C-reactive protein gene haplotypes and risk of coronary heart disease: the Rotterdam Study

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Aims C-reactive protein is associated with risk of cardiovascular disease. However, whether C-reactive protein is a marker of severity of cardiovascular disease or actually is involved in its pathogenesis remains unknown. We investigated the relation between C-reactive protein haplotypes, representing the comprehensive variation of the C-reactive protein gene, and coronary heart disease.

Methods and results The Rotterdam Study is a prospective population-based study among men and women aged 55 years and older. C-reactive protein was associated with risk of coronary heart disease, with a multivariable adjusted hazard ratio of 1.9 (95% CI 1.5–2.4) for the highest vs. the lowest quartile. Four C-reactive protein haplotypes were present with overall frequencies of 32.8, 31.7, 29.5, and 5.9%. C-reactive protein serum levels were significantly different according to C-reactive protein haplotypes. C-reactive protein haplotypes were not associated with coronary heart disease.

Conclusion Steady-state C-reactive protein serum level is influenced by C-reactive protein gene haplotypes. Although elevated C-reactive protein level has lately been found to be a consistent and relatively strong risk factor for cardiovascular disease, our study does not support that the common variation in the C-reactive protein gene has a large effect on the occurrence of coronary heart disease.

KEYWORDS Coronary heart disease; Inflammation; C-reactive protein; Genetics; Epidemiology

Introduction

C-reactive protein is associated with cardiovascular disease.1 However, whether C-reactive protein is merely a marker of severity of cardiovascular disease or actually is involved in its pathogenesis remains unknown. Genetic markers offer a possibility to study this.

Evidence has emerged that C-reactive protein may play a pathogenic role in cardiovascular disease.2 If this is true, genetic variants associated with high C-reactive protein level may be associated with greater risk of cardiovascular disease. The genes involved in this regulation remain ill-defined. The C-reactive protein gene is likely to play a part, as several studies have demonstrated associations between single nucleotide polymorphisms (SNPs) in the C-reactive protein gene and C-reactive protein level.3–13 Two recent studies have identified comprehensive sets of common C-reactive protein gene haplotypes and found associations of these haplotypes with C-reactive protein level.14,15 One of these studies has also examined the association between these haplotypes and myocardial infarction or ischaemic stroke in a nested case–control study within the Physicians’ Health Study cohort,15 but the association between C-reactive protein variants and baseline C-reactive protein did not correlate with the effects of those variants on clinical cardiovascular events in this study.

Seattle SNPs (part of the National Heart Lung and Blood Institute’s Programs for Genomic Applications) reports that four C-reactive protein gene haplotypes are present in populations of European descent. These haplotypes represent all common variation across the C-reactive protein gene in these populations. To further clarify the role of C-reactive protein in coronary heart disease, we set out to investigate the relation between these four C-reactive protein gene haplotypes, C-reactive protein serum level, and coronary heart disease prospectively in all participants of the large, population-based Rotterdam Study.

Participants and methods

Study population and baseline data collection

The present study is part of the Rotterdam Study, a population-based cohort study aimed at assessing the occurrence of and risk factors for chronic diseases in the elderly. Objectives and methods of the Rotterdam Study have been described in detail elsewhere.16 The Rotterdam Study cohort includes 7983 men and women aged 55 years and over (78% of the eligible population), living in a
well-defined suburb of the city of Rotterdam, The Netherlands. The Medical Ethics Committee of Erasmus Medical Center, Rotterdam, approved the study. Participants gave written informed consent and permission to retrieve information from treating physicians. This study complies with the Declaration of Helsinki.

Baseline data were collected from 1990 until 1993, as described previously. A trained interviewer visited all participants at home and collected information on current health status, medical history, drug use, and smoking, using a computerized questionnaire. In addition, in 7129 participants, established cardiovascular risk factors were measured at the research center.

