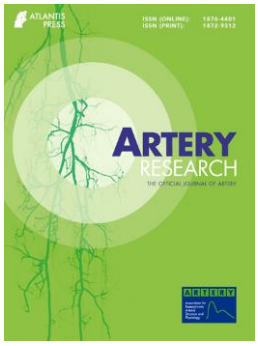

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# Genetic variation in the C-reactive protein gene and arterial stiffness: The Rotterdam Study

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**KEYWORDS**
C-reactive protein;
Polymorphism;
Arterial stiffness;
Pulse wave velocity;
Distensibility coefficient;
Pulse pressure

**Summary** *Background and aim:* Arterial stiffness increases with age and has been found to predict cardiovascular disease. C-reactive protein (CRP) is an inflammation marker and has been found to be associated with arterial stiffness and risk of cardiovascular disease. Genetic factors account for part of the variance in CRP level. We studied the association of the total common variation in the CRP gene by polymorphisms 1184 C/T, 2042 C/T, 2911 C/G and haplotypes with arterial stiffness within the Rotterdam study.
*Methods:* The study ($n = 3615$) was embedded in the Rotterdam Study, a prospective, population-based study among subjects aged 55 years and older. Associations of genotypes and haplotypes with CRP level and measures of arterial stiffness were examined using linear regression and analyses of variance. Measures of arterial stiffness included aortic pulse wave velocity, carotid distensibility and pulse pressure. Analyses were adjusted for age, sex, mean arterial pressure, heart rate, known cardiovascular risk factors and measures of atherosclerosis.
*Results:* CRP level was significantly associated with pulse wave velocity ($p < 0.001$) and pulse pressure ($p < 0.05$), also after adjusting for cardiovascular risk factors. CRP level was also associated with the 1184 C/T (T-allele: higher level), the 2042 C/T (T-allele: lower level) and 2911 C/G (G-allele: higher level) polymorphisms (all $p < 0.001$). Genotype and haplotype

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analyses showed no consistent associations of genetic variation with pulse wave velocity, carotid distensibility and pulse pressure.
*Conclusions:* No consistent associations of the CRP polymorphisms 1184 C/T, 2042 C/T, 2911 C/G and corresponding haplotypes were found with measures of arterial stiffness.


## Introduction

Arterial stiffness increases with age and has been associated with hypertension, diabetes mellitus, end-stage renal disease and atherosclerosis.[1–6] Arterial stiffness has been found to predict cardiovascular events in various populations.[7–10] The extent of the increase in stiffness may also depend on genetic variation.

Arterial stiffness has been found to be associated with inflammatory markers such as C-reactive protein (CRP).[11–16] However, it is not clear whether the association is causal, or whether the effect is mediated via atherosclerosis. After all, inflammation has been established as a marker of atherosclerosis and the atherosclerotic process itself affects arterial stiffness. Study of genetic variation in the inflammatory pathway in relation to arterial stiffness may provide more insight into the underlying mechanisms. Heritability estimates for levels of CRP vary from 27% up to 40%, which suggests genetic variation to have an effect on bioavailability of CRP.[17,18] If genetic variation in CRP can be found to be related to arterial stiffness, this would support evidence for a causal role of CRP in the loss of elasticity of the vessel wall.

Generally a significant association implies only a correlation between variation in a gene and an outcome; it does not enable us to draw conclusions on potential causal relations.[19] However, if variation in the CRP gene specifically affects levels, then differences in levels have effectively been allocated at random at the moment of conception, i.e. ''Mendelian randomization''.[20] An association between this genetic variation and an outcome would then provide convincing evidence for a causal relation between a risk factor and the outcome.

The CRP gene is located on chromosome 1 (1q21–q23). Seattle SNPs (part of the National Heart Lung and Blood Institute's Programs for Genomic Applications) reports that four CRP gene haplotypes are present in populations of European descent. These haplotypes represent all common variation across the CRP gene in these populations and can be inferred from three tagging polymorphisms, 1184 C/T, 2042 C/T and 2911 C/G.

In order to clarify the role of genetic variation in the CRP gene in the development of arterial stiffness, we set out to investigate CRP polymorphisms 1184 C/T, 2042 C/T and 2911 C/G and concurrent haplotypes in relation to arterial stiffness within the Rotterdam Study.

