Genetic Loci Associated With C-Reactive Protein Levels and Risk of Coronary Heart Disease

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Context Plasma levels of C-reactive protein (CRP) are independently associated with risk of coronary heart disease, but whether CRP is causally associated with coronary heart disease or merely a marker of underlying atherosclerosis is uncertain.

Objective To investigate association of genetic loci with CRP levels and risk of coronary heart disease.

Design, Setting, and Participants We first carried out a genome-wide association ($n=17\,967$) and replication study ($n=13\,615$) to identify genetic loci associated with plasma CRP concentrations. Data collection took place between 1989 and 2008 and genotyping between 2003 and 2008. We carried out a mendelian randomization study of the most closely associated single-nucleotide polymorphism (SNP) in the *CRP* locus and published data on other *CRP* variants involving a total of 28 112 cases and 100 823 controls, to investigate the association of *CRP* variants with coronary heart disease. We compared our finding with that predicted from meta-analysis of observational studies of CRP levels and risk of coronary heart disease. For the other loci associated with CRP levels, we selected the most closely associated SNP for testing against coronary heart disease among 14 365 cases and 32 069 controls.

Main Outcome Measure Risk of coronary heart disease.

Results Polymorphisms in 5 genetic loci were strongly associated with CRP levels (% difference per minor allele): SNP rs6700896 in *LEPR* (-14.8%; 95% confidence interval [CI], -17.6% to -12.0%; $P=6.2\times10^{-22}$), rs4537545 in *IL6R* (-11.5%; 95% CI, -14.4% to -8.5%; $P=1.3\times10^{-12}$), rs7553007 in the *CRP* locus (-20.7%; 95% CI, -23.4% to -17.9%; $P=1.3\times10^{-38}$), rs1183910 in *HNF1A* (-13.8%; 95% CI, -16.6% to -10.9%; $P=1.9\times10^{-18}$), and rs4420638 in *APOE-CI-CII* (-21.8%; 95% CI, -25.3% to -18.1%; $P=8.1\times10^{-26}$). Association of SNP rs7553007 in the *CRP* locus with coronary heart disease gave an odds ratio (OR) of 0.98 (95% CI, 0.94 to 1.01) per 20% lower CRP level. Our mendelian randomization study of variants in the *CRP* locus showed no association with coronary heart disease: OR, 1.00; 95% CI, 0.97 to 1.02; per 20% lower CRP level, compared with OR, 0.94; 95% CI, 0.94 to 0.95; predicted from meta-analysis of the observational studies of CRP levels and coronary heart disease (z score, -3.45; $P<.001$). SNPs rs6700896 in *LEPR* (OR, 1.06; 95% CI, 1.02 to 1.09; per minor allele), rs4537545 in *IL6R* (OR, 0.94; 95% CI, 0.91 to 0.97), and rs4420638 in the *APOE-CI-CII* cluster (OR, 1.16; 95% CI, 1.12 to 1.21) were all associated with risk of coronary heart disease.

Conclusion The lack of concordance between the effect on coronary heart disease risk of *CRP* genotypes and CRP levels argues against a causal association of CRP with coronary heart disease.

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CORONARY HEART DISEASE (CHD) is the leading cause of death worldwide.[1] Inflammation plays a key role in the pathogenesis of CHD at every stage from initiation to progression and rupture of the atherosclerotic plaque.[2] C-reactive protein (CRP), an acute-phase protein synthesized primarily by the liver, is currently the most widely used biomarker of inflammation.[3] Ob-servational studies have consistently demonstrated that higher plasma levels of CRP are associated with higher risk of CHD,[4,5] and measurement of CRP has been advocated as a means of improving risk prediction.[6] There is

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considerable interest in whether CRP has a causal role in CHD[7,8] or whether CRP is merely a marker of underlying atherosclerosis. Although previous studies have addressed this question, it is unclear whether they included a sufficient number of cases to have adequate statistical power to confirm or refute associations of CHD with genetically determined differences in CRP levels.[8] Resolution of this question will improve understanding of inflammatory mechanisms in atherosclerosis.

The mendelian randomization concept has been used to investigate possible causal relationships of an intermediate trait (such as CRP levels) with disease, taking advantage of the random allocation of alleles at conception.[9] If the intermediate trait is causally linked to disease, then genetic variants influencing the trait should also influence disease risk.[10] Mendelian randomization studies should be unaffected by confounding from environmental factors, eg, smoking, and reverse causation bias, ie, where the disease itself (atherosclerosis) influences the trait (CRP levels).[9]

The aims of the present study were to conduct a genome-wide association study to identify common genetic variants associated with CRP levels and use mendelian randomization to improve understanding of the possible causal relationship of CRP levels with CHD.

METHODS
Study Design and Rationale
The study involved 4 interrelated components. First, a genome-wide association and replication study was performed to identify genetic loci associated with CRP levels, and at each locus, the most closely associated single-nucleotide polymorphism (SNP) was selected. Second, a mendelian randomization study for the most associated SNP in the *CRP* locus in our data together with published data on *CRP* variants with CHD let us assess the potential causal association of CRP with CHD. Third, we compared the finding from the mendelian randomization study with that predicted from meta-analysis of the relationship of CRP levels with CHD from observational stud-

ies. Fourth, we carried out genetic association between CHD and the most associated SNPs in genetic loci outside the *CRP* locus using the concept of CRP as an intermediate phenotype,[10] to identify putative pathways linking inflammation with CHD.

Population Cohorts
Genome-wide and Replication Study for CRP. Genome-wide association to identify variants related to CRP levels, measured using high-sensitivity assays, was carried out in 17 967 participants from 5 studies: the London Life Sciences Population (LOLIPOP) study (n=5502), a population-based cohort of European and Indian Asian men and women aged 35 to 75 years and living in West London, United Kingdom[11] (data collection, 2001-2007; genotyping, 2003-2008); the 1966 Northern Finnish Birth Cohort (NFBC) (n=4761), a prospective birth cohort of persons born in 1966 in the 2 northernmost provinces of Finland[12,13] (data collection, 1997-1998; genotyping, 2007-2008); the Lausanne Cohort (CoLaus) (n=5226), a cross-sectional study of a random sample of European men and women aged 35 to 75 years and living in Lausanne, Switzerland[14] (data collection, 2003-2006; genotyping, 2006-2007); the Genetic Epidemiology of Metabolic Syndrome (GEMS) study (n=1781), a case-control study of dyslipidemic cases (age, 20-65 years) matched with normolipidemic controls by sex and recruitment site[15] (data collection, 2003-2006; genotyping, 2006-2007); and the Data from an Epidemiological Study on the Insulin Resistance syndrome (DESIR) study (n=697), a longitudinal French general population cohort of persons aged 30 to 64 years recruited through the French social security system[16] (data collection, 1994-2004; genotyping, 2006-2007). Replication of SNPs associated with CRP levels, identified in the genome-wide association study, was performed in a further 13 615 LOLIPOP participants who were not included in the genome-wide association study and were free of known CHD.