Measurement of C-reactive protein
At baseline, non-fasting blood was collected. All tubes were stored on ice before and after blood sampling. High-sensitivity C-reactive protein was determined in serum, which was stored at $-20^\circ$C until performance of the C-reactive protein measurements in 2003–04. C-reactive protein was measured using Rate Near Infrared Particle Immunoassay (Immage$^\text{TM}$ Immunochemistry System, Beckman Coulter, USA). This system measures concentrations ranging from 0.2 to 1440 mg/L, with a within-run precision $\pm 5.0\%$, a total precision $\pm 7.5\%$, and a reliability coefficient of 0.995.

Genotyping
The Seattle SNPs Program for Genomic Applications has identified 31 SNPs in the C-reactive protein gene and has established that, based on SNPs with overall frequencies above 5%, four common C-reactive protein gene haplotypes are present in 23 unrelated individuals of European descent from the CEPH pedigrees (http://pga.gs.washington.edu/data/crp, 'visual haplotype' option). These four haplotypes can be identified by 'haplotype tagging' SNPs. By genotyping three haplotype tagging SNPs we were able to infer all four haplotypes and consequently to describe the common variation across the C-reactive protein gene (Figure 1). These three tagging SNPs were chosen partly based on their presence in existing literature and on their proximity to the C-reactive protein gene. Other SNPs were also eligible, as a range of SNP trios across the C-reactive protein gene captures the four most common haplotypes among European participants.

All participants were genotyped for the 1184 C>T, 2042 C>T, and 2911 C>G SNPs of the C-reactive protein gene. The polymorphisms are described in relation to the start of the coding sequence of exon 1 using the Human Map 2004 (hg 17) assembly (http://genome.ucsc.edu). These polymorphisms have also been described at http://www.ncbi.nlm.nih.gov/SNP under identification numbers rs1130864 (1184 C>T), rs1205 (2042 C>T), and rs3093068 (2911 C>G).

DNA was extracted according to standard procedures. DNA was solubilized in double-distilled water and stored at $-20^\circ$C until used for DNA amplification. Genotypes were determined in 2 ng genomic DNA with the Taqman allelic discrimination assay (Applied Biosystems, Foster City, CA, USA). Primer and probe sequences were optimized using the SNP assay-by-design service of Applied Biosystems (http://store.appliedbiosystems.com). Reactions were performed with the Taqman Prism 7900HT 384 wells format. Haplotype alleles present in the population were inferred by means of the haplo.em function of the program Haplo Stats (http://cran.r-project.org/src/contrib/Descriptions/haplo.stats.html), which computes maximum likelihood estimates of haplotype probabilities. Haplotype reconstruction resulted in seven haplotypes, but the fifth, sixth, and seventh haplotypes were present in $<0.001\%$ of the alleles and were therefore not used in the analyses. Haplotype alleles were coded as haplotype numbers 1 through 4 in the order of decreasing frequency in the population: coding from 1184 C>T, 2042 C>T, and 2911 C>G, haplotype 1 = C-T-C, 2 = T-C-C, 3 = C-C-C, and 4 = C-C-G (Figure 1).

![Figure 1](https://academic.oup.com/eurheartj/article/27/11/1331/457016/data/2711153/1457016)
Follow-up procedure

Follow-up started at the baseline examination and for the present study lasted until January 1, 2002. Information on fatal and nonfatal cardiovascular endpoints was obtained from general practitioners (GPs) and letters and discharge reports from medical specialists. Two research physicians independently coded all reported events according to the International Classification of Diseases, 10th edition (ICD-10). In case of disagreement, consensus was reached. A medical expert in cardiovascular disease, whose judgment was considered final, reviewed all events.

We defined incident coronary heart disease as myocardial infarction, coronary artery bypass grafting (CABG), percutaneous transluminal coronary angioplasty (PTCA), and cardiac death. In identifying myocardial infarctions, all available information, which included ECG, cardiac enzyme levels, and the clinical judgment of the treating specialist, was used. We defined cardiac death as death from myocardial infarction or other ischaemic heart disease (ICD-10: I20–I25), sudden cardiac death (I46), sudden death undefined (R96), or death from heart failure (I50).

