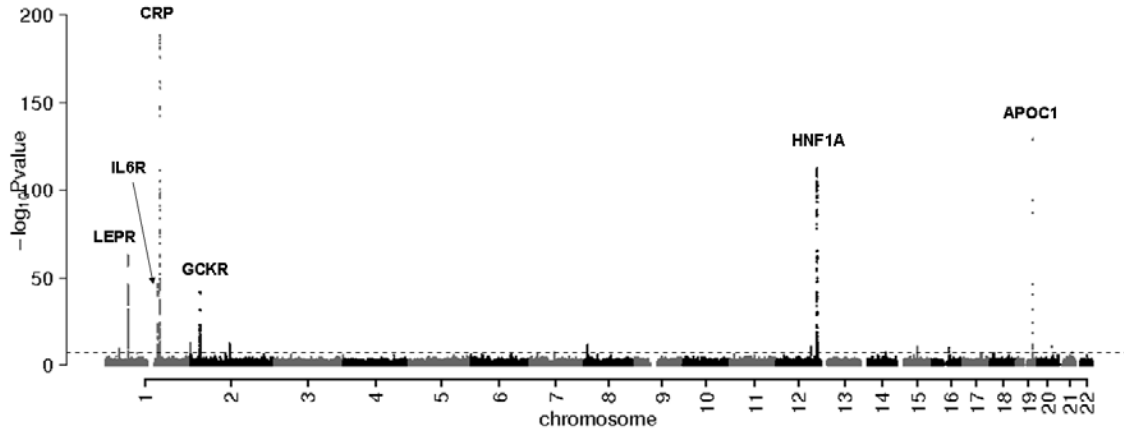
Supplementary Figure III. Regional plots of loci associated with CRP levels. The association p-values ($-\log_{10}$ transformed, indicated by the left y-axis) for SNPs in a 400kb region are plotted against their chromosome positions (NCBI build 36) on the x-axis. Each diamond represents a SNP with the color indicating the linkage disequilibrium (estimated using HapMap CEU sample) between the SNP and the lead SNP. The lead SNP is plotted twice, in dark blue, representing the p-value in the discovery panel and in gray, representing the combined p-value of discovery and replication panels. Shown in light blue are the estimated recombination rates with values indicated by the right y-axis. The bottom panel displays the genes in the region based on the UCSC Genome Browser March 2006 assembly, with the arrow to right (left) indicate indicating +(-) strand.

Supplementary Figure I.

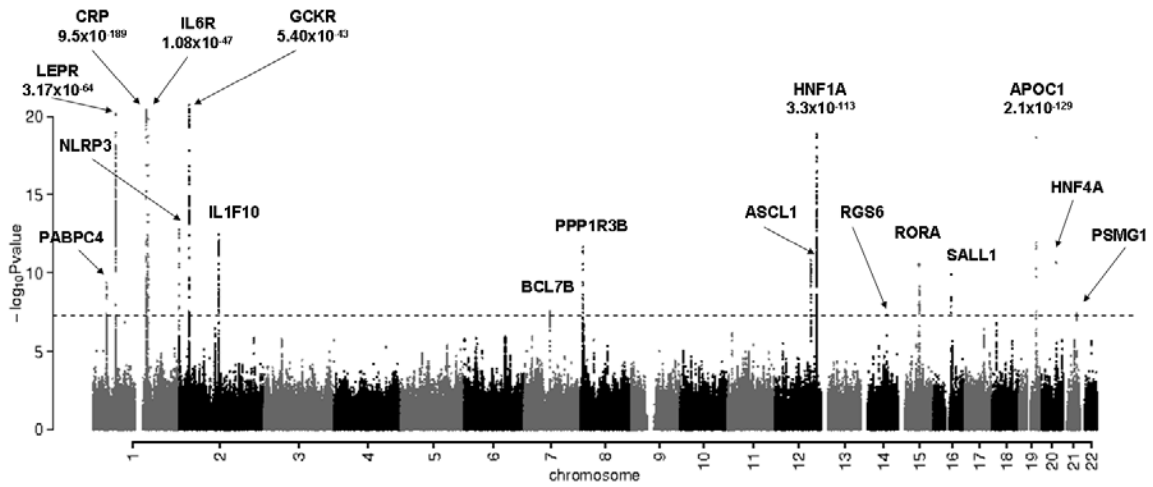


Supplementary Figure II.

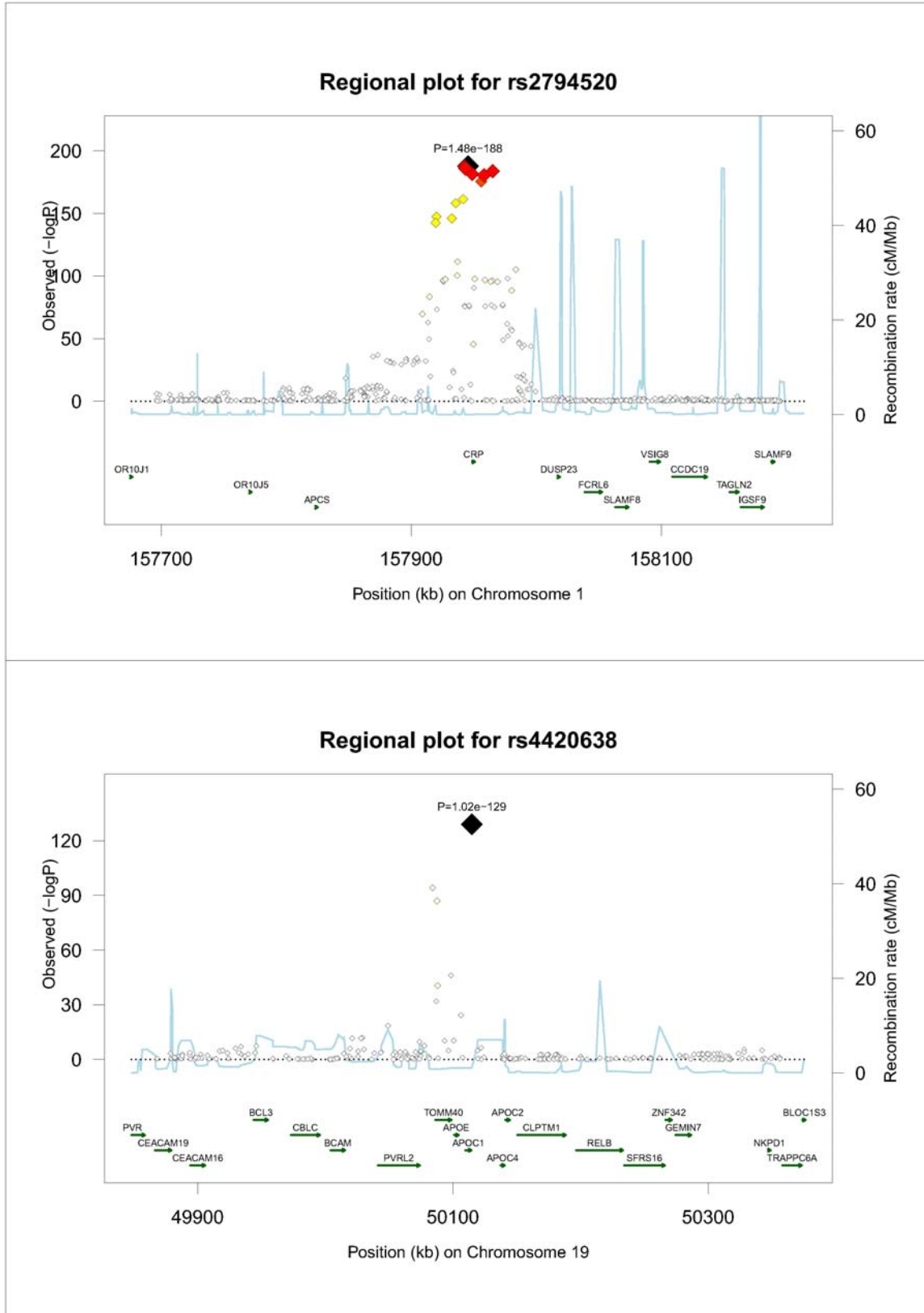
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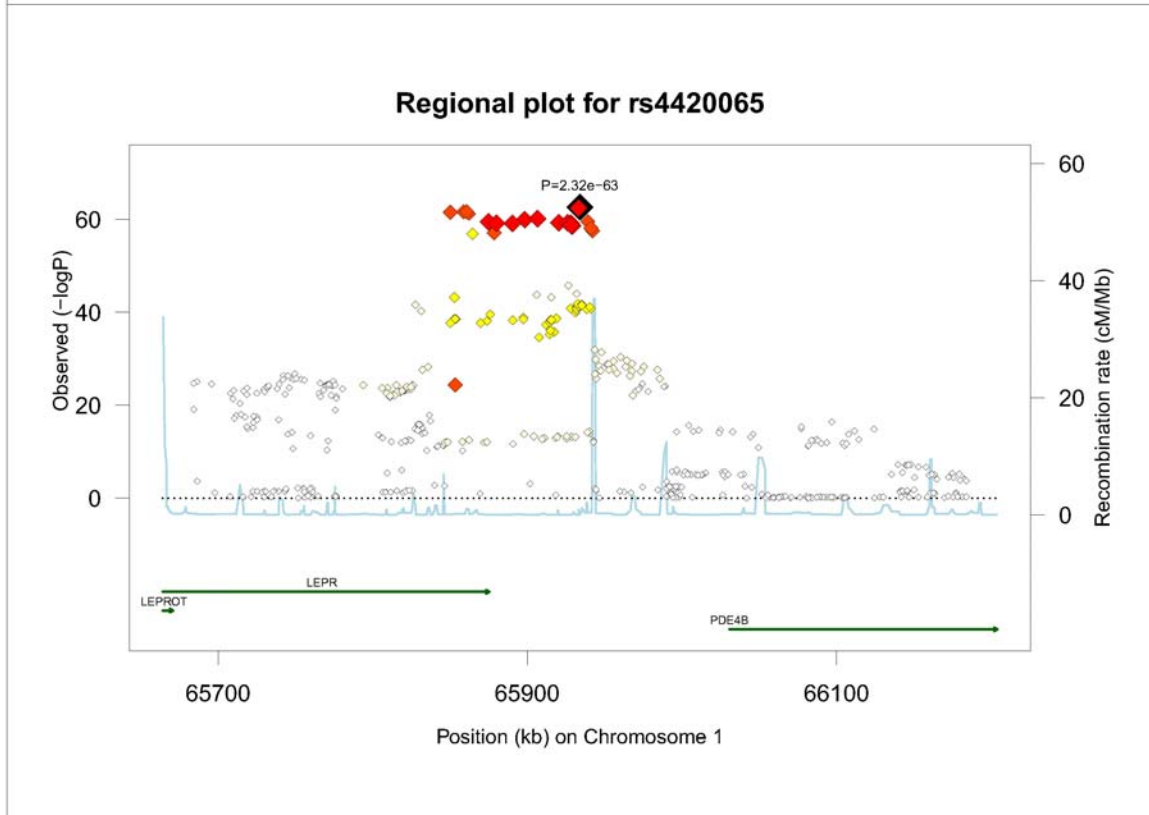
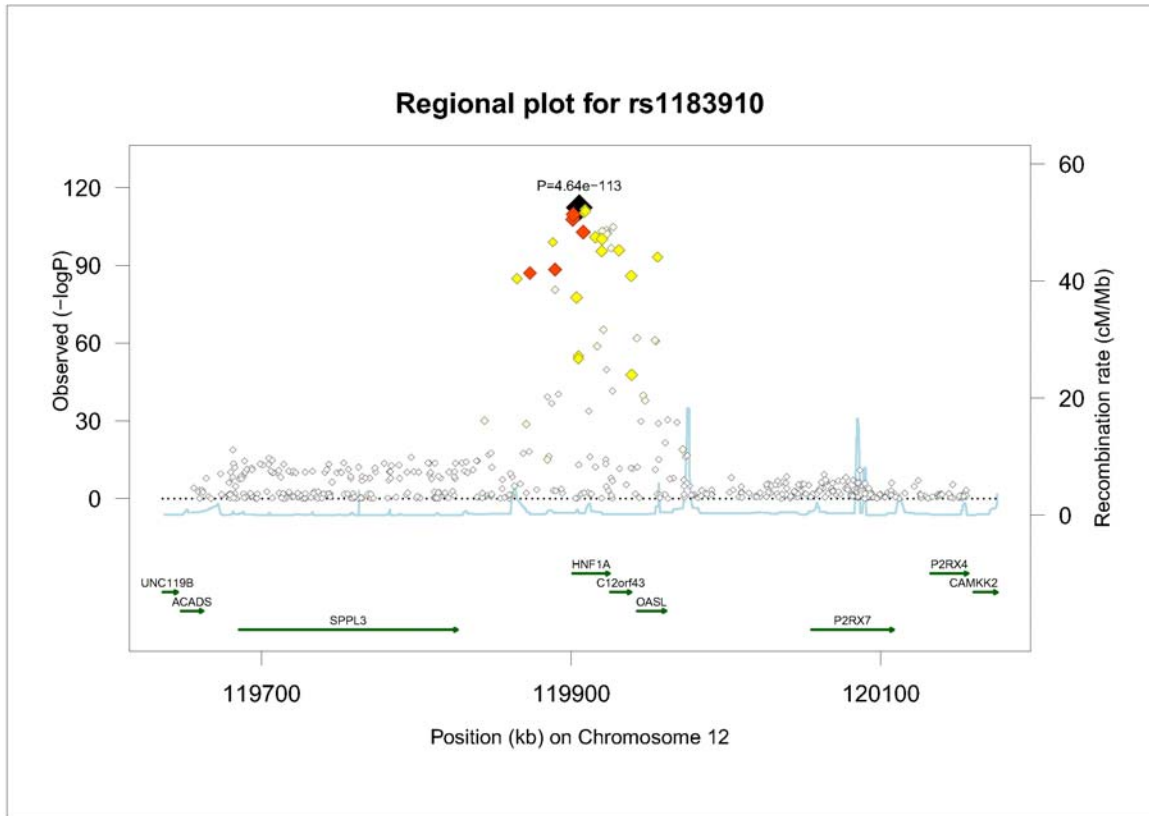