## Methods

The Rotterdam Study is an ongoing prospective cohort study including 7983 participants of 55 years and older. Its general aims are to investigate determinants of chronic diseases, including cardiovascular disease, dementia and osteoporosis.[21] During the first phase of this study (1990–1993), all inhabitants of a Rotterdam suburban area (Ommoord), aged 55 years and over, were invited to participate in this study. The response rate was 78%. The third examination phase took place from 1997 to 1999, during which measurements of arterial stiffness were performed. Approval of the Medical Ethics Committee of the Erasmus University Rotterdam was obtained for the Rotterdam Study. From all participants written informed consent was acquired. A more in-depth description of the design of the Rotterdam Study was published previously.[21]

### Measurement of CRP levels

Serum levels of CRP were determined in blood samples obtained during the third examination phase of the Rotterdam Study and stored at −80 °C. High-sensitivity CRP measurements were performed using rate near-infrared particle immunoassay (Immage Immunochemistry System; Beckman Coulter, San Diego, CA).

### Genotyping and haplotyping

The Seattle SNPs Program for Genomic Applications has identified 31 SNPs in the CRP gene and has established that, based on SNPs with overall frequencies above 5%, four common CRP gene haplotypes are present in 23 unrelated individuals of European descent from the CEPH pedigrees (http://pga.gs.washington.edu/data/crp). These four haplotypes are identified by ''haplotype tagging'' SNPs. By genotyping three haplotype tagging SNPs we were able to infer all four haplotypes and consequently to describe the total common variation across the CRP gene. These three tagging SNPs were chosen partly based on their presence in existing literature and on their proximity to the CRP gene. Genotyping of the CRP 1184 C/T, 2042 C/T and 2911 C/G polymorphisms [also described in relation to the start of the coding sequence of exon 1 using the Human May 2004 (hg 17) assembly (http://genome.ucsc.edu), and also at http://www.ncbi.nlm.nih.gov/projects/SNP/ under identification numbers rs1130864 (1184C/T), rs1205 (2042C/T) and rs3093068 (2911C/G)] was performed using samples stored earlier at −80 °C. DNA was isolated using standard procedures. Genotypes were determined in 2-ng genomic DNA with the Taqman allelic discrimination assay (Applied Biosystems, Foster City, CA). Primer and probe sequences were optimized by using the SNP assay-by-design service of Applied Biosystems (for details, see http://store.appliedbiosystems.com). Reactions were performed with the Taqman Prism 7900HT 384 wells format in 2 μL reaction volume.

Haplotypes were estimated using PHASE software (http://archimedes.well.ox.ac.uk).[22] Haplotypes with a frequency of <0.001% were not used in the analyses. The remaining four haplotypes were coded from 1 to 4 in decreasing order of their population frequency (coding from 1184C/T, 2042C/T and 2911C/G, haplotype 1 = C-T-C, 2 = T-C-C, 3 = C-C-C and 4 = C-C-G) (Fig. 1).

## Arterial stiffness

In this study three measures of arterial stiffness were used: the carotid–femoral pulse wave velocity (PWV) as a measure of aortic stiffness and the distensibility coefficient (DC) of the common carotid artery as a measure of common carotid arterial stiffness. In addition, pulse pressure (PP) was assessed as an indicator of arterial stiffness. All measures were obtained on the same day, during the same session, during the third follow-up examination.

Carotid–femoral PWV (m/s) was measured using an automatic device (Complior, Colson) and was calculated as the ratio between the distance traveled by the pulse wave and the foot-to-foot time delay.

Common carotid artery distensibility was assessed by measuring the vessel wall motion of the right common carotid artery using a duplex scanner (ATL Ultramark IV, operating frequency 7.5 MHz) connected to a vessel wall movement detector system.[23,24] The cross-sectional arterial wall DC (1/MPa) was calculated as a measure of arterial stiffness.[25] A decreased DC implies increased carotid stiffness.

PP (mmHg) was defined as the difference between systolic and diastolic blood pressure, using the mean systolic and diastolic blood pressure of two measurements obtained by measuring blood pressure on the right arm using a random-zero sphygmomanometer.