Mendelian Randomization and Genetic Association Studies With CHD. Variants related to CRP levels were tested for association with CHD among 14 365 CHD cases and 32 069 controls. The participating studies comprised the Precocious Coronary Artery Disease study (PROCARDIS) (n=8328), a case-control study of premature CHD before age 66 years[17] (data collection, 1999-2006; genotyping, 2008); the International Studies of Infarct Survival (ISIS) (n=3624), comprising men aged 30 to 54 years and women aged 30 to 64 years with nonfatal myocardial infarction (MI) and their spouse controls[18,19] (data collection, 1989-1992; genotyping, 2008); the British Heart Foundation Family Heart Study, comprising individuals with MI or coronary revascularization before the age of 66 years and at least 1 first-degree relative with premature CHD, who were also studied as part of the Wellcome Trust Case Control Consortium (WTCCC) (n=3249-4863)[20] (data collection, 1998-2006; genotyping, 2006-2008); the German MI Family Studies (GerMIFS I: n=2519; GerMIFS II: n=2520), comprising persons with MI before the age of 60 years and at least 1 first-degree relative with premature CHD and matched controls[20,21] (data collection, 1996-2008; genotyping, 2006-2008); the INTERHEART study, a multinational case-control study of persons presenting with first MI (n=4043),[22] (data collection, 1999-2003; genotyping, 2008); and the LOLIPOP study (n=20 475) (data collection, 2001-2007; genotyping, 2008).

Genotyping
Genome-wide association scans were performed using the Affymetrix 500K mapping array (Affymetrix Inc, Santa Clara, California), the Illumina 317K array (Illumina Inc, San Diego, California), and Perlegen Sciences customized arrays (Perlegen Sciences Inc, Mountain View, California). To combine data across genotyping platforms, imputation was done using a hidden Markov model algorithm implemented in MACH version 1.0 (Center for Statistical Genetics, University of Michigan, Ann Arbor) (in LOLIPOP) or IMPUTE version 0.5.0 (Genetics Software Suite, University of Oxford, Oxford,

United Kingdom) (in other studies) and phased haplotypes from National Center for Biotechnology Information (NCBI) build 35, dbSNP build 125. For the European data sets, the HapMap CEU sample was used for reference haplotypes; Indian Asian data sets were imputed based on a combination (mixed) of HapMap populations. Imputed SNPs with minor allele frequency (MAF) less than 0.01 or a low-quality score ($r^2 < 0.30$ in MACH or information score <0.50 in IMPUTE) were removed. This left approximately 1.4 million directly genotyped or imputed autosomal SNPs per participant with data available in all samples. Genotyping for replication testing and for evaluation against CHD was performed using KASPar (KBiosciences Ltd, Hoddesdon, Hertfordshire, United Kingdom) (in LOLIPOP), Sequenom (Sequenom Inc, San Diego) (in INTERHEART and PROCARDIS), TaqMan (Applied Biosystems, Foster City, California) (in ISIS and WTCCC), or Affymetrix mapping arrays (in GerMIFS I and II and WTCCC).

Statistical Analyses

Genome-wide Association and Replication Study for CRP. Genome-wide SNP associations for CRP were tested in multiple linear regression analyses using an additive genetic model. CRP levels were log-transformed to achieve approximate normal distribution and analyzed as a quantitative trait with adjustment for age and sex (analysis of residuals showed good adherence to normality assumptions). To account for heterogeneity in population structure, principal components derived from EIGENSTRAT version 2.0 (Reich Laboratory, Harvard University, Cambridge, Massachusetts) were included as covariates in age- and age-sex–adjusted analyses for CoLaus, GEMS, NFBC, and LOLIPOP Indian Asian Illumina analyses (the number of principal components included varied from 4 to 10, depending on the popula-

Figure 1. Manhattan Plot of Results From the Combined Analysis of Genome-Wide Association Data
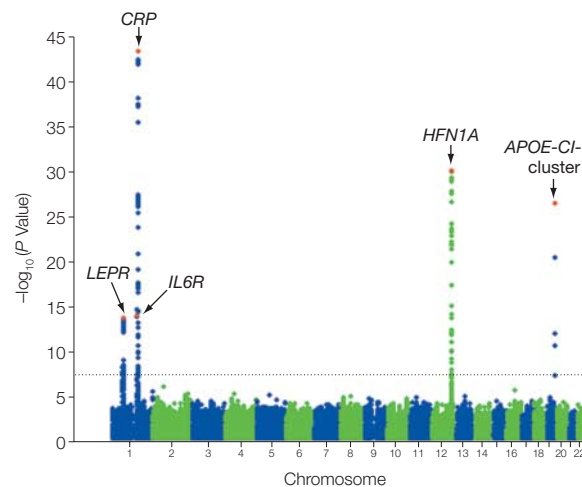
The horizontal dotted line represents genome-wide significance (5×10^{-8}). The red dots are the most associated single-nucleotide polymorphism at each of the *LEPR*, *IL6R*, *CRP*, and *HNF1A* loci and the *APOE-CI-CII* cluster.