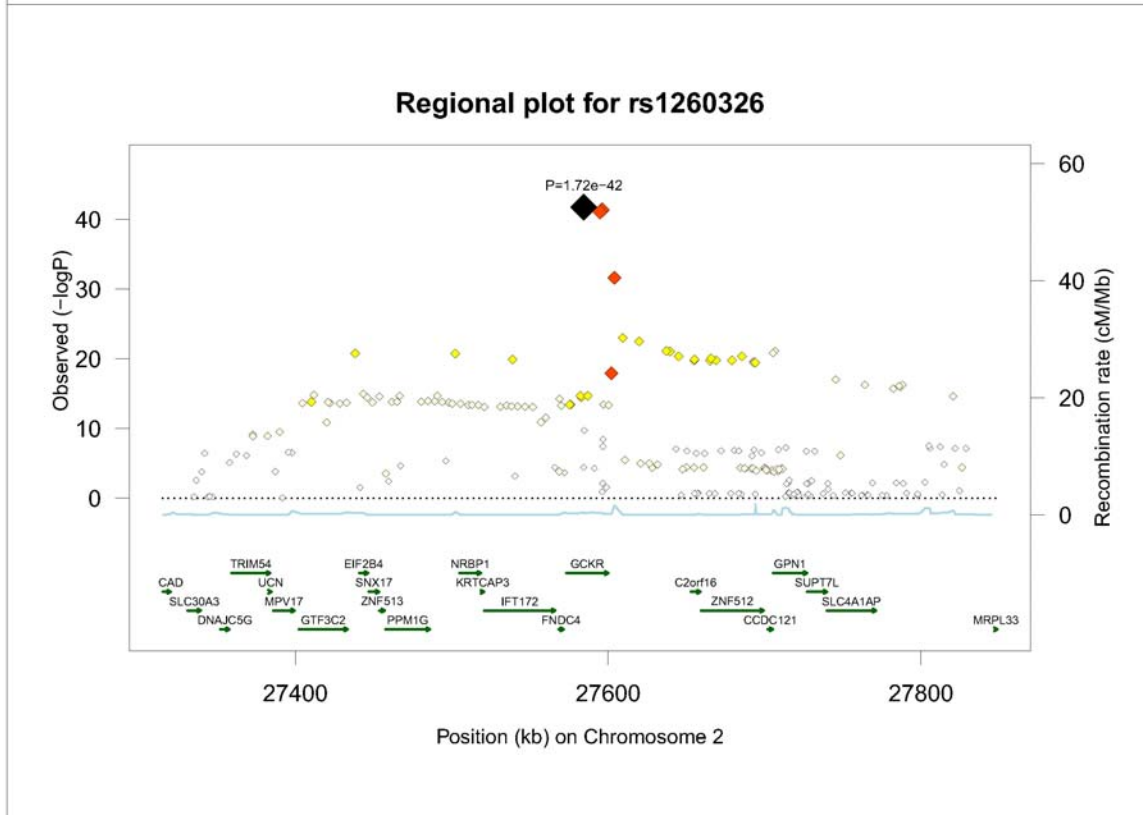
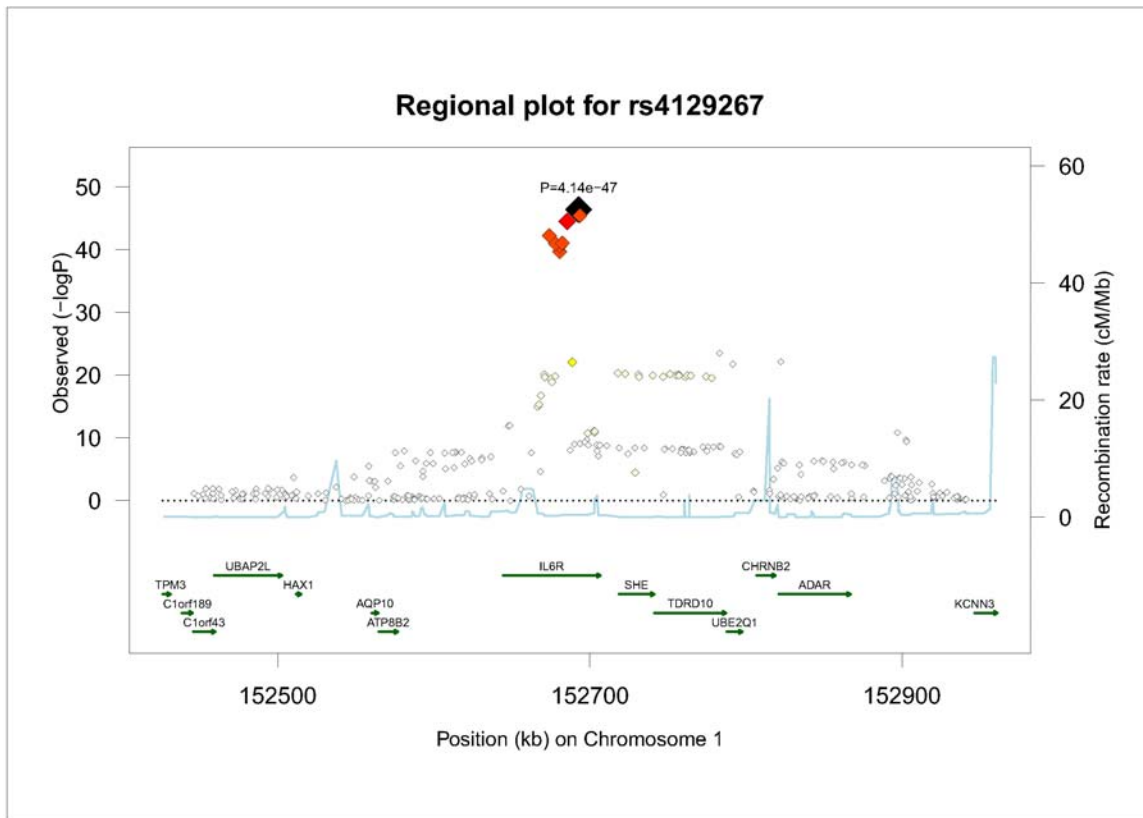
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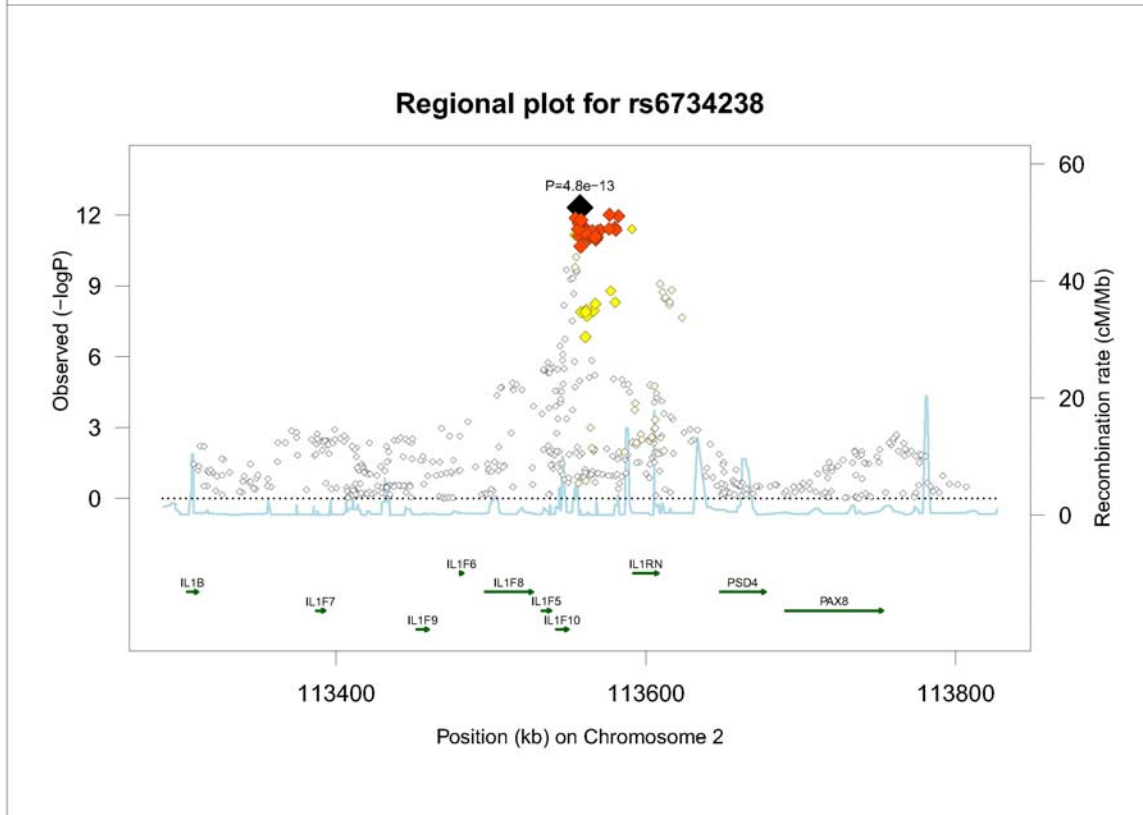
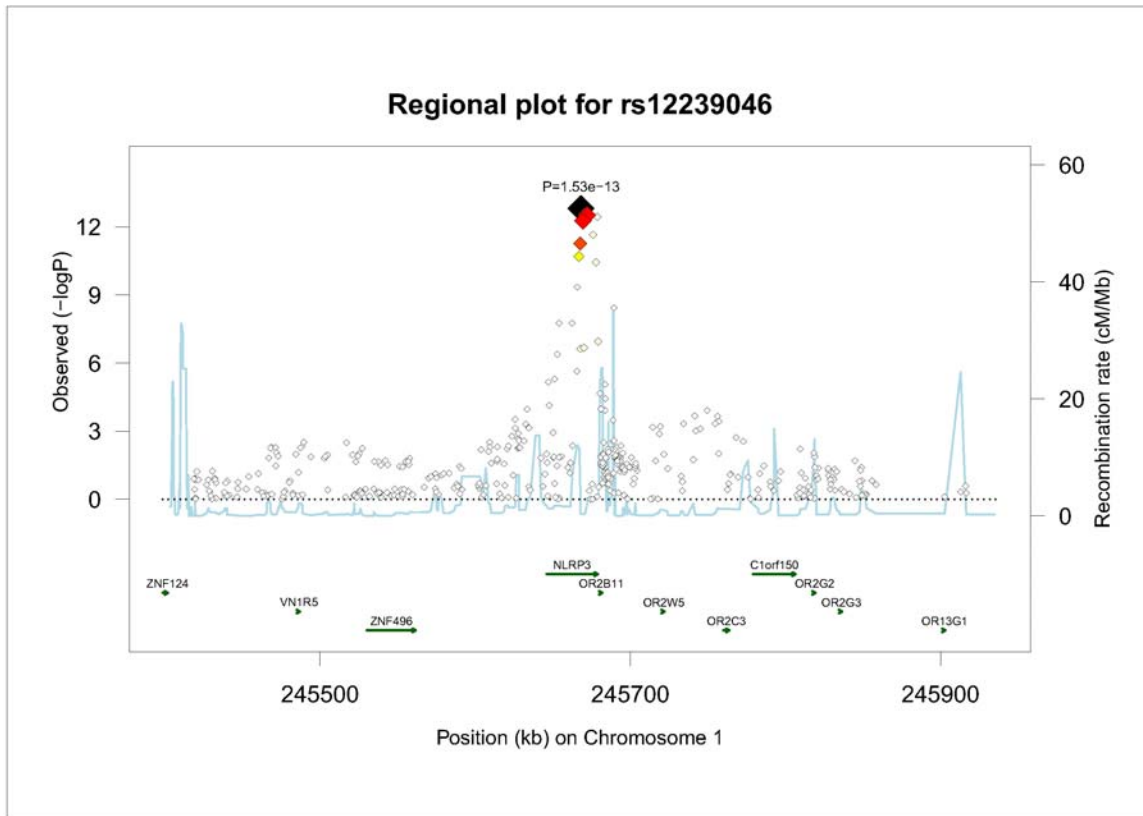