Details on all measures of stiffness have been described previously.[6,7]

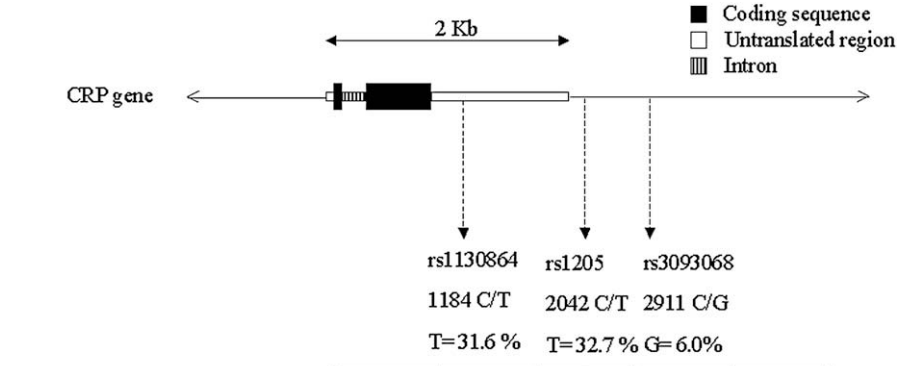
## Clinical characteristics

Information on cardiovascular risk factors was collected during the third follow-up examination. Data on drug use and smoking habits were obtained during the home interview.

Smoking was classified as never, former or current smoking. At the research center, blood pressure was measured twice on the right arm using a random-zero sphygmomanometer. The average of the two blood pressure values was used in the analyses. Length and weight were measured and body mass index (weight/height$^2$) (BMI) was calculated. Serum total cholesterol and high-density lipoprotein (HDL) cholesterol values were determined by an automated enzymatic procedure (Boehringer Mannheim System). Diabetes mellitus (DM) was defined as use of antidiabetic medication and/or a fasting serum glucose level of equal to or above 7.0 mmol/L.[26] Evaluation of the atherosclerotic status of participants was accomplished using ultrasonography (carotid intima-media thickness [IMT]) and radiography (aorta calcification); these methods have been extensively described previously.[27,28]

## Population for analysis

The Rotterdam Study comprises 7893 subjects, of which 6007 subjects were successfully and completely genotyped for the CRP polymorphisms. Generally 84% of the subjects participated in the third phase of the study, 14% refused to participate, 1.9% were not able to participate (not otherwise specified) and 0.2% had died. A total of 4024 subjects underwent the physical examination of the third phase. PWV was measured in 3550 subjects; 69 subjects (1.9%) were excluded from the analyses because of poor quality of the PWV recordings, leaving 3481 subjects (3023 successfully genotyped for all polymorphisms). Common carotid distensibility was measured in 3098 subjects (2672 successfully genotyped for all polymorphisms). PP measurements could




Haplotypes in population of European descent (n=6007)

| Haplotype | 1184 | 2042 | 2911 | frequency |
|---|---|---|---|---|
| 1 | C | T[a] | C | 32.8% |
| 2 | T[a] | C | C | 31.7% |
| 3 | C | C | C | 29.5% |
| 4 | C | C | G[a] | 5.9% |
| Others | | | | < 0.001% |

[a] Tagging SNP for that haplotype

**Figure 1** CRP gene and haplotypes. Reproduced with permission from the European Heart Journal (Kardys et al.[30]).

be determined for all subjects participating in the third phase (3601 successfully genotyped for all polymorphisms). For 2402 completely genotyped subjects, full data on PWV, the distensibility coefficient and pulse pressure were available. For 3615 completely genotyped subjects data were available on one or more measures of arterial stiffness. CRP levels were successfully measured during the third phase in 3824 subjects. Missing information on measures of arterial stiffness was almost entirely due to logistic reasons.

### Statistical analyses

Chi-square tests were performed to test for deviations from Hardy–Weinberg equilibrium. Missing data on clinical characteristics were imputed using Expectation-Maximization algorithms available in SPSS. For CRP measurements all values above mean plus three times the standard deviation were excluded, as correction for outliers. Natural-log transformed (ln-transformation) CRP levels were used to normalize the distribution of this variable. Haplotype analyses were allele-based: each haplotype allele is considered individually in the analyses. The most common haplotype allele is the reference (i.e., haplotype 1). Analyses on the associations of CRP levels and arterial stiffness were performed using linear regression. Analyses on genotypes and haplotypes in relation to CRP levels and arterial stiffness were performed using linear regression and analyses of variance. The analyses were adjusted for age and sex (and for PWV and DC also for mean arterial pressure [MAP] and heart rate), and additionally for systolic blood pressure (only when MAP was not in the model), BMI, HDL and total cholesterol levels, smoking, diabetes mellitus, and in the full model also for measures of atherosclerosis. A $p$-value of 0.05 and smaller was considered significant in all analyses. The statistical analyses were performed using SPSS version 11.0.1 for MS-Windows.