Population for analysis

C-reactive protein serum levels were available for 6658 participants. C-reactive protein measurements were lacking for participants who did not visit the research center (854) and for participants of whom no blood was available due to logistic reasons (471). After excluding participants with coronary heart disease at baseline (870), defined as a history of myocardial infarction, PTCA, or CABG, 5788 participants were left for the analysis of the association between C-reactive protein serum levels and coronary events.

DNA was available for 6571 participants. Genotyping of all three polymorphisms was successful in 6007 participants. For 584 of these participants, C-reactive protein serum levels were available. After excluding participants with coronary heart disease at baseline, 5231 participants were left for the analysis of the association between C-reactive protein haplotypes and coronary events.

Data analysis

Linear regression was used to investigate the association between C-reactive protein serum levels and established cardiovascular risk factors. After log-transformation of C-reactive protein, the residuals were normally distributed with a constant variance.

Subsequently, participants with coronary heart disease at baseline were excluded, and Cox proportional hazards analysis was used to determine the relative risks of coronary heart disease and myocardial infarction associated with increasing C-reactive protein quartiles (cut-points 0.9, 1.8, and 3.5 mg/L). The proportional hazards assumption was tested by drawing log minus log plots of the survival function. We adjusted for age and sex (model 1), and subsequently for age, sex, body mass index (BMI), systolic blood pressure, diastolic blood pressure, total cholesterol, HDL-cholesterol, smoking, and diabetes mellitus (model 2).

Hardy-Weinberg equilibrium of the three C-reactive protein gene polymorphisms was tested using a $\chi^2$ test. Differences in serum C-reactive protein levels (log-transformed) and established cardiovascular risk factors for the three polymorphisms were examined using analysis of covariance, adjusting for age and sex, categorizing the participants by their genotypes. We used the Bonferroni correction to account for multiple testing (three genotypes). All the above analyses were performed using SPSS 11.0 for Windows.

To test the associations of C-reactive protein gene haplotypes with cardiovascular risk factors, we used the program Haplo Stats http://cran.r-project.org/src/contrib/Descriptions/haplo.stats.html. The probability for each haplotype pair in each individual was assigned and then an individual’s phenotype was directly modelled as a function of each inferred haplotype pair, weighed by their estimated probability, to account for haplotype ambiguity. The haplo.score function of Haplo Stats was used to test the associations. Details on the background and theory of score statistics can be found in Schaid et al. We adjusted for age and sex and we computed global simulation P-values and simulation P-values for each haplotype. The number of simulations was set as 1000.

As haplo.score does not provide the magnitude of the effect of each haplotype, the association between C-reactive protein gene haplotypes and C-reactive protein serum level, coronary heart disease and myocardial infarction was investigated using the haplo.glm function of Haplo Stats. This approach is based on a generalized linear model, and computes the regression of a trait on haplotypes and other covariates. For the analysis regarding the disease outcomes, the haplotype that was found to be associated with the lowest serum C-reactive protein levels served as the reference category. First, we adjusted for age and sex, and second, we additionally adjusted for BMI, systolic blood pressure, diastolic blood pressure, total cholesterol, HDL-cholesterol, smoking, and diabetes mellitus. Haplo.em, haplo.score, and haplo.glm were all implemented in the Haplo Stats software using the R language.

Values for cardiovascular covariates were missing in <4% of participants. These missing values were handled by single imputation using the expectation-maximization algorithm in SPSS 11.0. All tests were two-sided.

Results

Table 1 shows baseline characteristics of the total cohort and their associations with C-reactive protein serum level. All studied characteristics, except for total cholesterol, were significantly associated with C-reactive protein serum level.

The mean follow-up time was 8.1 years (SD 3.0 years). Among participants without history of coronary heart disease, 584 (8.8%) participants experienced incident coronary heart disease during follow-up, including 224 myocardial infarctions. Hazard ratios for coronary heart disease and myocardial infarction increased significantly across quartiles of C-reactive protein (Table 2). When we repeated the analysis without excluding participants with coronary heart disease at baseline, the results did not change materially.