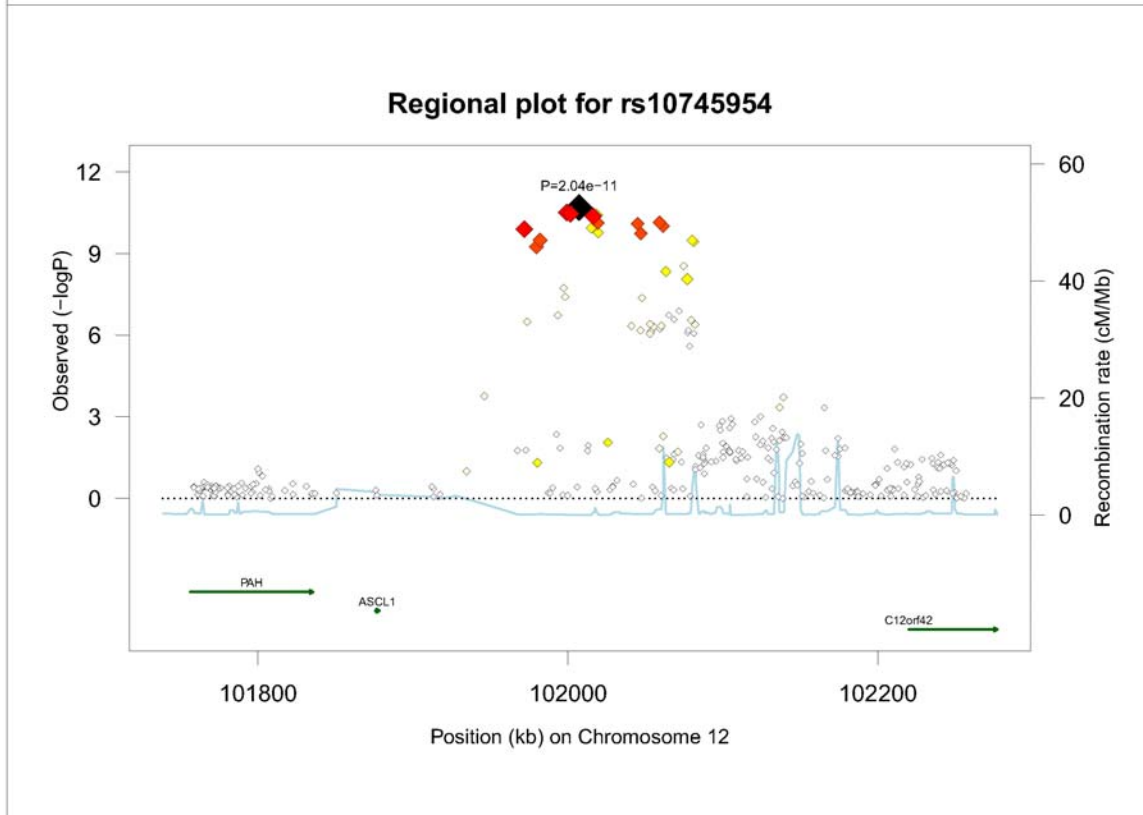
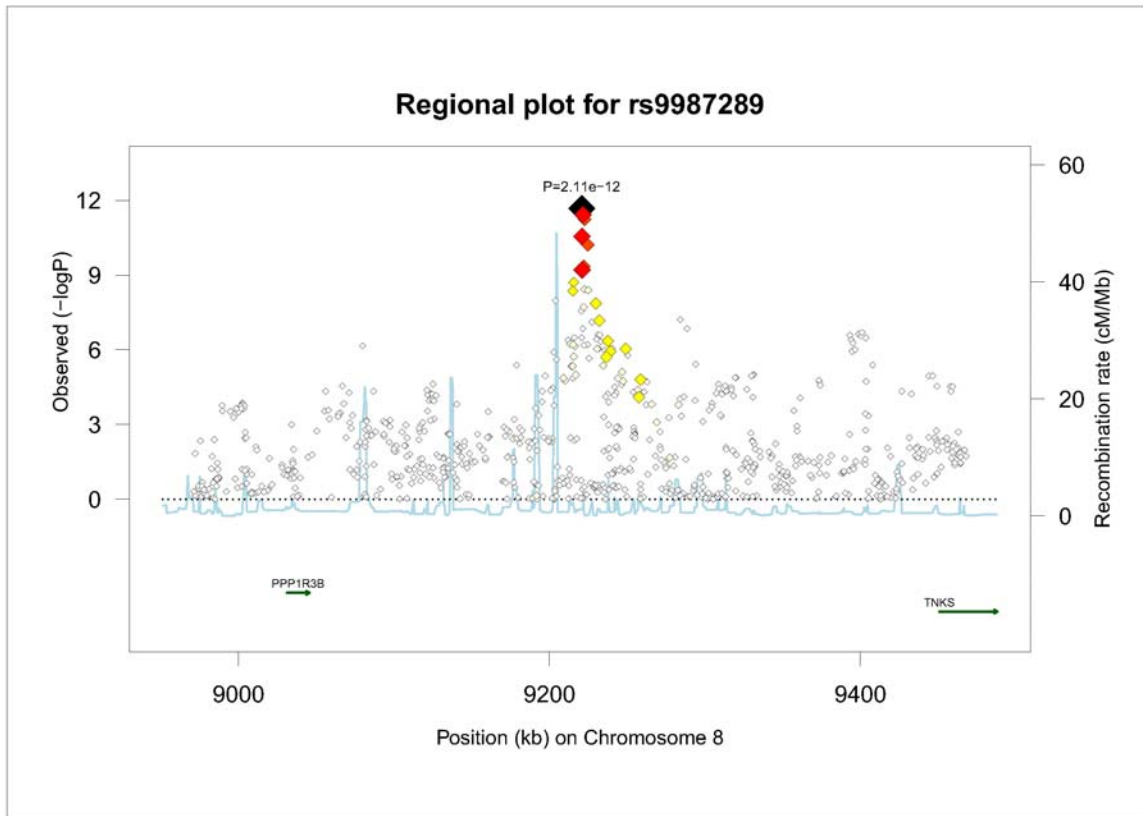
Supplementary Figure III.

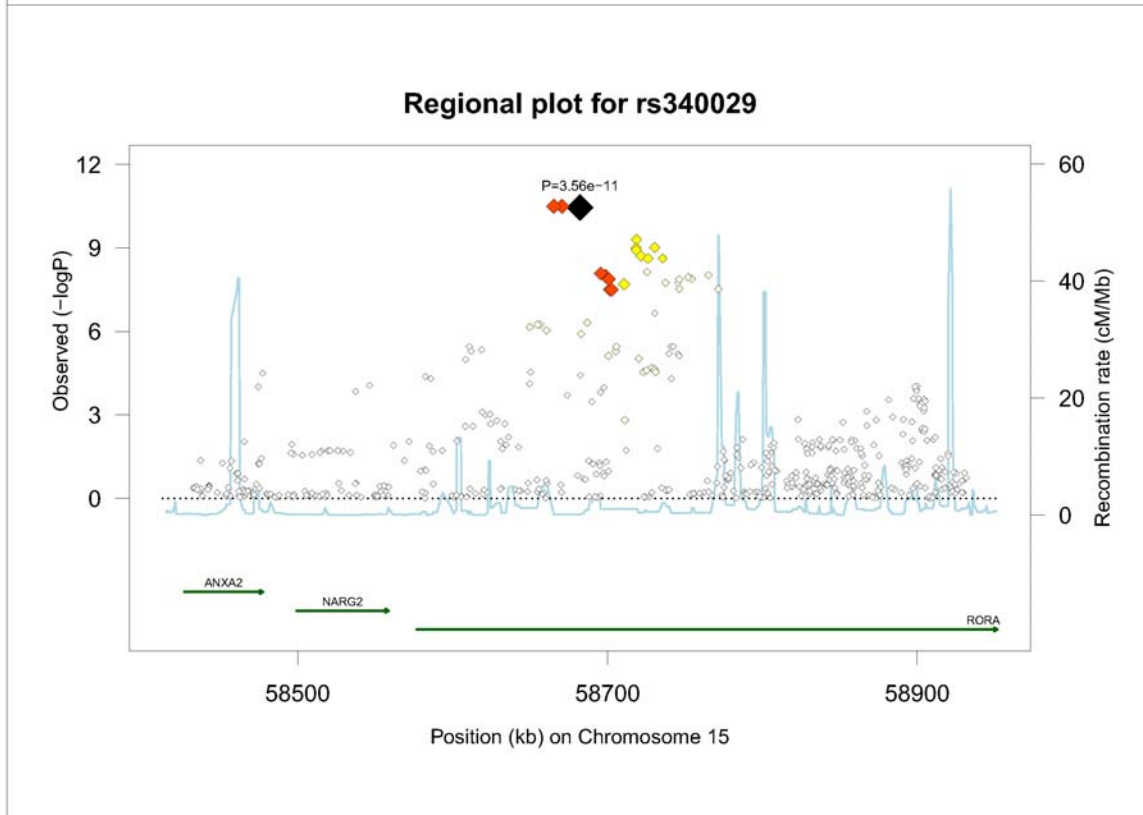
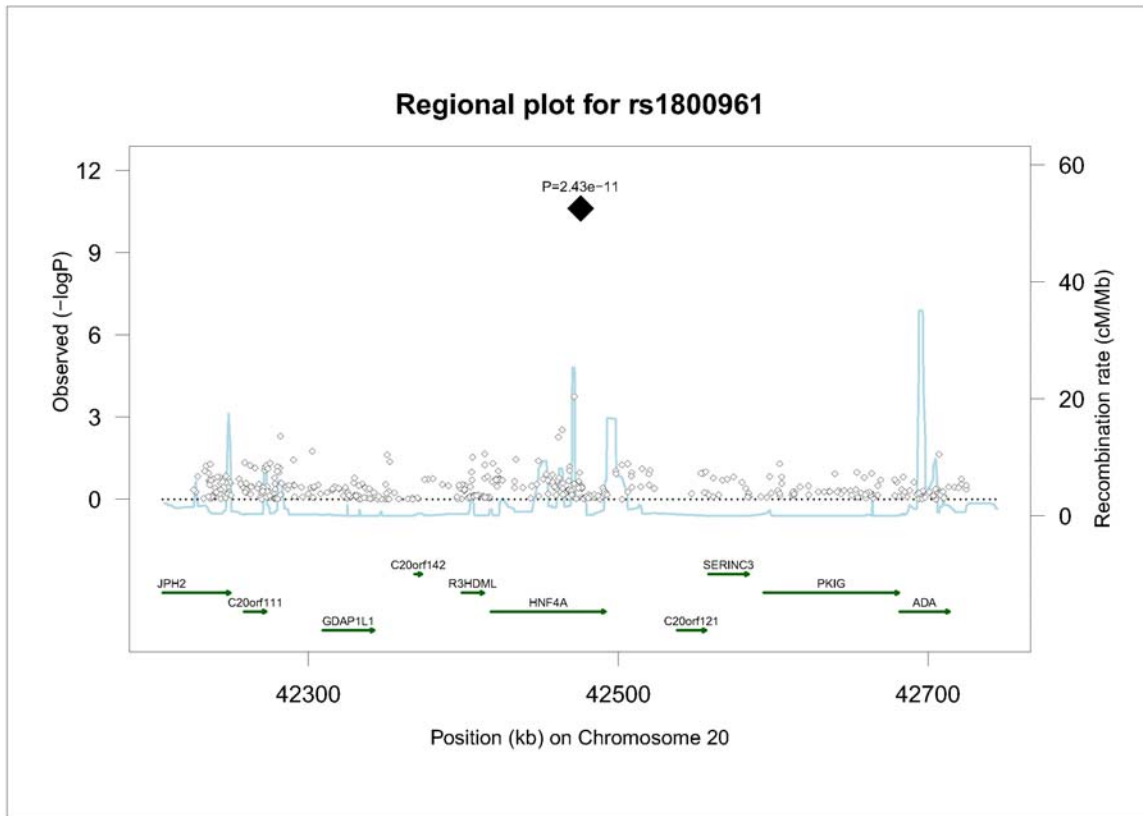