Table 1. Genomic Context, Alleles, Minor Allele Frequency, Association Test Results for Most Associated SNP at Each Locus, and Effect on CRP Levels[a]

	Single-Nucleotide Polymorphism				
	rs6700896	**rs4537545**	**rs7553007**	**rs1183910**	**rs4420638**
Genomic context					
Chromosome	1	1	1	12	19
Position	65862370	152685503	157965173	119905190	50114786
Locus	*LEPR*	*IL6R*	*CRP*	*HNF1A*	*APOE-CI-CII* cluster
Alleles					
Reference	C	C	G	C	A
Minor	T	T	A	T	G
Minor allele frequency[b]					
Europeans	0.38	0.43	0.33	0.32	0.19
Indian Asians	0.46	0.31	0.29	0.39	0.12
Genome-wide association					
P value	3.1×10^{-14}	1.8×10^{-14}	7.6×10^{-44}	1.2×10^{-30}	4.5×10^{-27}
Replication					
CRP effect,% (95% CI)[c]	−14.8 (−17.6 to −12.0)	−11.5 (−14.4 to −8.5)	−20.7 (−23.4 to −17.9)	−13.8 (−16.6 to −10.9)	−21.8 (−25.3 to −18.1)
P value	6.2×10^{-22}	1.3×10^{-12}	1.3×10^{-38}	1.9×10^{-18}	8.1×10^{-26}

Abbreviations: CI, confidence interval; CRP, C-reactive protein; SNP, single-nucleotide polymorphism.
[a] Association tests based on National Center for Biotechnology Information (NCBI) build 36, dbSNP build 126.
[b] Minor allele frequency from people genotyped in replication sample.
[c] Effect size is % change and 95% confidence interval in CRP per copy of minor allele under an additive genetic model adjusted for age, sex, and ethnicity in the replication sample (n = 13 615). Based on geometric mean CRP (1.93 mg/L) in the replication sample, estimated absolute CRP effects are as follows: for rs6700896, −0.29 mg/L; for rs4537545, −0.22 mg/L; for rs7553007, −0.40 mg/L; for rs1183910, −0.27 mg/L; and for rs4420638, −0.42 mg/L. (To convert CRP to nmol/L, multiply by 9.524.)

tion structure of the specific cohort). For other LOLIPOP data sets, genomic control factors were used to correct for any inflation. No principal components were included for DESIR because the population was recruited from a geographically restricted area.

Statistical software used for genome-wide associations comprised SNPTEST (Genetics Software Suite) version 1.1.3 (in DESIR), and version 1.1.4 (in CoLaus, GEMS, and NFBC); and MACH2QTL version 1.0 (Center for Statistical Genetics) (in LOLIPOP). Results of the separate genome-wide association studies were combined using weighted z scores, and a fixed- rather than random-effects model to maximize discovery, since random-effects estimates are associated with larger variance. Quantile-quantile plots showed good adherence to null expectations (λ for combined data=1.0625). We used $P < 5 \times 10^{-8}$ to designate genome-wide significance, taking account of the approximately 1 million independent tests for common variants across the genome.[23] For 5 genetic loci associated with CRP levels at genome-wide significance, we selected the single most closely associated SNP (ie, smallest P value) for replication against CRP.

Using Quanto[24] version 1.2 for quantitative traits, we estimated that the genome-wide association study had 80% power to detect SNPs associated with 0.2% of population variation in CRP levels, or an 11% difference in CRP level per allele copy, at MAF 0.3 and genome-wide level of significance ($P < 5 \times 10^{-8}$).

Mendelian Randomization Study of Genetic Variants in the *CRP* Locus and CHD. We analyzed the relationship of SNP rs7553007 in the *CRP* locus, the SNP most strongly associated with CRP levels in our data, for association with CHD risk using logistic regression under an additive genetic model as part of a mendelian randomization study. To identify published data on the relationship of *CRP* variants with CHD, 2 electronic databases (Medline and EMBASE) were searched up to and including November 2008 for all prospective studies (including cohort, nested case-control, and case-cohort studies) and case-control studies, with no threshold sample size. For the search, the MeSH terms *C-reactive protein* and *polymorphism, single nucleotide* or *polymorphism, genetic* or *haplotype* in combination with *coronary disease* or *heart disease* were used, and

the search was limited by the terms *human* and *English language*. We also scanned reference lists of previous reports.

Eight studies of 18 cohorts were identified. Two studies[25,26] examining 9 cohorts reported results for a single SNP (SNP rs1130864); for these studies, odds ratios (ORs) were reported under a recessive model comparing homozygotes (TT) for the minor allele with CT/CC genotype, and mean effect on CRP levels was obtained from published ratio of geometric means (1.21).[25,26] For the remaining studies,[8,27-31] we selected SNP rs1205 on the basis of MAF greater than 0.1 and largest per-allele effect size on CRP levels (−0.35 mg/L) reported in the study of *CRP* variants and CRP levels by Verzilli et al.[32] (To convert CRP to nmol/L, multiply by 9.524.) We used a per-allele OR of rs1205 with CHD where available[27,29] or where this could be estimated directly from the data[8,31]; otherwise, ORs were estimated from averaging published effect sizes for minor allele homozygotes (TT) and heterozygotes (CT) compared with wild type (CC).[28,30] We estimated standard errors of the effect sizes of *CRP* variants on CHD from the reported 95% confidence intervals (CIs), assuming normality.

Table 2. Relationships Between Most Associated SNPs in the 5 Genetic Loci Associated With CRP Levels and Cardiovascular Risk Factors in Replication Sample (LOLIPOP, n = 13 615) From Regression Analyses Adjusted for Age, Sex, and Ethnic Group Under an Additive Genetic Model[a]

Cardiovascular Risk Factors	rs6700896 (*LEPR*)	*P* Value	rs4537545 (*IL6R*)	*P* Value	rs7553007 (*CRP*)	*P* Value	rs1183910 (*HNF1A*)	*P* Value	rs4420638 (*APOE-CI-CII*)	*P* Value
Weight, kg	−0.44 (−0.78 to −0.10)	.01	0.19 (−0.16 to 0.54)	.29	0.10 (−0.27 to 0.46)	.60	0.03 (−0.31 to 0.37)	.87	0.16 (−0.31 to 0.64)	.49
Body mass index	−0.12 (−0.23 to −0.00)	.05	0.01 (−0.11 to 0.13)	.83	0.00 (−0.12 to 0.13)	.98	−0.04 (−0.16 to 0.07)	.46	0.01 (−0.15 to 0.18)	.86
Systolic BP, mm Hg	0.03 (−0.38 to 0.43)	.89	0.07 (−0.34 to 0.49)	.74	−0.02 (−0.45 to 0.41)	.93	0.02 (−0.39 to 0.43)	.93	−0.38 (−0.94 to 0.19)	.19
Diastolic BP, mm Hg	0.10 (−0.14 to 0.35)	.39	0.14 (−0.11 to 0.39)	.27	−0.19 (−0.45 to 0.07)	.15	−0.04 (−0.28 to 0.21)	.77	−0.09 (−0.43 to 0.24)	.58
Cholesterol, mg/dL	0.18 (−0.81 to 1.18)	.72	0.00 (−1.03 to 1.02)	.99	−0.78 (−1.84 to 0.29)	.15	1.24 (0.23 to 2.25)	.02	6.27 (4.88 to 7.66)	1.0×10^{-18}
Triglycerides, mg/dL	−0.34 (−2.85 to 2.18)	.79	−2.21 (−4.80 to 0.39)	.10	0.12 (−2.57 to 2.82)	.93	−0.78 (−3.33 to 1.77)	.55	11.05 (7.54 to 14.56)	7.0×10^{-10}
HDL cholesterol, mg/dL	0.15 (−0.14 to 0.44)	.30	−0.10 (−0.40 to 0.20)	.52	−0.01 (−0.32 to 0.30)	.94	0.30 (0.01 to 0.59)	.05	−1.17 (−1.57 to −0.76)	1.6×10^{-8}
LDL cholesterol, mg/dL	0.14 (−0.69 to 0.97)	.75	0.41 (−0.45 to 1.27)	.35	−0.65 (−1.54 to 0.24)	.15	1.22 (0.38 to 2.07)	.004	5.77 (4.60 to 6.93)	3.5×10^{-22}
Diabetes mellitus	1.05 (0.97 to 1.13)	.26	0.94 (0.86 to 1.02)	.12	1.03 (0.94 to 1.12)	.53	1.05 (0.97 to 1.14)	.21	1.01 (0.90 to 1.13)	.91