Genotype distributions for the three haplotype tagging SNPs were in Hardy-Weinberg equilibrium. Both using the Seattle SNPs website and the HapMap website (http://www.hapmap.org), the SNPs were found to lie in one linkage disequilibrium block. The 1184 T allele was present in 31.6% of 12 014 chromosomes, the 2042 T allele in 32.7%, and the 2911 G allele in 6.0%. Figure 2 shows differences in serum C-reactive protein levels according to the genotypes of the three C-reactive protein polymorphisms. For all three polymorphisms we observed an allele dose effect. No associations were present between genotypes and established cardiovascular risk factors (see online supplementary material, webtables 1, 2, and 3). Genotypes were not associated with coronary heart disease and myocardial infarction (see online supplementary material, webtable 4).

Haplotype alleles were present in the following frequencies: haplotype 1 (C-T-C) in 32.8%, haplotype 2 (T-C-C) in 31.7%, haplotype 3 (C-C-C) in 29.5%, haplotype 4 (C-C-G) in 5.9%, and remaining haplotypes (T-C-G, T-T-C and C-T-G) in less than 0.001%.

Haplotype 4 was associated with lower BMI ($P = 0.03$), haplotype 1 with higher systolic blood pressure ($P = 0.04$), and haplotype 3 with higher percentage of prevalent myocardial infarction ($P = 0.04$). No other associations
with cardiovascular risk factors were present (see online supplementary material, webtable 5). All haplotypes provided significantly higher C-reactive protein levels than haplotype 1 (see online supplementary material, webtable 6). The effect of haplotype on C-reactive protein level, calculated from the regression coefficients, is displayed in Figure 3. Additional adjustment for BMI, systolic blood pressure, diastolic blood pressure, total cholesterol, HDL-cholesterol, smoking, and diabetes mellitus did not materially change the results.

In Table 3, age- and sex-adjusted odds ratios (OR) for coronary heart disease and for myocardial infarction are displayed for different C-reactive protein haplotypes. As haplotype 1 was associated with the lowest serum C-reactive protein levels, it served as the reference. For both outcomes, the OR for all haplotypes were around one. Additional adjustment for BMI, systolic blood pressure, diastolic blood pressure, total cholesterol, HDL-cholesterol, smoking, and diabetes mellitus did not materially change the point estimates, and neither did repeating the analysis without excluding participants with coronary heart disease at baseline.

**Discussion**

In this population-based study, elevated C-reactive protein serum level was a strong and independent marker of increased risk of coronary heart disease and myocardial infarction in participants without a history of coronary heart disease. C-reactive protein haplotypes were associated with C-reactive protein serum levels. However, C-reactive protein haplotypes were not associated with coronary heart disease and myocardial infarction. The approach we used in this study has also been termed 'Mendelian randomization'. This approach has been used

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**Table 1** Baseline characteristics of the population and age- and sex-adjusted regression coefficients for cardiovascular risk factors, describing the increase in log-C-reactive protein per unit increase in each risk factor

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total (n = 6658)</th>
<th>Regression coefficient (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)a</td>
<td>69.6 ± 9.2</td>
<td>0.020 (0.017, 0.022)</td>
<td>10^{-45}</td>
</tr>
<tr>
<td>Womenb</td>
<td>3970 (60%)</td>
<td>-0.137 (-0.188, -0.086)</td>
<td>10^{-7}</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.3 ± 3.7</td>
<td>0.065 (0.058, 0.071)</td>
<td>10^{-79}</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>139 ± 22</td>
<td>0.005 (0.004, 0.006)</td>
<td>10^{-16}</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>74 ± 12</td>
<td>0.003 (0.002, 0.006)</td>
<td>10^{-4}</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>6.6 ± 1.2</td>
<td>0.006 (-0.015, 0.027)</td>
<td>0.6</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.3 ± 0.4</td>
<td>-0.470 (-0.540, -0.399)</td>
<td>10^{-38}</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>704 (11%)</td>
<td>0.321 (0.239, 0.402)</td>
<td>10^{-14}</td>
</tr>
</tbody>
</table>

* Adjusted for sex.
* Adjusted for age.