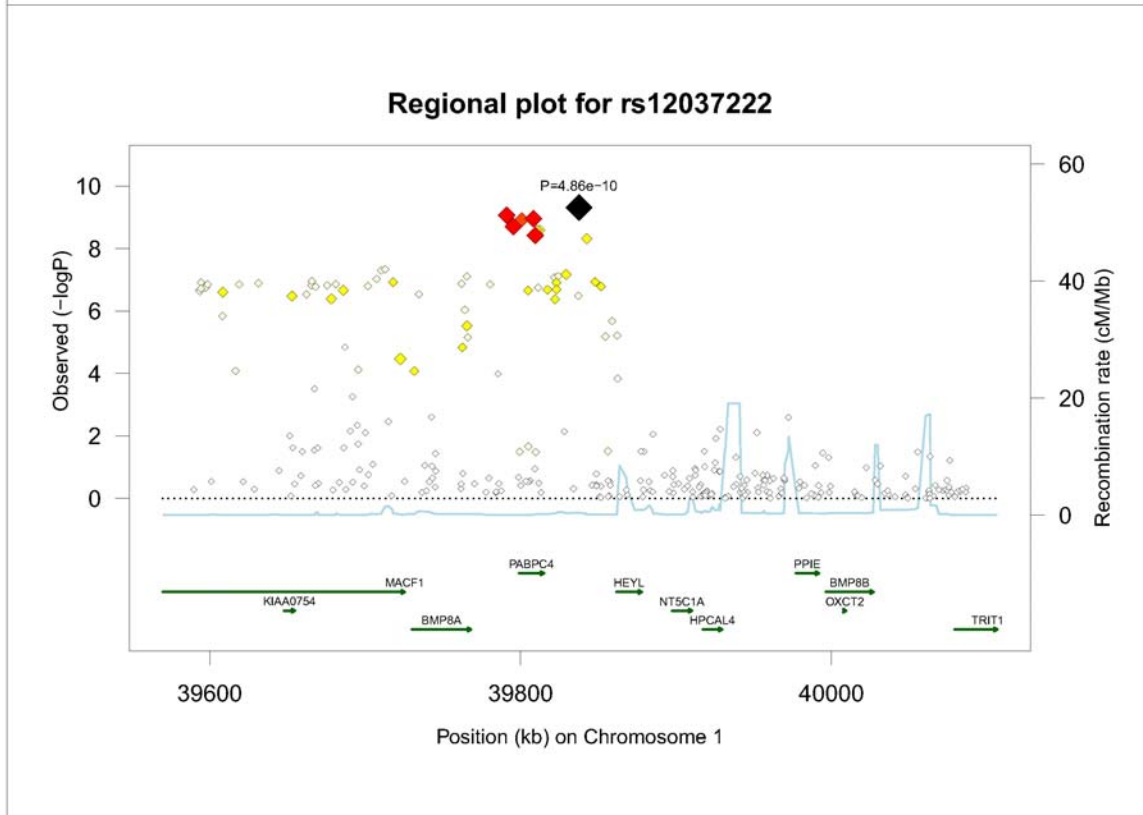
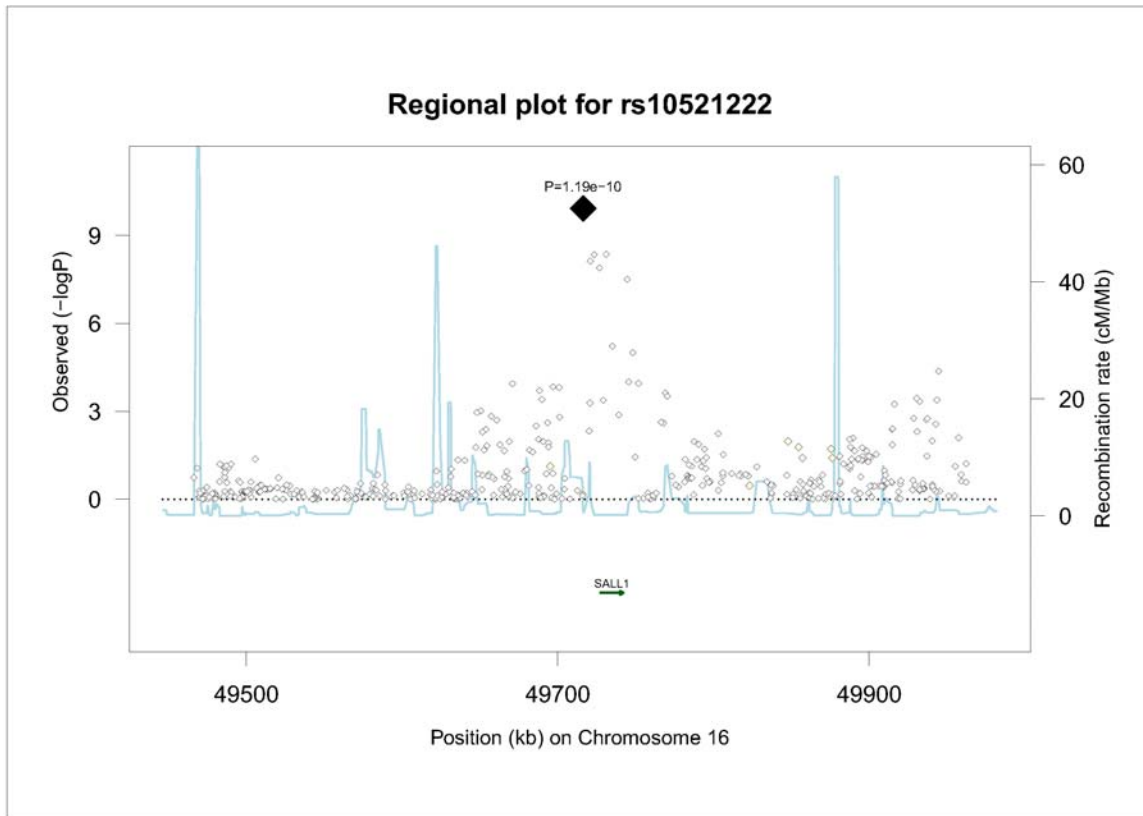


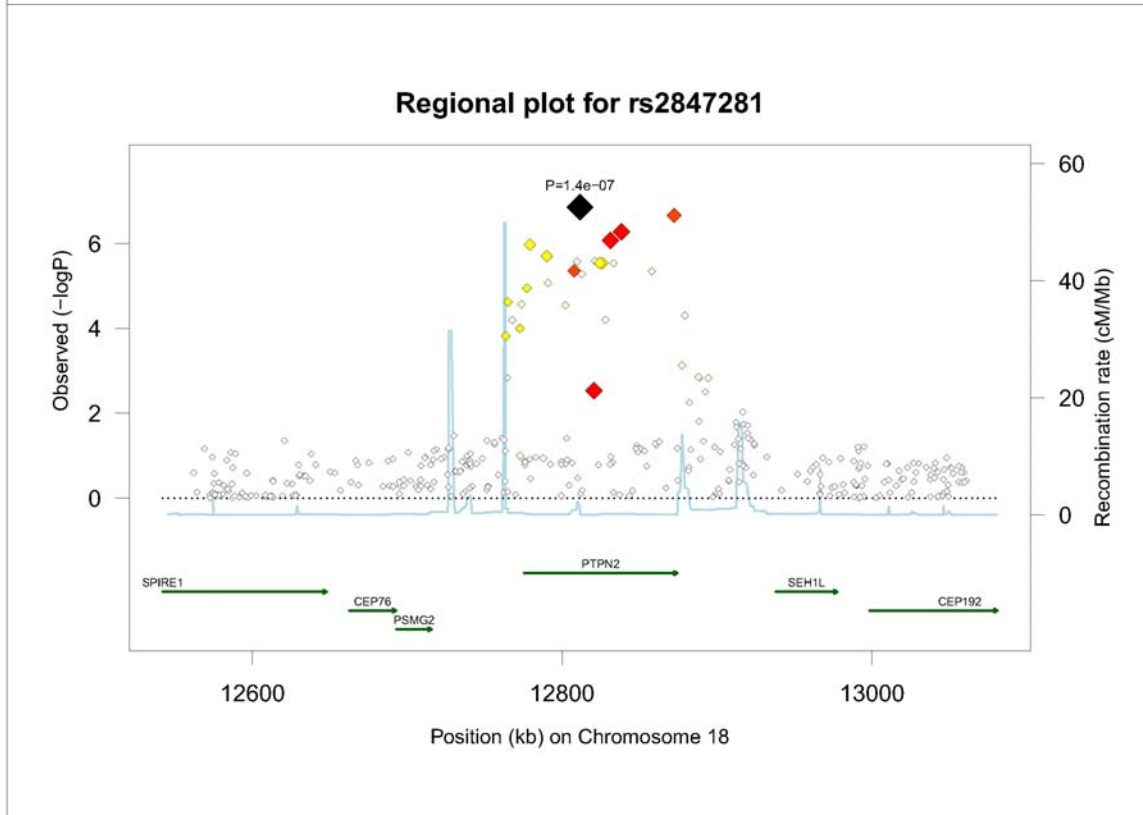
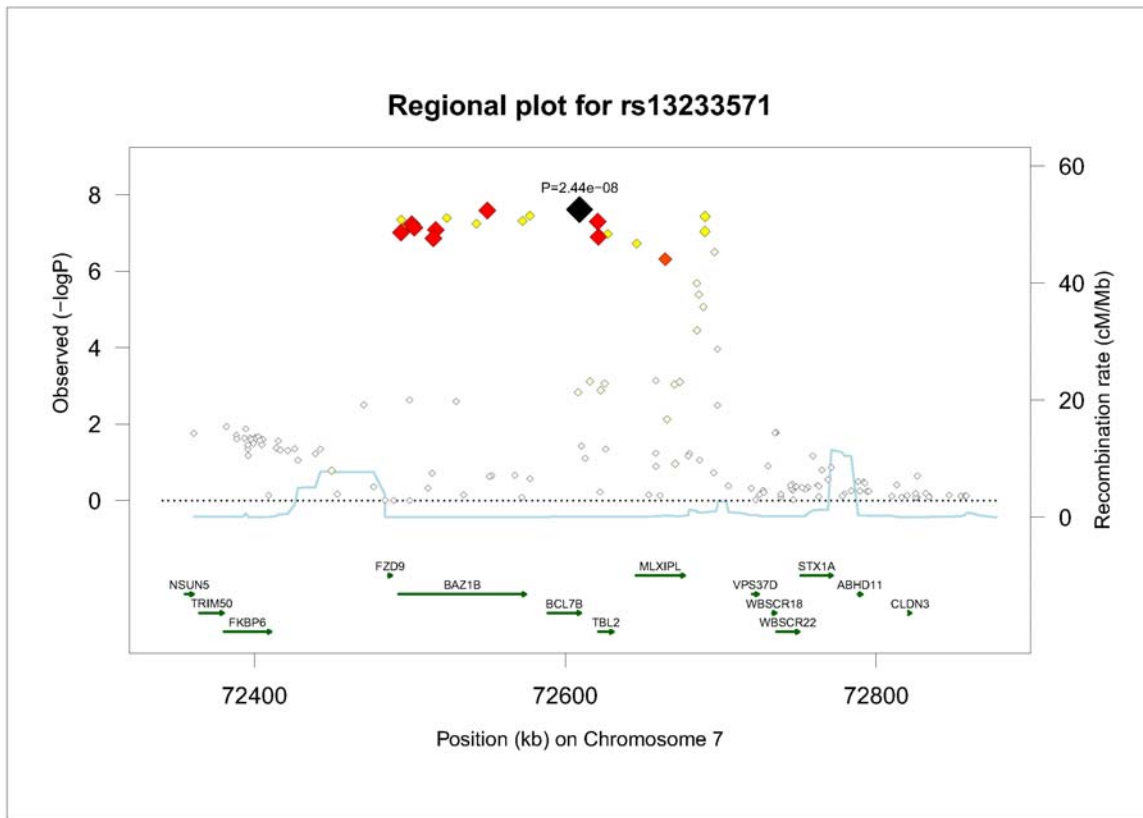