## Results

General characteristics of the subjects are described in Table 1. Genotype and allele proportions were in Hardy–Weinberg equilibrium. Haplotypes are described in Fig. 1.

### CRP level and arterial stiffness

The CRP level was positively associated with pulse wave velocity ($p < 0.001$), also after full adjustment (Table 2). The CRP level was inversely associated with the distensibility coefficient ($p < 0.001$), but only in the age- and sex-adjusted analyses. The CRP level was positively associated with pulse pressure ($p < 0.001$), which remained significant after additional adjustment for cardiovascular risk factors, but was borderline significant after additional adjustment for measures of atherosclerosis ($p = 0.06$).

### CRP level by genotype and haplotype

Serum CRP levels according to the genotype of the three CRP polymorphisms are shown in Fig. 2. For all three polymorphisms we observed an allele dose effect (all $p < 0.001$).

**Table 1** General characteristics of the study population.

| Characteristic | Overall | Imputed |
|---|---|---|
| Total number | 3615 | |
| Age, years | 72.4 ± 7.0 | – |
| Male sex, % | 43 | – |
| Body mass index, kg/m$^2$ | 27 | 2.1% |
| Systolic blood pressure, mmHg | 144 ± 21 | 2.5% |
| Diastolic blood pressure, mmHg | 75 ± 11 | 2.5% |
| Mean arterial pressure, mmHg | 107 ± 13 | – |
| Total cholesterol, mmol/L | 5.8 ± 1.0 | 2.0% |
| HDL-cholesterol, mmol/L | 1.4 ± 0.4 | 2.3% |
| Smoking | | 2.0% |
| % Current | 30 | |
| Former | 38 | |
| Never | 32 | |
| Diabetes, % | 9 | <0.1% |
| Pulse wave velocity[a], m/s | 13.6 ± 3.0 | – |
| Distensibility coefficient[b], 1/MPa | 10.4 ± 4.3 | – |
| Pulse pressure, mmHg | 68 ± 18 | – |
| Intima media thickness, mm | 0.77 ± 0.13 | 17.8% |
| CRP[c], mg/L | 2.4 (1.2–4.5) | – |

Continuous values are depicted as mean ± SD.
[a] Based on all participants with PWV information available ($n = 3023$).
[b] Based on all participants with DC information available ($n = 2672$).
[c] Median (interquartile range).

Differences in CRP level according to haplotypes are shown in Table 3. In comparison to the reference haplotype 1, the other haplotypes had a significantly higher level of CRP (all $p < 0.001$). Haplotypes 2, 3 and 4 all contained one or more of the alleles associated with an increased CRP level in the genotype analyses.

### CRP gene and pulse wave velocity

No significant associations between genotypes of the 1184 C/T or 2024 C/G polymorphisms and pulse wave velocity were found. Those with the 2911-GG genotype had a higher PWV compared with CG heterozygotes ($p = 0.03$), but no significant trend was found, $p = 0.66$. The haplotype-based analyses yielded no significant relations with arterial stiffness (Table 4).

### CRP gene and distensibility coefficient

Subjects with the 1184 TT genotype had a lower distensibility coefficient than heterozygotes ($p = 0.04$), also after adjustment for cardiovascular risk factors. The association, however, did not remain significant after additional adjustment for atherosclerosis. For polymorphisms 2042 C/G and 2911 C/T no significant differences in the distensibility coefficient between the genotypes were found. In the haplotype-based analyses no significant differences were found (Table 4).

### CRP gene and pulse pressure

No significant associations were found of 1184 C/T ($p = 0.38$), 2042 C/T ($p = 0.96$) or 2911 C/G ($p = 0.49$)

**Table 2** Relation between CRP level and arterial stiffness.

| | Pulse wave velocity ($n = 3252$) | | Distensibility coefficient ($n = 2876$) | | Pulse pressure ($n = 3721$) | |
|---|---|---|---|---|---|---|
| | $\beta$-coefficient (SE) | $p$ | $\beta$-coefficient (SE) | $p$ | $\beta$-coefficient (SE) | $p$ |
| Model 1 | 0.206 (0.04) | <0.001 | −0.222 (0.063) | <0.001 | 1.103 (0.279) | <0.001 |
| Model 2 | 0.168 (0.046) | <0.001 | −0.098 (0.067) | 0.141 | 0.809 (0.289) | 0.005 |
| Model 3 | 0.143 (0.045) | 0.002 | −0.081 (0.066) | 0.221 | 0.538 (0.283) | 0.058 |

$\beta$-coefficient: regression coefficient based on ln-transformed C-reactive protein levels.
Model 1: adjusted for age, sex (and for pulse wave velocity and the distensibility coefficient also for mean arterial pressure and heart rate).
Model 2: model 1 + BMI, total and HDL cholesterol, smoking, diabetes mellitus.
Model 3: model 2 + measures of atherosclerosis.

with pulse pressure. The haplotype analyses also yielded no significant associations (Table 4).