**Table 2** Hazard ratios for coronary heart disease and myocardial infarction for quartiles of C-reactive protein in participants without history of coronary heart disease at baseline

<table>
<thead>
<tr>
<th></th>
<th>Events/participants</th>
<th>Hazard ratio (95% CI)</th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Coronary heart disease</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 1 (≤0.9)</td>
<td>92/1450</td>
<td>1.0 (reference)</td>
<td>1.0 (reference)</td>
<td></td>
</tr>
<tr>
<td>Quartile 2 (&gt;0.9–1.8)</td>
<td>133/1450</td>
<td>1.4 (1.1–1.8)</td>
<td>1.3 (1.0–1.7)</td>
<td></td>
</tr>
<tr>
<td>Quartile 3 (&gt;1.8–3.5)</td>
<td>158/1446</td>
<td>1.7 (1.3–2.1)</td>
<td>1.5 (1.1–1.9)</td>
<td></td>
</tr>
<tr>
<td>Quartile 4 (&gt;3.5)</td>
<td>201/1442</td>
<td>2.2 (1.7–2.8)</td>
<td>1.9 (1.5–2.4)</td>
<td></td>
</tr>
<tr>
<td>P for trend</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Myocardial infarction</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 1 (≤0.9)</td>
<td>32/1450</td>
<td>1.0 (reference)</td>
<td>1.0 (reference)</td>
<td></td>
</tr>
<tr>
<td>Quartile 2 (&gt;0.9–1.8)</td>
<td>62/1450</td>
<td>2.0 (1.3–3.0)</td>
<td>1.8 (1.1–2.7)</td>
<td></td>
</tr>
<tr>
<td>Quartile 3 (&gt;1.8–3.5)</td>
<td>67/1446</td>
<td>2.2 (1.4–3.3)</td>
<td>1.8 (1.2–2.8)</td>
<td></td>
</tr>
<tr>
<td>Quartile 4 (&gt;3.5)</td>
<td>63/1442</td>
<td>2.1 (1.4–3.2)</td>
<td>1.7 (1.1–2.7)</td>
<td></td>
</tr>
<tr>
<td>P for trend</td>
<td>0.07</td>
<td>0.20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Model 1: adjusted for age and sex.
Model 2: adjusted for age, sex, BMI, systolic blood pressure, diastolic blood pressure, total cholesterol, HDL-cholesterol, smoking, and diabetes mellitus.
recently in studies of C-reactive protein, hypertension, and metabolic syndrome.\(^8,\text{22}\) It deals with residual confounding, as alleles of the C-reactive protein gene that influence C-reactive protein level are transmitted from parent to offspring at random, and factors that could confound associations of C-reactive protein level with cardiovascular disease should be evenly distributed in those who do, and those who do not, have alleles that cause high C-reactive protein level. Furthermore, it deals with reverse causation, because genotype is determined before onset of disease.\(^23\)

The present study uses haplotypes describing the total common variation of the C-reactive protein gene. So far, two other studies have used this approach. First, Carlson \textit{et al.}\(^{14}\) defined all common genetic variation across the C-reactive protein gene region by resequencing the region in a multiethnic variation discovery panel (24 African-Americans and 23 European-Americans), and selected SNPs for genotyping in a larger panel (CARDIA study), in which associations between common haplotypes and C-reactive protein levels were investigated. Carlson \textit{et al.} used a population that was partly of European descent and partly of African descent, and used all haplotypes that occur in these populations. We used a population of European descent; therefore, the studies are not strictly comparable. In approximation, Carlson \textit{et al.}’s haplotypes 4, 5, and 7 concur with our haplotypes 3, 2, and 4, respectively, and Carlson \textit{et al.}’s haplotype 1 and 2 taken together concur with our haplotype 1. Remaining haplotypes in the Carlson \textit{et al.} study were only present in African-Americans. Carlson \textit{et al.} found that their haplotypes 5 and 7 were associated with high C-reactive protein levels, haplotype 1 and 2 with the lowest levels, and haplotype 4 with intermediate levels. These results are in agreement with ours. Furthermore, Carlson \textit{et al.} did a promoter transcriptional analysis of the C-reactive protein gene, which suggested

![Figure 2](https://academic.oup.com/eurheartj/article-lookup/10.1093/eurheartj/ehs088)

**Figure 2** Geometric means and 95% CI of serum levels of C-reactive protein (mg/L) for three C-reactive protein gene polymorphisms. *P*-value < 10\(^{-3}\).