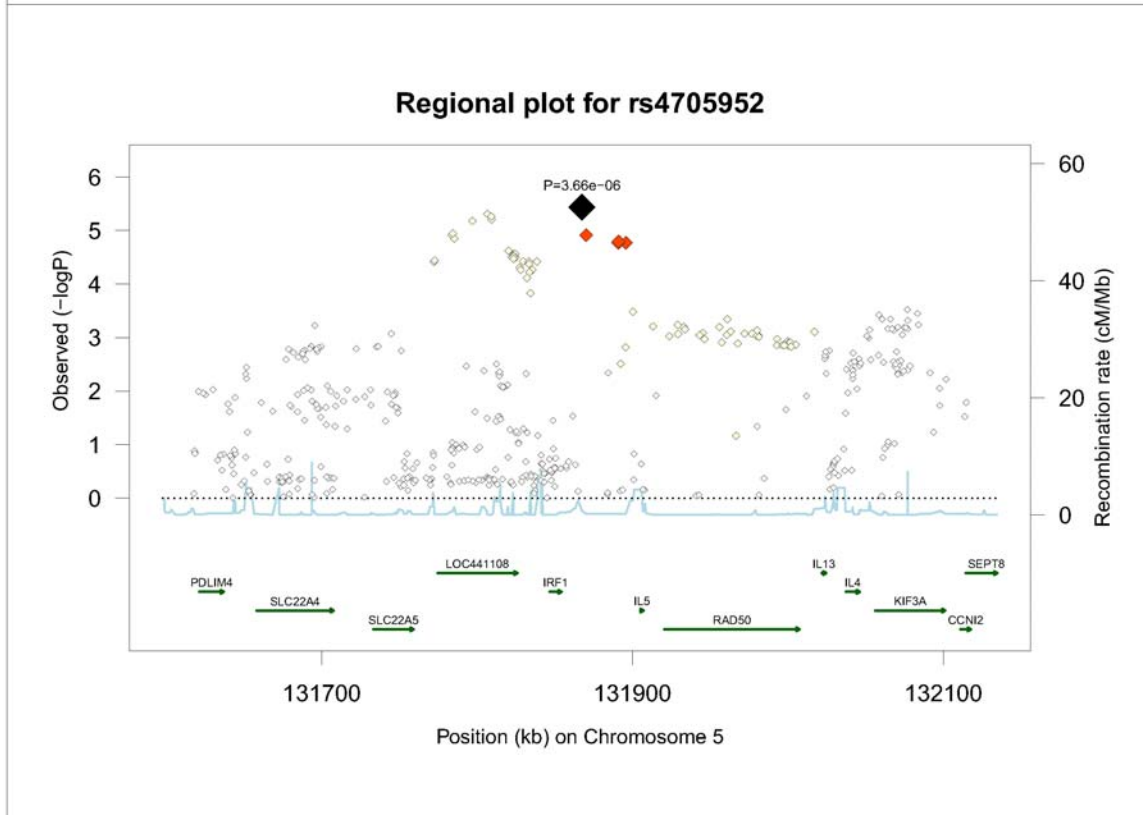
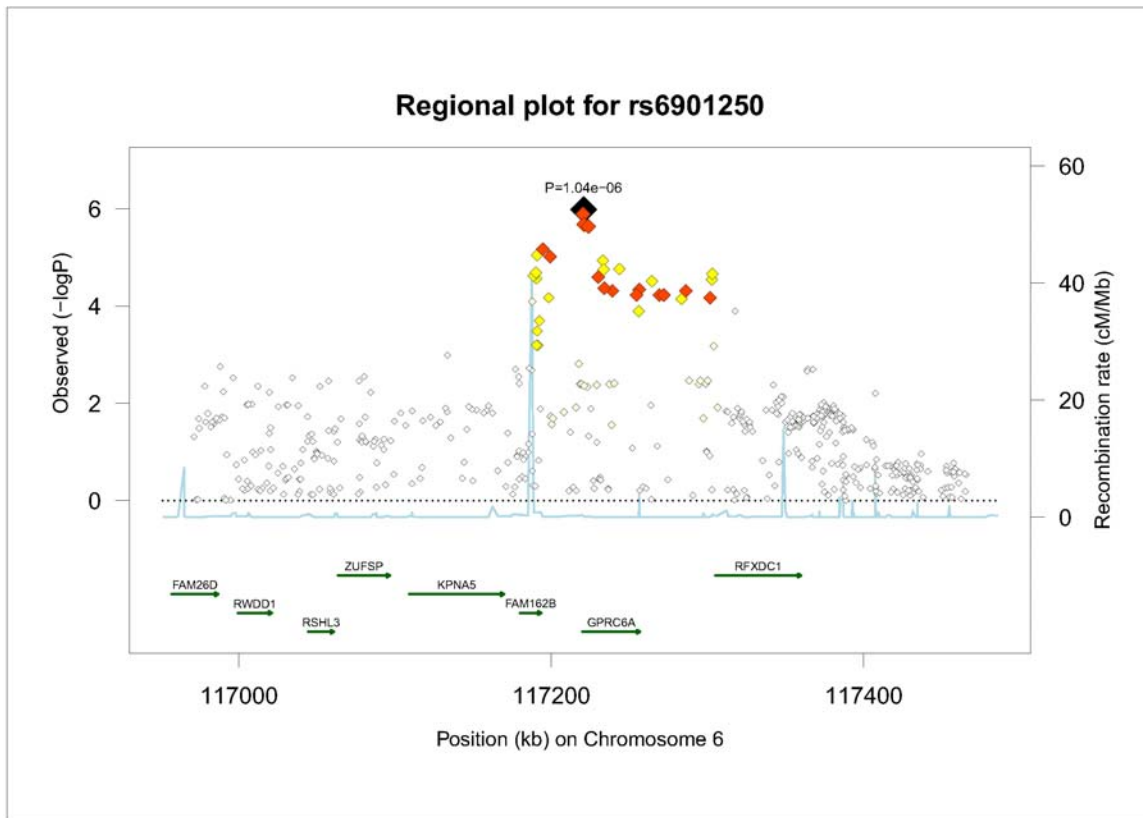












1 **Supplementary tables**

2 Supplementary table I: Characteristics of the Study Participants

Panel	Study	Country	Sample size	λ	CRP, median(25th, 75th), mg/L	Age, mean (SD), y	Men, (%)
Discovery	AGES	Iceland	3218	1.038	1.90 (1.00,3.90)	76.4 (5.5)	42.0
	ARIC	USA	6431	1.014	2.29 (1.04, 5.04)	63.1 (5.6)	46.8
	B58C	UK	1461	0.995	1.00 (0.51, 2.33)	44.9 (0.4)	50.0
	CHS	USA	3265	1.013	2.26 (1.15, 3.99)	72.3 (5.4)	39.0
	ERF	Netherlands	1792	1.030	1.92 (0.68, 2.75)	48.6 (14.3)	42.9
	FHS	USA	6899	0.999	0.62 (1.48, 3.71)	49.4 (13.8)	46.7
	Health2000	Finland	2080	1.000	0.73 (0.23, 2.01)	50.5 (11.1)	48.6
	KORA	Germany	1587	1.014	1.34 (0.65, 2.73)	52.4 (10.1)	50.1
	MICROS	Italy	1215	1.328	1.60 (0.73 3.54)	45.0 (16.8)	43.2
	NFBC66	Finland	5125	1.009	0.81 (0.38, 1.96)	31 (0)	48.0
	ORCADES	UK	715	1.129	1.24 (0.56,2.68)	53.6 (15.7)	46.5
	RS	Netherlands	5567	1.019	0.91 (1.88, 3.67)	69.4 (9.1)	58.9
	SHIP	Germany	4103	1.034	1.25 (0.68, 3.01)	50.3 (16.4)	49.1
	VIS	Croatia	774	1.120	1.26 (0.64,2.72)	56.6(15.6)	41.6
	WGHS	USA	21953	1.068	0.20 (0.08, 0.44)	54.7 (7.1)	0
Replication	BLSA	USA	481		1.35 (0.67,2.92)	69 (13.6)	54.1
	CARLA	Germany	1695		1.8 (0.9, 3.5)	64.4 (10.1)	54
	Finrisk	Finland	1893		1.15 (0.58, 2.6)	55.6 (12.1)	53.6
	HAPI	USA	840		0.9 (0.4, 2.0)	43.7 (14)	53.6
	Health ABC	USA	1658		1.48 (0.94, 2.72)	73.8 (2.8)	52.8
	InCHIANTI	Italy	1202		2.40 (1.15,5.48)	68.3 (15.5)	44.5
	Nesda	Netherlands	1706		1.27 (0.56, 3.07)	42.5 (12.5)	31.8
	NTR	Netherlands	1598		1.50 (0.70, 3.10)	45.8 (14.0)	37.1
	SardINIA	Italy	4295		1.22(0.58, 2.79)	43.6(17.6)	43.8
	TwinsUK	UK	1172		1.42 (0.60,3.17)	55.4 (12.4)	0