Abbreviations: BP, blood pressure; CRP, C-reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SNP, single-nucleotide polymorphism.
[a] Data presented as unit change (continuous traits) or odds ratio (diabetes mellitus) and 95% confidence interval per copy of minor allele.
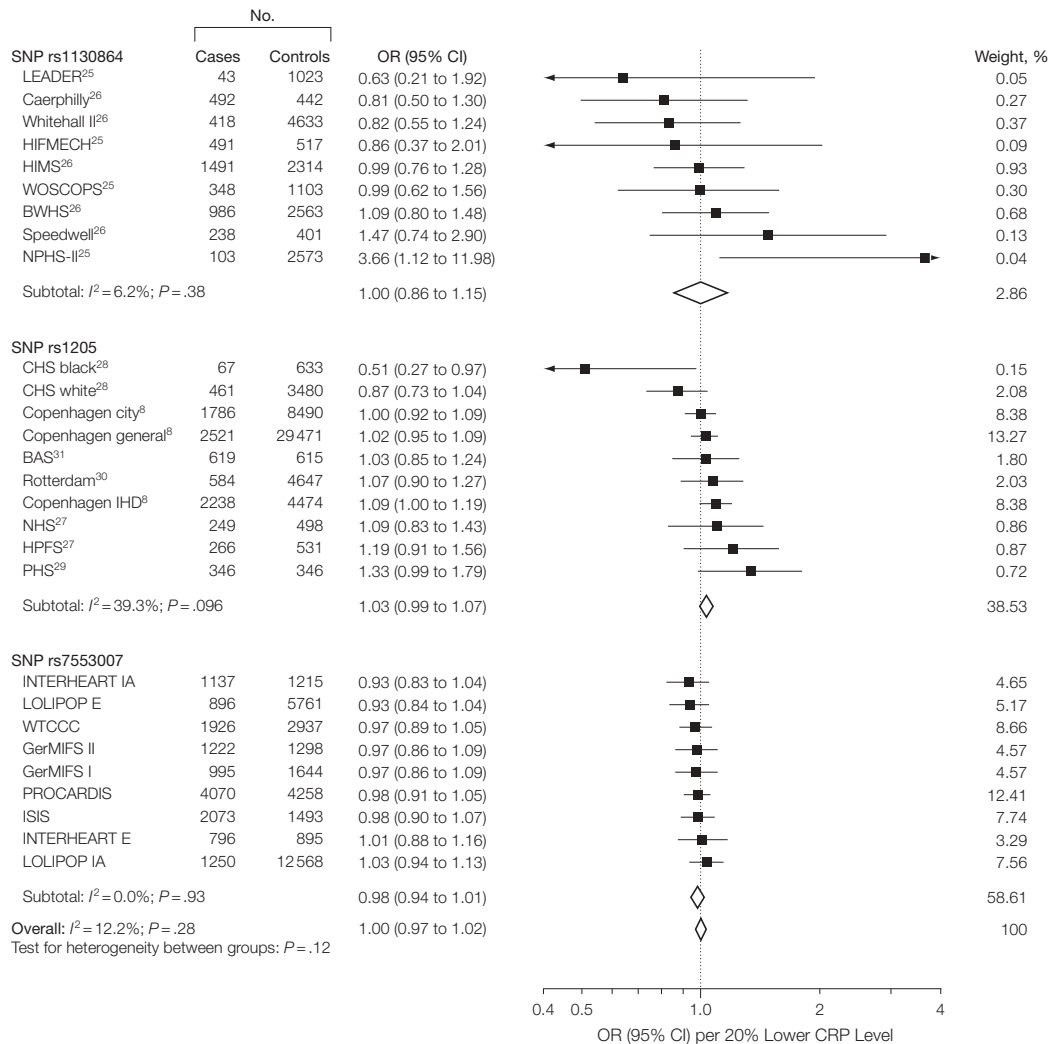
Estimated ORs for *CRP* variants on CHD were converted to a common scale by standardizing to 20% lower CRP, ie, the approximate effect per minor allele of rs7553007 on CRP levels. Results were combined across studies by SNP and across the 3 SNPs weighted by the inverse of variance. We used 95% CIs and assessed heterogeneity with standard χ^2 statistics, expressed as I^2, the proportion of variability between studies due to heterogeneity.[33] In the absence of heterogeneity, we used a fixed-effects model.

We also investigated the 3-way associations between *CRP* genetic variants, CRP levels, and CHD in prospective studies that reported all 3 sets of data in the same cohorts.[25-27,30] We did not include retrospective (case-control) studies since these could be biased by treatment effects.[34]

Comparison of the Result of the Mendelian Randomization Study With Meta-analysis of the CRP-CHD Relationship. We compared the result from our mendelian randomization study with that predicted from a meta-analysis of the obser-vational studies of CRP levels and CHD.[4,5,8,26,35-61] This was obtained from a systematic review of the CRP-CHD relationship published by Shah et al,[5] updated with all studies published from August 2007 until November 2008. For the search, the MeSH terms *C-reactive protein* and *CRP* in combination with *coronary*, *coronary heart disease*, *CHD*, and *CVD* were used, and the search was limited by the terms *human* and *English language*. Studies in which total mortality was the only outcome reported were excluded; if more than 1 article was published on the same cohort

Figure 2. Results of Mendelian Randomization Experiment of rs7553007 in the *CRP* Locus (Present Study) With rs1130864 and rs1205 From Published Studies

Numbers totaled 28 112 cases with coronary heart disease and 100 823 controls. Effects are given as odds ratios (ORs) and 95% confidence intervals (CIs) per 20% lower C-reactive protein (CRP) level. E indicates European; IA, Indian Asian; SNP, single-nucleotide polymorphism.