![Figure 3](https://academic.oup.com/eurheartj/article-lookup/10.1093/eurheartj/ehs088)

**Figure 3** Relative effects of C-reactive protein gene haplotypes on C-reactive protein serum levels (mg/L). *P*-value < 10\(^{-3}\). Regression coefficients were estimated with haplo.glm for each haplotype, adjusted for age and sex. Coefficients reflect difference in mean ln(C-reactive protein) per copy relative to haplotype 1, the most frequent haplotype. Values were transformed back to normal scale. Haplotypes 2–4 were all significantly higher than haplotype 1.

<table>
<thead>
<tr>
<th>OR (95% CI)</th>
<th>Coronary heart disease</th>
<th>Myocardial infarction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haplotype 1 (C-T-C)</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>Haplotype 2 (T-C-C)</td>
<td>0.93 (0.79–1.10)</td>
<td>0.98 (0.76–1.25)</td>
</tr>
<tr>
<td>Haplotype 3 (C-C-C)</td>
<td>1.00 (0.85–1.17)</td>
<td>1.16 (0.91–1.48)</td>
</tr>
<tr>
<td>Haplotype 4 (C-C-G)</td>
<td>0.84 (0.63–1.13)</td>
<td>1.02 (0.67–1.56)</td>
</tr>
</tbody>
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![Table 3](https://academic.oup.com/eurheartj/article-lookup/10.1093/eurheartj/ehs088)

**Table 3** Age- and sex-adjusted OR for coronary heart disease and myocardial infarction for C-reactive protein haplotypes in participants without history of coronary heart disease.
that the C-reactive protein haplotype–phenotype associations are at least partially attributable to functional changes at promoter sites rs3093062 (SNP 1421 in their paper) and rs3091244 (SNP 1440 in their paper).

Secondly, Miller et al.15 resequenced 192 individuals to ascertain a comprehensive set of common variants in the C-reactive protein gene, studied their association with C-reactive protein level in (subsets of) three cohorts, and also studied their association with myocardial infarction or ischaemic stroke in a nested case–control study within the Physicians’ Health Study cohort. Interestingly, after resequencing this large number of individuals, Miller et al. found a haplotype pattern similar to the pattern of Seattle SNPs. Miller et al.’s haplotypes 1, 2, and 5 concur with haplotypes 3, 2, and 4 in our study, respectively. There were only two minor differences: Miller et al.’s haplotypes 3 and 4 together constitute our haplotype 1, and we did not determine Miller et al.’s haplotype 6, but the mean frequency of this haplotype was only 2.1%.15 Miller et al.’s haplotypes 2 and 5 were associated with higher C-reactive protein levels and haplotypes 3 and 4 with lower C-reactive protein levels; these results are again in agreement with ours. Also, Miller et al. found that the minor allele of SNP rs2794521 was associated with reduced risk of atherothrombotic events. However, this SNP was associated with higher C-reactive protein level, so the association between the C-reactive protein variant and baseline C-reactive protein did not correlate with the effect of this variant on clinical cardiovascular events. We did not determine this SNP in our study. Our study has the advantage that we had data available on all cases and non-cases in a large, population-based cohort.