3 Supplementary table I: Characteristics of the Study Participants (continued)

Panel	Study	Body mass index, mean (SD), kg/m ²	Waist circumference, mean(SD), cm	Systolic blood pressure, mean (SD), mm Hg	Total/HDL cholesterol, mean(SD)	Fasting glucose, mean(SD), mg/dL	Triglycerides, mean(SD), mg/dL
Discovery	AGES	27.1 (4.4)	100.8 (12.2)	142.6 (20.3)	3.8 (1.1)	224.4 (43.5)	107.5 (59.1)
	ARIC	28.4 (5.3)	101.6 (14.3)	126.1 (18.5)	4.5 (1.5)	107.4 (30.4)	151.9 (86.9)
	B58C	27.4 (4.9)	92.15(13.3)	126.7(15.3)	4.0 (1.1)	NA	185.1(133.7)
	CHS	26.3 (4.5)	93.1 (12.8)	135.2 (21.1)		107.6 (29.0)	140.2 (76.2)
	ERF	26.7 (4.3)	86.82 (13.1)	139.5 (20.5)	4.6 (1.3)	82.6 (17.5)	118.7 (70.0)
	FHS	27.4 (5.5)	96.0 (15.1)	121.2 (17.1)	3.9 (1.4)	99.2 (23.2)	125.2 (90.8)
	Health2000	27.6 (4.5)	93.9 (12.7)	134.3 (19.9)	4.9 (1.7)	97.2 (9.0)	141.7 (88.6)
	KORA	27.3 (4.1)	90.3 (12.4)	133.2 (18.2)	4.8 (1.8)	85.9 (18.2)	114.1 (56.7)
	MICROS	25.5 (4.8)	NA	132.3 (21.1)	3.96 (1.3)	91.8 (12.6)	106.3 (62.0)
	NFBC66	24.7 (4.2)	83.8 (12.1)	125.6 (14.0)	3.5 (1.1)	NA	180.7 (143.1)
	ORCADES	27.7(4.8)	93.7(14.0)	130.2 (19.3)	3.6 (0.9)	97.9 (17.6)	118.8(59)
	RS	26.3 (3.7)	90.6 (11.1)	139.2 (22.3)	5.2 (1.6)	NA	NA
	SHIP	27.3 (4.8)	89.4 (13.9)	137.1 (21.5)	4.3 (1.6)	NA	143.1 (129.2)
	VIS	27.3(4.3)	95.9(11.8)	137.4 (24.2)	3.9 (0.9)	102.6 (25.2)	137.4(80.8)
WGHS	25.9 (5.0)	NA	123.6 (13.7)	4.2 (1.3)	NA	143.7 (91.7)	
Replication	BLSA	26.6 (4.4)	91.9 (12.1)	124.6 (16.2)	3.5 (1.1)	92.8 (12.9)	111.1 (64.1)
	CARLA	28.4 (4.7)	100.0 (12.7)	143.9 (21.3)	4.2 (1.3)	NA	168.0 (133.9)
	Finrisk	27.0 (4.5)	91.9 (13.3)	NA	4.1 (1.3)	NA	132.9 (79.7)
	HAPI	26.6 (4.5)	87.3 (11.0)	121.5 (14.7)		76.8 (8.7)	91.7 (9.7)
	Health ABC	26.6 (4.1)	99.2 (12.3)	133.8 (20.0)	4.2 (1.3)	101.6 (29.1)	153.1 (88.1)
	InCHIANTI	27.2 (4.2)	91.3 (11.2)	145.7 (21.5)	4.1 (1.2)	94.4 (26.4)	125.9 (77.9)
	Nesda	27.8 (5.0)	89.5 (14.1)	134.1 (17.2)	3.4 (1.2)	93.2 (17.4)	115.6 (78.0)
	NTR	25.0 (3.9)	86.6 (12.3)	NA	3.9 (1.3)	100.1 (17.8)	120.5 (68.2)
	SardINIA	25.3(4.7)	84.8(13.3)	125.7 (18.6)	3.4(0.9)	90(23.2)	87.7(68.7)
	TwinsUK	25.3 (4.2)	NA	127.5 (48.4)	3.8 (1.3)	86.5 (21.4)	97.1 (55.7)

4 Supplementary table I: Characteristics of the Study Participants (continued)

Panel	Study	Current smoker, (%)	Diabetes, (%)	Hypertension, (%)	Lipid treatment, (%)	Hormone replacement, (%)	Prevalent CHD, (%)
Discovery	AGES	12.7	11.6	80.6	22.7	11.5	23.5
	ARIC	14.5	13.6	43.0	15.8	19.5	8.9
	B58C	23.0	1.9	4.2	NA	0.0	4.2
	CHS	11.3	11.7	36.7	4.3	8.5	NA
	ERF	39.4	2.6	19.9	12.6	24.7	3.6
	FHS	15.6	7.5	19.4	13.4	16.5	6.2
	Health2000	26.3	0.0	33.3	4.7	32.1	8.6
	KORA	18.0	3.6	17.5	4.4	7.9	NA
	MICROS	16.3	0.6	NA	NA	0.1	NA
	NFBC66	48.6	4.4	25.2	4.0	21.9	1.6
	ORCADES	8.5	3.0	21.2	11.5	NA	8.6
	RS	22.5	10.7	32.4	2.6	19.0	8.2
	SHIP	31.2	7.9	61.0	12.6	20.4	3.4
	VIS	28.0	6.7	25.0	3.2	NA	13.8
WGHS	11.7	2.5	24.5	3.3	43.7	NA	
Replication	BLSA	3.6	3.0	48.6	NA	NA	NA
	CARLA	19.5	15.5	78.9	NA	NA	8
	Finrisk	23.5	NA	57.2	7.8	NA	0.0
	HAPI	10.7	6.0	13.7	NA	NA	NA
	Health ABC	6.4	10.9	46.9	14.6	30.9	26.5
	InCHIANTI	18.7	9.9	73.4	7.2	NA	13.6
	Nesda	41.2	3.6	11.6	7.0	9.0	6.2*
	NTR	20.7	2.8	4.8	7.7	0.5	NA
	SardINIA	19.9	2.1	10.8	NA	5.9	17.5
	TwinsUK	33.1	1.6	13.3	6.0	9.3	1.5

5

6 Supplementary Table II - Genome-wide significant and suggestive loci with their gene annotations

SNP	Chromosome	Position	Coded Allele	Beta	SE	P-value	Closest Gene
rs2794520	1	157945440	C	0.193	0.007	9.5×10^{-189}	CRP
rs4420638	19	50114786	A	0.240	0.010	2.1×10^{-129}	APOC1
rs1183910	12	119905190	G	0.152	0.007	3.3×10^{-113}	HNF1A
rs4420065	1	65934049	C	0.111	0.007	3.2×10^{-64}	LEPR
rs4129267	1	152692888	C	0.094	0.007	1.1×10^{-47}	IL6R
rs1260326	2	27584444	T	0.089	0.007	5.4×10^{-43}	GCKR
rs12239046	1	245668218	C	0.048	0.007	1.6×10^{-13}	NLRP3
rs6734238	2	113557501	G	0.047	0.007	3.4×10^{-13}	IL1F10
rs9987289	8	9220768	G	0.079	0.011	2.3×10^{-12}	PPP1R3B*
rs10745954	12	102007224	A	0.043	0.006	1.6×10^{-11}	ASCL1*
rs1800961	20	42475778	C	0.120	0.018	2.3×10^{-11}	HNF4A
rs340029	15	58682257	T	0.044	0.007	2.6×10^{-11}	RORA
rs10521222	16	49716211	C	0.110	0.017	1.3×10^{-10}	SALL1
rs12037222	1	39837548	A	0.047	0.008	4.5×10^{-10}	PABPC4
rs13233571	7	72609167	A	0.054	0.010	2.8×10^{-8}	BCL7B
rs2836878	21	39387404	G	0.040	0.007	4.0×10^{-8}	DSCR2*
rs4903031	14	72088989	A	-0.046	0.008	4.6×10^{-8}	RGS6
rs13962	1	157442151	A	-0.057	0.011	6.6×10^{-8}	DARC
rs469772	1	91302893	T	-0.042	0.008	1.4×10^{-7}	ZNF644
rs2847281	18	12811593	A	-0.034	0.007	1.7×10^{-7}	PTPN2
rs1030023	2	102098706	T	0.034	0.007	3.5×10^{-7}	IL1R1
rs1292067	17	55285428	T	-0.033	0.007	3.9×10^{-7}	TUBD1
rs6486122	11	13318100	T	0.033	0.007	6.7×10^{-7}	ARNTL
rs10189761	2	636364	A	0.040	0.008	1.2×10^{-6}	TMEM18

rs6901250	6	117220718	A	-0.034	0.007	1.2×10^{-6}	GPRC6A
rs1880241	7	22725994	A	0.030	0.006	1.2×10^{-6}	IL6
rs195522	6	116351709	A	-0.032	0.007	1.2×10^{-6}	FRK
rs12130660	1	152354882	T	0.085	0.018	1.3×10^{-6}	NUP210L
rs1962073	8	10297963	A	0.031	0.007	1.3×10^{-6}	MSRA
rs1441170	2	213741808	A	-0.031	0.006	1.4×10^{-6}	IKZF2
rs9271605	6	32699426	A	0.039	0.008	1.4×10^{-6}	HLA-DQA1
rs4985288	6	327246	T	-0.044	0.009	1.7×10^{-6}	IRF4
rs1317140	3	49853656	A	0.032	0.007	1.7×10^{-6}	TRAIP
rs4410870	8	11193530	C	0.031	0.007	1.7×10^{-6}	MTMR9
rs12680682	8	38856003	T	0.054	0.011	1.7×10^{-6}	PLEKHA2
rs3787428	20	60020185	A	0.046	0.010	1.8×10^{-6}	TAF4
rs8133426	21	32033763	T	0.072	0.015	1.8×10^{-6}	SFRS15
rs7372878	3	199257080	A	0.067	0.014	1.9×10^{-6}	LMLN
rs5755993	22	34648865	A	-0.076	0.016	2.2×10^{-6}	RBM9
rs10460119	18	56256381	C	-0.032	0.007	2.2×10^{-6}	MC4R*
rs1558902	16	52361075	A	0.032	0.007	2.2×10^{-6}	FTO
rs6073972	20	44023705	C	0.039	0.008	2.9×10^{-6}	ZNF335
rs11047132	12	23899702	T	-0.054	0.012	3.1×10^{-6}	SOX5
rs16889362	6	37073683	A	0.067	0.015	3.4×10^{-6}	FGD2
rs3862790	11	72162823	T	-0.037	0.008	3.5×10^{-6}	STARD10
rs17648140	15	49481784	A	0.031	0.007	3.9×10^{-6}	GLDN
rs3888586	12	119152369	A	0.038	0.008	4.0×10^{-6}	PXN
rs4705952	5	131867517	A	0.038	0.008	4.1×10^{-6}	IRF1
rs10086637	8	78206017	A	-0.062	0.014	4.5×10^{-6}	PXMP3*
rs12890135	14	51269244	A	-0.040	0.009	4.5×10^{-6}	FRMD6

rs17476223	4	149096412	A	-0.090	0.020	5.3×10 ⁻⁶	ARHGAP10
rs10802285	1	234378509	T	0.038	0.008	6.6×10 ⁻⁶	GPR137B
rs3771452	2	70790369	T	-0.030	0.007	7.6×10 ⁻⁶	ADD2
rs261068	5	169253267	A	0.033	0.007	7.6×10 ⁻⁶	DOCK2
rs4767822	12	118404921	T	0.059	0.013	7.8×10 ⁻⁶	CCDC60
rs7069110	10	8735184	A	-0.029	0.007	8.5×10 ⁻⁶	GATA3*
rs1107426	1	31475112	A	0.278	0.063	9.0×10 ⁻⁶	WDR57
rs6463598	7	6903863	A	-0.056	0.013	9.1×10 ⁻⁶	C7orf28B*
rs2635727	6	50928899	T	-0.032	0.007	9.1×10 ⁻⁶	TFAP2B
rs10869954	9	79481827	A	0.028	0.006	9.1×10 ⁻⁶	GNA14
rs13374371	1	65468777	T	-0.048	0.011	9.2×10 ⁻⁶	AK3L1
rs543678	1	10665764	T	0.144	0.033	9.2×10 ⁻⁶	CASZ1
rs707939	6	31834667	A	0.029	0.007	9.7×10 ⁻⁶	MSH5
rs7876	11	60654488	A	0.028	0.006	9.9×10 ⁻⁶	VPS37C