Figure 3. Associations of SNPs rs1130864 and rs1205 With CRP Levels, CRP Levels With CHD, and SNPs With CHD

A Associations of rs1130864 and rs1205 with CRP levels

SNP rs1130864	No. Cases	Controls	% Change (95% CI)	Weight, %
Speedwell[26]	238	401	−14.1 (−35.9 to 15.2)	4.61
HIMS[26]	1491	2314	−13.5 (−22.7 to −3.3)	32.28
BWHS[26]	986	2563	−12.7 (−23.0 to −0.9)	24.73
NPHS-II[25]	103	2573	−12.3 (−23.7 to 0.9)	19.87
Caerphilly[26]	492	442	−8.8 (−25.5 to 11.8)	8.70
WOSCOPS cases[25]	348		−6.9 (−31.8 to 26.9)	3.51
WOSCOPS controls[25]		1103	−1.3 (−20.8 to 23.0)	6.30
Subtotal: I^2=0.0%; P=.97			−11.7 (−17.2 to −6.2)	100
SNP rs1205				
NHS[27]	249	498	−24.0 (−35.5 to −10.5)	10.48
HPFS[27]	266	531	−22.0 (−32.7 to −9.6)	12.15
Rotterdam[30]	584	4647	−14.0 (−18.5 to −9.3)	77.37
Subtotal: I^2=39.6%; P=.19			−16.1 (−20.1 to −12.0)	100

% Change in CRP Level per Allele Copy

B Associations of CRP levels with CHD

SNP rs1130864	No. Cases	Controls	OR (95% CI)	Weight, %
NPHS-II[25]	103	2573	0.90 (0.84 to 0.96)	3.67
Speedwell[26]	238	401	0.95 (0.91 to 0.99)	7.70
BWHS[26]	986	2563	0.96 (0.93 to 0.99)	13.89
Caerphilly[26]	492	442	0.96 (0.93 to 1.00)	8.45
WOSCOPS[25]	348	1103	0.97 (0.93 to 1.00)	9.44
HIMS[26]	1491	2314	0.98 (0.96 to 1.00)	42.98
Subtotal: I^2=32.7%; P=.19			0.97 (0.95 to 0.98)	86.12
SNP rs1205				
HPFS[27]	266	531	0.92 (0.87 to 0.97)	5.79
NHS[27]	249	498	0.96 (0.91 to 1.02)	4.58
Rotterdam[30]	584	4647	0.98 (0.92 to 1.05)	3.50
Subtotal: I^2=19.7%; P=.29			0.95 (0.92 to 0.98)	13.88
Overall: I^2=26.7%; P=.21			0.96 (0.95 to 0.98)	100
Test for heterogeneity between groups: P=.32				

OR (95% CI) per 20% Lower CRP Level

C Associations of rs1130864 and rs1205 with CHD

SNP rs1130864	No. Cases	Controls	OR (95% CI)	Weight, %
Caerphilly[26]	492	442	0.81 (0.50 to 1.30)	4.47
HIMS[26]	1491	2314	0.99 (0.76 to 1.28)	15.25
WOSCOPS[25]	348	1103	0.99 (0.62 to 1.56)	4.84
BWHS[26]	986	2563	1.09 (0.80 to 1.48)	11.05
Speedwell[26]	238	401	1.47 (0.74 to 2.90)	2.20
NPHS-II[25]	103	2573	3.66 (1.12 to 11.98)	0.73
Subtotal: I^2=25.1%; P=.25			1.04 (0.88 to 1.22)	38.53
SNP rs1205				
Rotterdam[30]	584	4647	1.07 (0.90 to 1.27)	33.20
NHS[27]	249	498	1.09 (0.83 to 1.43)	14.08
HPFS[27]	266	531	1.19 (0.91 to 1.56)	14.19
Subtotal: I^2=0.0%; P=.79			1.10 (0.97 to 1.25)	61.47
Overall: I^2=0.0%; P=.49			1.08 (0.97 to 1.19)	100
Test for heterogeneity between groups: P=.59				

OR (95% CI) per 20% Lower CRP Level

Prospective studies were included that reported all 3 analyses. CHD indicates coronary heart disease; CI, confidence interval; CRP, C-reactive protein; OR, odds ratio; SNPs, single-nucleotide polymorphisms.

or population, the most recent one was used in the meta-analysis.

Five new population studies were identified from 4 reports.[8,26,35,36] Risk ratios for CHD associated with CRP levels (logarithmically transformed) were extracted from each study. Two studies were excluded: one[62] that cited risk ratios per unit increase in CRP (ie, not logarithmically transformed) and one[63] that did not provide CIs for the association of CRP with CHD. The studies reported risk ratios based on different comparisons of CRP (tertiles, quintiles, or quartiles) or as differences in risk for a given increase in CRP level; these were converted to a common unit of 1 SD change.

The risk ratio per standard deviation change was converted to per 20% lower CRP by multiplying coefficients (and 95% CIs) on the logarithmic scale by −0.223, assuming that a 20% reduction in CRP is equivalent to a 0.223 SD reduction in log CRP.

Multivariate-adjusted risk ratios, controlled for conventional cardiovascular risk factors, were used when available; 2 studies reported risk ratios adjusted for age only and age and smoking only.[37,38] Because of significant heterogeneity, random-effects meta-analysis was used to combine the risk ratios from the individual studies. The overall OR for CHD was used to test the observed vs predicted association of CRP

variants with CHD, standardized to 20% lower CRP level.

Genetic Association of Variants Outside the *CRP* Locus and CHD. The relationships with risk of CHD of the most associated SNP in the 4 genetic loci other than the *CRP* locus in our data were analyzed by logistic regression under an additive genetic model. Results were combined across studies by SNP using a fixed-effects model weighted by the inverse of variance because there was no significant heterogeneity. By use of the Genetic Power Calculator,[64] we estimated that the genetic association study with CHD had 80% power to detect an OR of 1.04 per allele copy at $P < .05$ and MAF 0.3.