Remaining studies on C-reactive protein gene haplotypes and risk of cardiovascular events have mostly been conducted in smaller numbers of high-risk patients.24–26 The haplotypes used in these studies have been constructed without consideration of the patterns of variation across the locus as a whole. Remaining studies that have examined the association of C-reactive protein gene haplotypes with C-reactive protein levels6,7,12,26,27 are not comparable to our study, because of different polymorphisms used to reconstruct the haplotypes and different populations used in terms of health status, ethnicity, or age. The results of these studies are diverse, some finding associations with C-reactive protein levels, and some not. Interesting to note is the finding of Szalai et al.,27 that haplotypes reconstructed from the −409G/A (rs3093032) and −390C/T/A (rs3091244) C-reactive protein gene promoter polymorphisms affect transcription factor binding, alter transcriptional activity, and associate with differences in baseline serum C-reactive protein level. According to Seattle SNPs, the latter polymorphism is present in all participants with haplotypes 2 and 4 in our study, and therefore it may result in functional differences between the haplotypes in our study, leading to different serum C-reactive protein levels. Several, mostly smaller, studies have demonstrated associations between various C-reactive protein SNPs and C-reactive protein levels.3–11 These studies were different in design and were conducted in various populations, and are therefore not similar to ours.

In this study, we found an independent association between serum C-reactive protein levels and coronary heart disease. Since our earlier report on the role of C-reactive protein in the prediction of myocardial infarction in the Rotterdam Study, which was then investigated by means of a nested case–control study,28 data from four more years of follow-up have become available and C-reactive protein has been determined in the total cohort. This may in part explain the difference with the previous results that showed a lack of association after multivariable adjustment.

An issue that warrants consideration in this study is that C-reactive protein measurements were lacking for 854 participants who did not visit the research center at baseline. These participants generally had a higher age and worse general health. However, the association that we found between C-reactive protein serum level and coronary heart disease is in line with the results from previous studies,3 and this suggests that although we cannot entirely exclude the presence of selection bias, it is not likely that it has substantially influenced the results.

Although serum C-reactive protein levels were found to influence risk of coronary heart disease and C-reactive protein gene haplotypes were found to influence steady-state serum C-reactive protein levels, no association could be demonstrated between C-reactive protein haplotypes and coronary heart disease. Power calculation for the present study shows that, with a power of 80% and an alpha of 0.05, in reference to haplotype 1, (the most common haplotype, frequency 32.8%), we were able to demonstrate relative risks for coronary heart disease of at least 1.21 (for haplotype 2, frequency 31.7%).29 Therefore, either there indeed is no association between C-reactive protein gene haplotypes and coronary heart disease, or, otherwise, the relative risk is of relatively small magnitude. Application of the instrumental variables approach22 to our data is in compliance with the latter; the expected relative risks of coronary heart disease for haplotypes 2, 3, and 4, as estimated from the association between C-reactive protein serum level with coronary heart disease and the association between haplotypes and C-reactive protein serum level were 1.03 (95% CI 0.90–1.20), 1.02 (95% CI 0.88–1.18), and 1.05 (95% CI 0.82–1.36), respectively, as compared with haplotype 1. We were not able to demonstrate estimates of such small magnitude in the present study. Therefore, the door may still be open for a pathophysiological role of C-reactive protein in the development of cardiovascular disease.

Another explanation of the absence of an association in the present study is that baseline C-reactive protein levels are not solely determined by the variation in the C-reactive protein gene, but also by its interaction with several transcription factors induced by regulatory cytokines such as IL-6 and IL-1β which may have a larger influence on serum C-reactive protein levels. Furthermore, high C-reactive protein levels may exert their harmful effects in the acute phase of a coronary event, with high peak C-reactive protein levels leading to enhanced infarct size and more complications such as arrhythmias.31 As high C-reactive protein responders may not necessarily have high baseline serum C-reactive protein levels, this aspect needs to be studied by means of a study design different from ours.

In conclusion, this study confirms that steady-state C-reactive protein serum level is predictive of coronary heart disease. Furthermore, it demonstrates that
steady-state C-reactive protein serum level is influenced by C-reactive protein haplotypes. Although elevated C-reactive protein level has lately been found to be a consistent and relatively strong risk factor for cardiovascular disease, our study does not support that the common variation in the C-reactive protein gene has a large effect on the occurrence of coronary heart disease.

Supplementary material

Supplementary material is available at European Heart Journal online.

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References