* More than 60kb

11 Supplementary Table III - Association of seven formerly reported loci with CRP levels in the discovery panel and combined with the replication results
 12 without data from WGHS

SNP	Coded allele	Discovery		Discovery + replication	
		Beta* (SE)	P-value	Beta* (SE)	P-value
rs2794520	C	0.186 (0.008)	5.3×10^{-125}	0.147 (0.006)	4.1×10^{-128}
rs4420638	A	0.237 (0.011)	1.6×10^{-95}	0.232 (0.011)	6.4×10^{-104}
rs1183910	G	0.143 (0.008)	6.4×10^{-73}	0.137 (0.007)	2.6×10^{-84}
rs4420065	C	0.095 (0.008)	1.7×10^{-34}	0.075 (0.006)	5.4×10^{-36}
rs4129267	C	0.094 (0.008)	3.8×10^{-35}	0.076 (0.006)	3.0×10^{-36}
rs1260326	T	0.082 (0.008)	3.9×10^{-27}	0.064 (0.006)	9.5×10^{-26}
rs10745954	A	0.024 (0.007)	1.5×10^{-03}	0.023 (0.007)	4.3×10^{-04}

13 * Beta coefficient represents one unit change in the natural log transformed CRP (mg/L) per copy increment in the coded allele

15 Supplementary Table IV - Interaction of 18 Loci with Age, Sex, BMI, and Smoking for CRP Levels

SNP	Effective allele	Age		Sex		BMI		Smoking	
		Beta (SE)	P-value	Beta (SE)	P-value	Beta (SE)	P-value	Beta (SE)	P-value
rs2794520	T	0.0007 (0.0006)	0.27	-0.0023 (0.0025)	0.37	0.0017 (0.001)	0.09	-0.0189 (0.0133)	0.15
rs4420638	A	0.0009 (0.0006)	0.13	-0.0045 (0.0045)	0.32	0.0048 (0.0015)	1.5×10 ⁻³	0.038 (0.0188)	0.04
rs1183910	A	-0.00001 (0.0006)	0.96	-0.0018 (0.0027)	0.51	-0.0012 (0.001)	0.23	-0.0051 (0.0131)	0.70
rs4420065	T	0.0015 (0.0006)	9.0×10 ⁻³	0.0031 (0.0025)	0.23	0.0047 (0.001)	2.4×10 ⁻⁶	0.0064 (0.0092)	0.49
rs4129267	T	-0.0002 (0.0005)	0.68	0.0004 (0.0025)	0.89	-0.002 (0.001)	0.04	0.0005 (0.0128)	0.97
rs1260326	T	0.0011 (0.0006)	0.05	0.0018 (0.0026)	0.50	0.0014 (0.001)	0.16	-0.0206 (0.0128)	0.11
rs12239046	T	-0.0001 (0.0005)	0.91	-0.0003 (0.0026)	0.92	-0.0027 (0.001)	6.6×10 ⁻³	-0.0019 (0.0127)	0.88
rs6734238	A	-0.0007 (0.0006)	0.19	-0.0018 (0.0025)	0.49	-0.0034 (0.001)	5.7×10 ⁻⁴	0.0143 (0.0121)	0.24
rs9987289	A	0.0013 (0.001)	0.19	-0.006 (0.0045)	0.18	0.0001 (0.0017)	0.94	-0.0412 (0.022)	0.06
rs10745954	A	-0.0013 (0.0005)	1.9×10 ⁻²	0.0014 (0.0025)	0.59	-0.0014 (0.001)	0.15	0.0204 (0.0123)	0.10
rs1800961	T	-0.0004 (0.0016)	0.83	0.001 (0.0067)	0.88	-0.0005 (0.0027)	0.86	0.0055 (0.0349)	0.87
rs340029	T	-0.0006 (0.0006)	0.27	0.0003 (0.0025)	0.91	-0.0003 (0.001)	0.80	-0.0081 (0.0131)	0.54
rs10521222	T	-0.0007 (0.001)	0.52	0.0042 (0.0077)	0.59	-0.001 (0.0025)	0.68	-0.0048 (0.0266)	0.86
rs12037222	A	-0.0004 (0.0007)	0.56	0.0081 (0.0028)	4.3×10 ⁻³	-0.0012 (0.0011)	0.28	0.0151 (0.0151)	0.32
rs13233571	T	0.0003 (0.0009)	0.77	-0.0013 (0.0037)	0.73	0.001 (0.0015)	0.51	0.0152 (0.0195)	0.44
rs2847281	A	0.0002 (0.0005)	0.77	-0.0007 (0.0025)	0.79	-0.0008 (0.001)	0.43	0.0079 (0.0126)	0.53
rs6901250	A	0.0004 (0.0003)	0.17	0.0014 (0.003)	0.63	0.0016 (0.0011)	0.14	-0.0049 (0.0137)	0.72
rs4705952	A	-0.0003 (0.0007)	0.66	0.0032 (0.0032)	0.31	0.00001 (0.0012)	0.97	-0.0158 (0.0166)	0.34

17 Supplementary Table V - Imputation and quality of imputation for the 18 genome-wide significant loci

SNP	AGES		ARIC		B58C		CHS		ERF		FHS		Health2000		KORA	
	Gen.	Quality	Gen.	Quality	Gen.	Quality	Gen.	Quality	Gen.	Quality	Gen.	Quality	Gen.	Quality	Gen.	Quality
rs2794520	0	1.00	1	1.00	0	0.99	1	1.01	1	1.00	0	1.00	1	0.98	0	0.97
rs4420638	1	0.57	1	0.98	1	0.14	0	0.37	0	0.57	1	NA	0	0.67	1	0.99
rs1183910	1	0.95	0	0.99	0	0.98	0	0.80	0	0.94	0	1.01	0	0.91	0	0.97
rs4420065	0	1.00	0	0.87	0	0.93	1	1.02	1	1.00	0	0.97	1	1.00	0	0.85
rs4129267	0	1.00	1	1.00	0	0.96	1	1.01	1	0.96	0	0.99	1	1.00	0	0.96
rs1260326	0	1.00	0	0.98	0	0.98	1	1.01	1	0.98	0	0.99	1	0.99	0	0.95
rs12239046	1	0.99	1	1.00	0	0.76	0	0.99	0	0.96	0	1.00	0	0.93	0	0.71
rs6734238	1	1.00	1	1.00	0	1.00	0	0.85	0	1.00	0	0.99	0	0.95	0	1.00
rs9987289	1	1.00	0	0.98	0	0.97	0	0.84	0	0.98	0	1.01	0	0.94	0	0.97
rs10745954	1	0.97	0	1.00	0	1.00	0	0.95	0	0.99	0	1.01	0	0.92	0	1.00
rs1800961	0	0.99	1	0.97	0	0.22	1	1.01	1	0.89	1	NA	1	0.98	0	0.19
rs340029	0	1.00	1	0.99	0	0.81	1	1.00	1	0.99	0	0.99	1	1.00	0	0.73
rs10521222	0	0.99	0	0.33	0	0.22	1	0.99	1	0.82	0	0.31	1	1.00	0	0.32
rs12037222	1	0.97	0	0.97	0	0.98	0	0.91	0	0.97	0	1.01	0	0.96	0	0.97
rs13233571	1	0.96	0	0.99	0	0.99	0	0.87	0	0.91	0	0.98	0	0.93	0	0.99
rs2847281	1	0.98	1	1.00	0	0.97	0	0.95	0	0.98	0	0.99	0	0.84	0	0.95
rs6901250	1	0.97	1	1.00	0	0.99	0	0.66	0	0.96	0	0.98	0	0.88	0	1.00
rs4705952	1	0.90	1	0.97	0	0.70	0	0.75	0	0.92	0	0.62	0	0.76	0	0.61

18 Gen. = Genotyped

19 Supplementary Table V - Imputation and quality of imputation for the 18 genome-wide significant loci (continued)

SNP	MICROS		NFBC66		TwinsUK		InCHIANTI		BLSA		HAPI		HABC		Finrisk	
	Gen.	Quality	Gen.	Quality	Gen.	Quality	Gen.	Quality	Gen.	Quality	Gen.	Quality	Gen.	Quality	Gen.	Quality
rs2794520	1	1.00	1	0.98	1	0.99	1	1.00	1	1.00	1	1.00	1	1.00	1	1.00
rs4420638	0	0.47	0	0.66	0	0.66	0	0.53	1	1.00	0	0.37	0	0.64	0	0.56
rs1183910	0	0.91	0	0.83	0	0.96	0	0.99	0	0.97	0	0.94	0	0.95	0	0.99
rs4420065	0	0.99	1	1.00	0	0.99	0	0.99	0	1.00	0	1.00	0	0.95	1	1.00
rs4129267	1	1.00	1	1.00	1	1.00	1	1.00	1	1.00	1	1.00	1	1.00	1	1.00
rs1260326	0	0.95	1	0.98	0	0.96	1	0.96	0	0.98	0	0.96	0	0.97	1	0.99
rs12239046	0	0.99	0	0.73	0	0.99	0	1.00	1	1.00	0	0.82	0	0.96	0	0.99
rs6734238	0	0.99	0	0.72	0	0.99	0	1.00	1	1.00	0	1.00	0	0.89	0	1.00
rs9987289	0	0.99	0	0.94	0	0.99	0	0.99	0	0.96	0	0.99	0	0.98	0	0.99
rs10745954	0	0.97	0	0.62	0	0.97	0	1.00	0	1.00	0	0.99	0	0.92	0	1.00
rs1800961	1	0.71	1	0.99	1	0.97	1	0.99	1	1.00	1	0.87	1	0.99	1	0.99
rs340029	1	1.00	1	1.00	1	1.00	1	1.00	1	1.00	1	1.00	1	1.00	1	1.00
rs10521222	1	0.98	1	1.00	1	0.98	1	0.98	0	0.33	1	0.98	1	0.98	1	0.98
rs12037222	0	0.98	0	0.88	0	0.98	0	0.98	0	0.97	0	0.98	0	0.97	0	0.99
rs13233571	0	0.99	0	0.91	0	0.96	0	0.98	0	0.99	0	0.98	0	0.91	0	0.98
rs2847281	0	0.99	0	0.83	0	0.99	0	0.98	1	1.00	0	0.99	0	0.97	0	1.00
rs6901250	0	0.96	0	0.79	0	0.98	0	0.97	1	1.00	0	0.98	0	0.87	0	0.98
rs4705952	0	0.89	0	0.74	0	0.94	0	0.91	1	1.00	0	0.93	0	0.83	0	0.86

20 Gen. = Genotyped

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Supplementary Table V - Imputation and quality of imputation for the 18 genome-wide significant loci (continued)