Figure 4. Meta-analysis of the Relationship of CRP Levels With CHD From Prospective Observational Studies

Study	Type	Cases	Controls	OR (95% CI)		Weight, %
HHS[37]	NCC	241	241	0.88 (0.83 to 0.94)		2.27
NPHS-II[5]	C	162	2317	0.90 (0.84 to 0.96)		1.87
Hisayama Study[36]	C	129	2460	0.90 (0.84 to 0.96)		1.97
Heinz Nixdorf women[40]	C	62	2148	0.90 (0.84 to 0.97)		1.69
PHS[41]	NCC	543	543	0.90 (0.86 to 0.94)		3.07
Zoetermeer[38]	C	31	372	0.90 (0.82 to 1.00)		0.94
MRFIT[42]	NCC	148	296	0.90 (0.85 to 0.97)		1.92
BRHS[43]	NCC	329	820	0.91 (0.86 to 0.96)		2.36
WHS[44]	C	464	15281	0.91 (0.87 to 0.95)		3.55
Copenhagen City Heart Study[8]	NCC	1786	8490	0.91 (0.88 to 0.94)		3.98
PRIME[45]	NCC	317	609	0.91 (0.86 to 0.97)		2.02
HPFS[46]	NCC	265	529	0.92 (0.87 to 0.97)		2.54
MONICA[47]	CC	382	1980	0.93 (0.89 to 0.97)		3.04
Edinburgh Artery Study[5]	C	147	815	0.93 (0.88 to 0.98)		2.64
WHI-OS[48]	NCC	304	304	0.93 (0.87 to 0.99)		1.92
Fletcher Challenge Study[49]	NCC	220	411	0.93 (0.87 to 1.00)		1.72
Busselton Health Study[35]	CC	253	441	0.94 (0.89 to 1.00)		2.33
EPIC-N[50]	NCC	1108	2164	0.95 (0.93 to 0.97)		5.02
Belstress[51]	NCC	446	892	0.95 (0.91 to 0.99)		3.21
PROSPER[52]	C	865	4815	0.95 (0.93 to 0.98)		4.34
Caerphilly and Speedwell[53]	C	351	2714	0.95 (0.92 to 0.99)		3.72
ARIC (87-93)[54]	NCC	242		0.95 (0.88 to 1.03)		1.33
Honolulu[55]	NCC	369	1348	0.96 (0.92 to 0.99)		3.90
Reykjavik[4]	NCC	2459	3969	0.96 (0.94 to 0.97)		5.72
CHS[56]	C	547	3424	0.96 (0.93 to 0.99)		4.45
BWHS[26]	C	136	3413	0.96 (0.93 to 0.99)		4.01
NHS[46]	NCC	239	469	0.96 (0.91 to 1.02)		2.18
WOSCOPS[57]	NCC	580	1160	0.97 (0.93 to 1.00)		3.36
Hoorn[58]	C	24	586	0.97 (0.87 to 1.08)		0.77
FHS[59]	C	160	4286	0.98 (0.93 to 1.02)		2.67
ARIC (90-95)[54]	NCC	373		0.98 (0.91 to 1.05)		1.60
HIMS[26]	C	631	3174	0.98 (0.96 to 1.00)		5.56
Rotterdam[60]	NCC	157	500	0.98 (0.92 to 1.05)		1.80
Heinz Nixdorf men[40]	C	218	1918	0.99 (0.95 to 1.03)		3.42
QCS[61]	C	210	1772	1.00 (0.96 to 1.04)		3.13
Overall: $I^2 = 49.4\%$; $P = .001$				0.94 (0.94 to 0.95)		100

OR (95% CI) per 20% Lower CRP Level

A random-effects estimate is presented; the fixed-effects estimate has an odds ratio (OR) of 0.95 (95% confidence interval [CI], 0.94-0.96). Effects are given as ORs and 95% CIs per 20% lower C-reactive protein (CRP) level. For cohort studies, number of "controls" represents the number of event-free individuals. Number of controls was not available for ARIC.[54] CHD indicates coronary heart disease; C, cohort; CC, case-cohort; NCC, nested case-control.

With the exception of the genome-wide association analyses described here, statistical analyses were done with Stata version 10 (StataCorp, College Station, Texas) or SAS version 9.1 (SAS Institute Inc, Cary, North Carolina). A significance level of $P < .05$ was used; all tests were 2-sided.

Ethics approval was obtained locally for each of the participating cohorts for analyses of genetic markers of cardiovascular disease risk. No additional ethics approvals were required for this study.

RESULTS
Genome-Wide Association and Replication Study for CRP

We found 160 SNPs to be associated with CRP levels at $P < 5 \times 10^{-8}$, distributed in the following 5 loci: *LEPR* (GeneID 3953, GenBank NC_000001.9, region 65658906 to 65875410), *IL6R* (GeneID 3570, GenBank NC_000001.9, region 152644293 to 152706812), *CRP* (GeneID 1401, GenBank NC_000001.9, region 157951003 to 157948703), *HNF1A* (GeneID 6927, GenBank

NC_000012.10, region 119900932 to 119924698) and *APOE-CI-CII* (GeneID 348, 341, 344, GenBank NC_000019.8, region 50100879 to 50104490). A Manhattan plot of results from the combined analysis of genome-wide association data is shown in FIGURE 1. Genomic context and *P* values for the most associated SNP at each of the 5 loci are shown in TABLE 1. The association of these SNPs with CRP levels was confirmed in replication testing (all $P \leq 10^{-10}$); for all 5 SNPs, the minor al-

Figure 5. Associations of SNPs in *LEPR* (rs6700896), *IL6R* (rs4537545), and *HNF1A* (rs1183910) Loci and *APOE-CI-CII* (rs4420638) Cluster in the Genetic Association Study With CHD
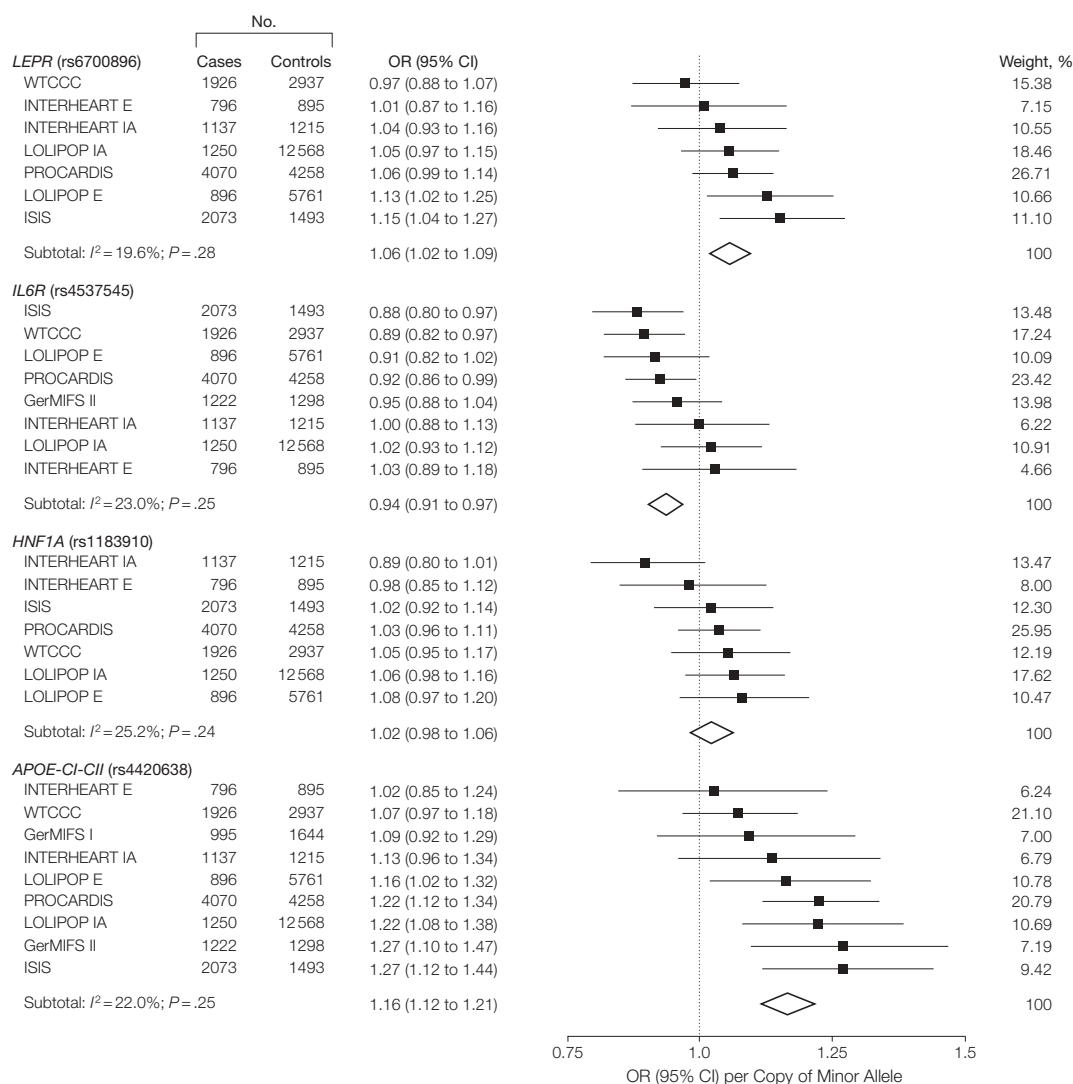
Effects are given as odds ratios (ORs) and 95% confidence intervals (CIs) per copy of minor allele. CHD indicates coronary heart disease; E, European; IA, Indian Asian; SNPs, single-nucleotide polymorphisms.

lele was associated with reduced levels of CRP (Table 1). For the most associated SNP in the *CRP* locus (rs7553007), CRP levels were lower by 21% (95% CI, −23.4% to −17.9%) per minor allele. For the other 4 SNPs, per-minor-allele differences in CRP levels ranged from −11.5% (95% CI, −14.4% to −8.5%) for SNP rs4537545 in *IL6R* to −21.8% (95% CI, −25.3% to −18.1%) for SNP rs4420638 in the *APOE-CI-CII* cluster (Table 1).

In the replication study (LOLIPOP), the percent variance of CRP explained ranged from 0.2% (SNP rs1183910 in *HNF1A*) to 1.3% (rs7553007 in the *CRP* locus). SNP rs4420638 in the *APOE-CI-CII* cluster was strongly associated with total cholesterol (6.27 mg/dL; 95% CI, 4.88 to 7.66 mg/dL; per minor allele), low-density lipoprotein (LDL) cholesterol (5.77 mg/dL; 95% CI, 4.60 to 6.93 mg/dL; per minor allele), triglycerides (11.05 mg/dL; 95% CI, 7.54 to 14.56 mg/dL; per minor allele) and high-density lipoprotein (HDL) cholesterol (−1.17 mg/dL; 95% CI, −1.57 to −0.76 mg/dL; per minor allele) (**TABLE 2**). SNP rs1183910 in *HNF1A* was associated with total cholesterol (1.24 mg/dL; 95% CI, 0.23 to 2.25 mg/dL; per minor allele), LDL cholesterol (1.22 mg/dL; 95% CI, 0.38 to 2.07 mg/dL; per minor allele), and HDL cholesterol (0.30 mg/dL; 95% CI, 0.01 to 0.59 mg/dL; per minor allele). (To convert total, LDL, and HDL cholesterol to mmol/L, multiply by 0.0259; to convert triglycerides to mmol/L, multiply by 0.0113.) SNP rs6700896 in *LEPR* was associated with weight (−0.44 kg; 95% CI, −0.78 to −0.10 kg; per minor allele) and body mass index (−0.12; 95% CI, −0.23 to −0.00; per minor allele; body mass index is calculated as weight in kilograms divided by height in meters squared). The remaining 2 SNPs were not significantly related to any of the phenotypes tested (Table 2).

Mendelian Randomization Study of Genetic Variants in the *CRP* Locus and CHD

FIGURE 2 shows the results of the mendelian randomization experiment of CHD with variants in the *CRP* locus: SNP rs7553007 (our data) and pub-

lished data for SNPs rs1130864 and rs1205 for 18 cohorts. SNP rs7553007 was not significantly associated with CHD; the estimated OR was 0.98 (95% CI, 0.94 to 1.01) per 20% lower CRP level. For rs1130864, the OR was 1.00 (95% CI, 0.86 to 1.15), and for rs1205, the OR was 1.03 (95% CI, 0.99 to 1.07). There was no association of CHD with *CRP* variants (per 20% lower CRP level) when results for all 3 SNPs were combined (OR, 1.00; 95% CI, 0.97 to 1.02) (Figure 2).

In the 3-way comparison of *CRP* genetic variants, CRP levels, and CHD risk reported in prospective studies, there was significant association of *CRP* variants with CRP levels, and CRP levels with CHD, but not *CRP* variants with CHD (**FIGURE 3**).