SNP	Nesda		NTR		ORCADES		RS		SHIP		VIS		WGHS	
	Gen.	Quality	Gen.	Quality	Gen.	Quality	Gen.	Quality	Gen.	Quality	Gen.	Quality	Gen.	Quality
rs2794520	1	0.98	0	0.98	1	0.99	1	1.00	1	1.00	1	1.00	1	1.00
rs4420638	1	0.58	0	0.54	0	0.66	0	0.53	1	1.00	0	0.37	0	0.64
rs1183910	1	0.99	0	0.99	0	0.96	0	0.99	0	0.97	0	0.94	0	0.95
rs4420065	1	0.99	0	0.99	0	0.99	0	0.99	0	1.00	0	1.00	0	0.95
rs4129267	1	0.99	0	1.00	1	1.00	1	1.00	1	1.00	1	1.00	1	1.00
rs1260326	1	0.96	0	0.97	0	0.96	1	0.96	0	0.98	0	0.96	0	0.97
rs12239046	0	1.00	1	1.00	0	0.99	0	1.00	1	1.00	0	0.82	0	0.96
rs6734238	1	1.00	0	1.00	0	0.99	0	1.00	1	1.00	0	1.00	0	0.89
rs9987289	1	0.97	0	0.99	0	0.99	0	0.99	0	0.96	0	0.99	0	0.98
rs10745954	1	1.00	0	1.00	0	0.97	0	1.00	0	1.00	0	0.99	0	0.92
rs1800961	1	0.68	0	0.68	1	0.97	1	0.99	1	1.00	1	0.87	1	0.99
rs340029	0	1.00	1	1.00	1	1.00	1	1.00	1	1.00	1	1.00	1	1.00
rs10521222	0	1.00	0	0.37	1	0.98	1	0.98	0	0.33	1	0.98	1	0.98
rs12037222	1	0.97	0	0.98	0	0.98	0	0.98	0	0.97	0	0.98	0	0.97
rs13233571	1	0.98	0	0.99	0	0.96	0	0.98	0	0.99	0	0.98	0	0.91
rs2847281	0	1.00	1	1.00	0	0.99	0	0.98	1	1.00	0	0.99	0	0.97
rs6901250	1	0.80	0	0.81	0	0.98	0	0.97	1	1.00	0	0.98	0	0.87
rs4705952	1	0.91	0	0.91	0	0.94	0	0.91	1	1.00	0	0.93	0	0.83

22

Gen. = Genotyped

23 Supplementary Table VI - Association of covariates with the effect on serum CRP of SNPs for which heterogeneity was shown

SNP	Age		Sex		BMI		Smoking	
	Beta (SE)	P-value	Beta (SE)	P-value	Beta (SE)	P-value	Beta (SE)	P-value
rs2794520	0.0021 (0.0014)	0.13	0.0004 (0.0012)	0.72	0.0046 (0.0174)	0.79	-0.0017 (0.0016)	0.27
rs4420065	0.0015 (0.001)	0.12	0.0012 (0.0006)	0.06	0.0065 (0.0121)	0.59	0 (0.0011)	0.96
rs4129267	0.001 (0.0008)	0.20	0.0006 (0.0006)	0.32	0.0046 (0.0102)	0.65	-0.0009 (0.0009)	0.30
rs1260326	0.0008 (0.0009)	0.40	-0.0001 (0.0007)	0.87	0.0204 (0.0119)	0.09	-0.0007 (0.001)	0.49
rs10745954	-0.0014 (0.0008)	0.06	-0.0014 (0.0003)	2.8×10^{-5}	0.0012 (0.0097)	0.90	0.0008 (0.001)	0.40

24 Supplementary Table VII - Details of methods and analysis for all participating cohorts

	FHS	CHS	ARIC	RS	KORA
N	6899	3265	6431	5567	1587
Sample	Serum	Plasma	Serum	Serum	Serum
Genotyping platform	Affymetrix 500K, MIPS 50K	Illumina HumanHap370CNV Duo	Affymetrix 6.0 Broad Institute of Harvard and Massachusetts Institute of Technology	Version 3 Illumina Infinium II HumanHap550	Affymetrix 500k
Genotyping center		General Clinical Research Center's Phenotyping/Genotyping Laboratory at Cedars-Sinai	Broad Institute of Harvard and Massachusetts Institute of Technology	Erasmus Medical Center	Helmholtz Zentrum München
Genotyping calling algorithm	Affymetrix BRLMM	BeadStudio	BeadStudio	BeadStudio	BRLMM
MAF filter (N filtered)	<0.01 (67006)	NA	NA	≤0.01 (24977)	NA
HWE pvalue filter (N filtered)	p<10 ⁻⁶ (20469)	p<10 ⁻⁵ (1829)	p<10 ⁻⁶ (if MAF >0.05) (21220)	p<10 ⁻⁵	NA
Call rate filter (N filtered)	<0.95 (23093)	≤0.97 (10220)	≤0.90 (26274)	≤0.90 (811)	≤0.90
Imputation quality filter (N filtered)	OEvar < 0.3 (61627)	OEvar < 0.01	NA	NA	NA
Number of genotyped SNPs in the analysis	425593	306655	589253	530683	490032
Imputation software	MACH	BIMBAM	MACH	MACH	MACH
Trait transformation	Natural logarithm	Natural logarithm	Natural logarithm	Natural logarithm	Natural logarithm
Adjustment	Age and sex	Age, sex, and study center	Age and sex	Age and sex	Age and sex
Analysis method	Linear mixed effect model	Linear regression	Linear regression	Linear regression	Linear regression

26 Supplementary Table VII - Details of methods and analysis for all participating cohorts (continued)

	AGES	B58C	ERF	MICROS	VIS
N	3218	1461	1792	1215	774
Sample	Serum	Citrated plasma	Plasma	Serum	EDTA plasma
Genotyping platform	Illumina Hu370CNV	Affymetrix 500K	Illumina HumHap 300 LGTC, Leiden	Version 2 Illumina HumanHap300	Illumina HumanHap300 (v1)
Genotyping center	National Institute on Ageing	Affymetrix	University Medical Centre	Helmholtz Center, Munich, Germany	
Genotyping calling algorithm	BeadStudio	CHIAMO	BeadStudio	BeadStudio	BeadStudio
MAF filter (N filtered)	<0.01 (17612 SNPs)	NA	<0.01	<0.01	<0.01
HWE p-value filter (N filtered)	p<10 ⁻⁶ (1595 SNPs)	NA	p<10 ⁻⁵	p<10 ⁻⁶	p<10 ⁻⁶
Call rate filter (N filtered)	≤0.97 (18,819 SNPS)	NA	≤0.90	≤0.98	≤0.98
Imputation quality filter (N filtered)	NA	NA	NA	NA	NA
Number of genotyped SNPs in the analysis	308340	419829	-	292917	305068
Imputation software	MACH	IMPUTE	MACH	MACH	MACH
Trait transformation	Natural logarithm (values +1)	Natural logarithm	Natural logarithm	Natural logarithm	Natural logarithm
Adjustment	Age and sex	Sex, date & time of sample collection, postal delay, nurse (which also adjusts for region of residence). No age-adjustment necessary as all cohort members are born in one week.	Age and sex	Age and sex	Age and sex
Analysis method	Linear regression	Linear regression	Linear regression	Score test for family-based association	Linear regression

27 Supplementary Table VII - Details of methods and analysis for all participating cohorts (continued)

	ORCADES	WGHS	SHIP	NFBC66	Health2000
N	715	21953	4103	5125	2080
Sample	EDTA plasma	Serum	Serum	Serum	Serum
Genotyping platform	Illumina HumanHap300 (v2)	Illumina HumanHap300 Duo+	Affymetrix SNP Array 6.0	Illumina HumanCNV370-duo BeadChip	Illumina Human610-Quad BeadChip
Genotyping center			University of Greifswald/Affymetrix Inc.	Broad institute	Sanger Institute
Genotyping calling algorithm	BeadStudio	Amgen lab BeadStudio 3.1	Birdseed V2	Broad institute BeadStudio	Sanger Institute Illuminus
MAF filter (N filtered)	<0.01	NA	NA	<0.01 (7666)	<0.02 (40847)
HWE pvalue filter (N filtered)	$p < 10^{-6}$	$p < 10^{-6}$	NA	$p < 10^{-6}$ (2614)	$p < 10^{-4}$ (5952)
Call rate filter (N filtered)	≤ 0.98	≤ 0.98	NA	≤ 0.98 (10922)	≤ 0.95 (5846)
Imputation quality filter (N filtered)	NA	NA	NA	NA	NA
Number of genotyped SNPs in the analysis	306,207	317,186	909,622	339,629	592,357
Imputation software	MACH	MACH	IMPUTE	MACH	MACH
Trait transformation	Natural logarithm	Natural logarithm	Natural logarithm	Natural logarithm	Natural logarithm
Adjustment	Age and sex	Age and sex	Age and sex	Age and sex	Age and sex
Analysis method	Linear regression	Linear regression	Linear regression	Linear regression	Linear regression

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30 Supplementary Table VII - Details of methods and analysis for all participating cohorts (continued)

	BLSA	AMISH	Finrisk	InCHIANTI	SardinIA
N	481	840	1893	1202	4295
Sample	Serum	Serum	Serum	Plasma	Serum
Genotyping platform	Illumina 550K	Affymetrix 500K University of Maryland Division of Endocrinology, Diabetes and Nutrition Birdseed V2	Illumina Human610- Quad BeadChip	Illumina 550K	Affymetrix SNP array 500K
Genotyping center	National Institute of Aging BeadStudio	National Institute of Aging Diabetes and Nutrition Birdseed V2	Sanger Institute Illuminus	National Institute of Aging BeadStudio	Lanusei, Sardinia BRLMM
Genotyping calling algorithm	<0.01 (23053)	NA	<0.02 (40847)	<0.01 (20646)	<0.05
MAF filter (N filtered)	p<10 ⁻⁴ (470)	NA	p<10 ⁻⁴ (5952)	p<10 ⁻⁵ (331)	p<10 ⁻⁶
HWE p-value filter (N filtered)	≤0.99 (23728)	NA	≤0.95 (5846)	≤0.97 (19009)	≤0.90
Call rate filter (N filtered)	NA	NA	NA	NA	OEvar < 0.3
Imputation quality filter (N filtered)	NA	338,598	554,988	NA	356,359
Number of genotyped SNPs in the analysis	MACH	MACH	MACH	MACH	MACH
Imputation software	Natural logarithm	Natural logarithm	Natural logarithm	Natural logarithm	Natural logarithm
Trait transformation	Adjustment	Age and sex	Age, Age ² , Sex	Age and sex	Age and sex
Adjustment	Analysis method	Linear regression	Linear regression	Linear regression	Linear regression in a variance-component framework
Analysis method	Linear regression	Linear regression	Linear regression	Linear regression	Linear regression in a variance-component framework

33 Supplementary Table VII - Details of methods and analysis for all participating cohorts (continued)

	Health ABC	NESDA	NTR	TwinsUK	CARLA
N	1658	1706	1596	1093	1695
Sample	Serum	Plasma	Serum	serum	Serum
Genotyping platform	Illumina 1M-Duo	Perlegen 600K	Affymetrix 600K, Perlegen	Illumina HumanHap300 Duo, HumanHap300, HumanHap550 and HumanHap610	ABI TaqMan Genotyping
Genotyping center				Wellcome Trust Sanger Institute; Le Centre National de Génotypage; Duke University; Finnish Institute of Molecular Medicine, Finnish Genome Center, University of Helsinki; CIDR	University of Greifswald, Institute for Pharmacology
Genotyping calling algorithm	CIDR Bead Studio	Perlegen Sciences Perlegen	Perlegen Perlegen Proprietary	Illuminus software	SDS 2.3
MAF filter (N filtered)	<0.01	<0.01	0.01 (41495)	<0.01	NA
HWE p-value filter (N filtered)	p<10 ⁻⁶	p<10 ⁻⁶	NA	p<10 ⁻⁴	NA
Call rate filter (N filtered)	≤0.95	≤0.95	≤0.95	≤0.95	NA
Imputation quality filter (N filtered)	NA	NA	NA	proper_info<0.5	NA
Number of genotyped SNPs in the analysis	NA	NA	64 for replication	Different numbers for different platforms	11 for replication
Imputation software	MACH	IMPUTE	IMPUTE	IMPUTE	NA
Trait transformation	Natural logarithm	Natural logarithm	Natural logarithm	Natural logarithm	Natural logarithm
Adjustment	Age and sex	Age and sex	Age and sex	Age, batch (only females in analysis)	Age and sex
Analysis method	Linear regression	Linear regression	Linear regression	Linear regression	Linear regression

34 OEvar = observed variance / expected variance

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