Comparison of the Result of the Mendelian Randomization Study With Meta-analysis of the CRP-CHD Relationship

The meta-analysis of observational studies of CRP levels and CHD gave a predicted OR of 0.94 (95% CI, 0.94 to 0.95) per 20% lower CRP level (**FIGURE 4**). This is significantly different from the estimated effect on CHD (OR, 1.00) of genetically determined differences in CRP levels obtained from our mendelian randomization study ($z = -3.45$, $P < .001$).

Genetic Association of Variants Outside the *CRP* Locus and CHD

Minor alleles of SNP rs6700896 in *LEPR* and rs4420638 in *APOE-CI-CII* cluster were associated with significantly increased risk of CHD (OR, 1.06; 95% CI, 1.02 to 1.09; and OR, 1.16; 95% CI, 1.12 to 1.21; respectively), and SNP rs4537545 in *IL6R* with a decreased risk of CHD (OR, 0.94; 95% CI, 0.91 to 0.97), while SNP rs1183910 in *HNF1A* was not significantly associated with CHD (**FIGURE 5**). The effects of SNPs rs6700896 in *LEPR* and rs4420638 in the *APOE-CI-CII* cluster on CHD were in the opposite direction to that predicted from the relationship of CRP levels with CHD (Figure 4); for rs4420638 the finding was consistent with the ef-

fects on blood lipid concentrations (Table 2).

COMMENT

The present genome-wide association study confirms the associations of common genetic variants in the *LEPR*, *IL6R*, *CRP*, and *HNF1A* loci and *APOE-CI-CII* cluster with CRP levels.[65,66] However, the minor allele of SNP rs7553007 and other variants in the *CRP* locus included in our mendelian randomization study were not associated with CHD risk.

The variants included in our mendelian randomization study are associated with approximately 20% lower CRP levels,[32] corresponding to a 6% reduction in CHD risk predicted by the meta-analysis of observational studies of CHD risk. The lack of association with CHD of genetic variants in the *CRP* locus suggests that the observational data linking CRP levels and CHD may be confounded by association with other CHD risk factors, or reflect a secondary inflammatory response associated with atherosclerosis (reverse causation), rather than indicate a causal relationship.

Our analysis of SNP rs7553007 with CHD risk includes more cases than all previously published studies of *CRP* variants and CHD combined,[8,25-31] yielding with the 2 published SNPs a total of more than 28 000 cases and 100 000 controls for the mendelian randomization study. The largest previous study, of 3 cohorts in Copenhagen, included 6545 cases.[8] In addition to rs1205 included in our mendelian randomization study, it provided data on the triallelic SNP rs3091244 and SNP rs3093077, both having variants with larger effects on CRP levels (20%-30%) than rs1205; however, both variants are rare, and neither was associated with CHD in the Copenhagen study.[8]

The JUPITER trial recently reported a benefit of treatment with rosuvastatin on CHD risk among men and women with elevated *CRP* levels (2.0 mg/L or higher) and with LDL cholesterol levels below 130 mg/dL.[34] In the treatment group, there was 54% reduction in rates of MI compared with placebo; LDL levels were reduced by 50% and CRP levels by 37%. Although the JUPITER trial demonstrated the benefits

of statin therapy in people with LDL levels below current treatment threshold, the results may simply reflect the benefits of lipid-lowering therapy in people who would not currently be considered for pharmacotherapy, rather than the benefits of CRP lowering per se.[67]

Investigation of genetic variants underlying an intermediate phenotype (such as CRP) has been advocated as a means of discovering new disease susceptibility loci.[10] In our study, minor alleles of SNPs rs6700896 in *LEPR* and rs4420638 in the *APOE-CI-CII* cluster showed significantly increased risk of CHD. However, both variants were associated with reduced levels of CRP (and for SNP rs6700896 in *LEPR*, lower body weight and body mass index), suggesting that the links with CHD are not mediated by CRP. *LEPR* has not previously been reported to increase risk of CHD, although associations of variants in the *APOE-CI-CII* cluster with CHD have been observed.[68,69] While the association of genetic variation in *APOE-CI-CII* with CHD can be explained in large part by its effects on blood lipids, this is not the case for SNP rs6700896 in *LEPR*. SNP rs6700896 is located in intron 19 of *LEPR*, the gene encoding the leptin receptor, a member of the class I cytokine receptor family.[70] *LEPR* is expressed in the hypothalamus and vascular endothelial cells,[71] and LEPR signaling has a role in appetite control, weight regulation, glucose homeostasis, blood pressure regulation, and angiogenesis.[70-72] The minor allele of SNP rs4537545 in *IL6R* was also associated with reduced CRP levels and reduced risk of CHD. SNP rs4537545 in *IL6R* is in high linkage disequilibrium ($r^2 = 0.96$ in the HapMap CEU reference population) with a nonsynonymous SNP (rs8192284, Asp358Ala) associated with increased *IL6R* expression and alterations of IL6R membrane binding,[39] providing a potential mechanism linking rs4537545 to biological function. Further studies will be needed to confirm *LEPR* and *IL6R* as new susceptibility loci for CHD.

Our study has limitations. Because of the relatively small effect of common genetic variants in *CRP* locus on CRP levels, a large sample size is needed to detect associations with CHD. To combine data across studies in our mendelian randomization study, we assumed a common effect on risk of CHD from different variants in the *CRP* locus by standardizing to a common difference on the log CRP scale. This approach is valid to the extent that the hypothesized effect of *CRP* variants on CHD risk reflects circulating levels of CRP. Although we found no association of *CRP* variants with CHD risk, it is not possible to exclude a small effect, despite the large sample size. However, we can effectively exclude the size of association predicted from observational data on the relationship of CRP levels to CHD. The mendelian randomization approach makes a number of assumptions concerning possible causality. These include the potential for pleiotropic effects of the genetic variants under study (or variants in high linkage disequilibrium with them) giving an alternative pathway to CHD or confounding through associations with disease risk factors.[9] Neither rs7553007 or the other 2 variants included from the *CRP* locus correlate with CHD risk factors,[8,26] satisfying an important condition for a valid mendelian randomization experiment.[9]

In summary, our mendelian randomization study of more than 28 000 cases and 100 000 controls found no association of variants in the *CRP* locus and CHD, arguing against a causal role for CRP in atherosclerosis. Moreover, this study suggests that development of therapeutic strategies targeting specific reductions in plasma levels of CRP are unlikely to be fruitful.

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