Overall, analyses specified by gender yielded no essentially different results (data not shown). Also, overall analyses using non-imputed data yielded no essentially different results (data not shown).

## Discussion

We studied the association of the CRP polymorphisms 1184 C/T, 2042 C/T, 2911 C/G and haplotypes with arterial stiffness within the Rotterdam study. We found the CRP level to be positively associated with pulse wave velocity and with pulse pressure, independent of cardiovascular risk factors. The minor alleles of the 1184 C/T and 2911 C/G polymorphisms were positively associated with the CRP level, and the minor allele of the 2042 C/T polymorphism inversely. Overall, no consistent significant differences between the genotypes and haplotypes of the CRP gene in pulse wave velocity, the distensibility coefficient or pulse pressure were found. The association between 1184 TT and the distensibility coefficient may very well be a type 1 error or due to multiple testing.

The CRP 1184 C/T, 2042 C/T and 2911 C/G polymorphisms were chosen because together, they represent

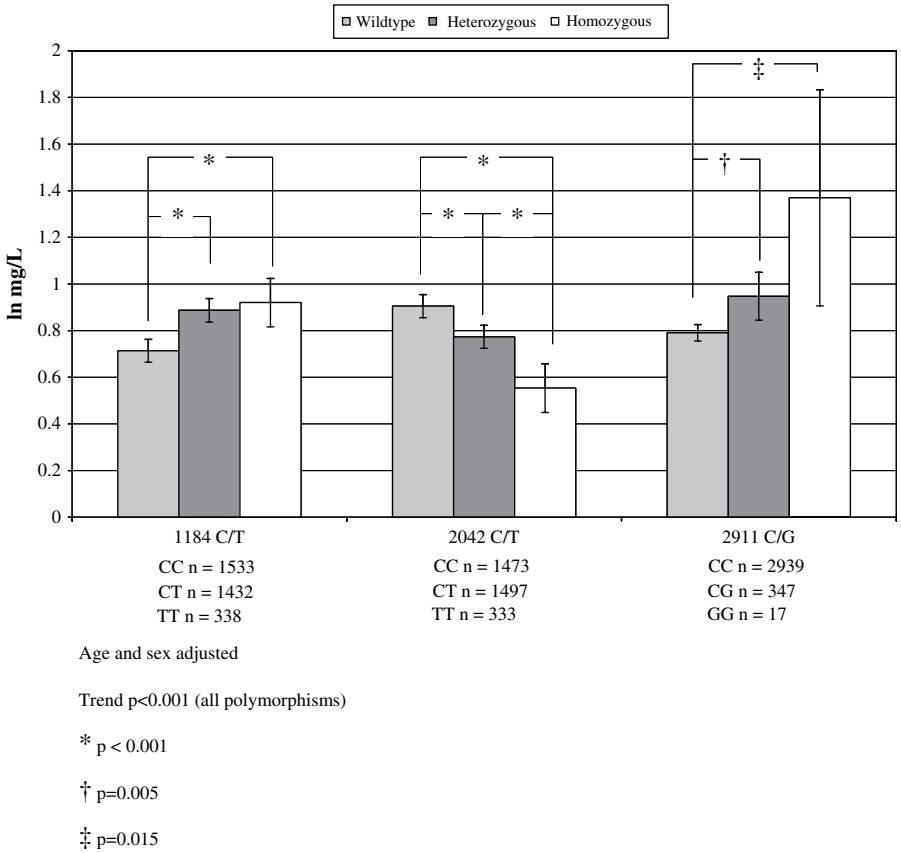



**Figure 2** CRP level by CRP genotype. Age- and sex-adjusted. Trend $p < 0.001$ (all polymorphisms). *$p < 0.001$; †$p = 0.005$; ‡$p = 0.015$.

**Table 3** CRP levels by haplotype of the CRP gene.

| Haplotype | 1184 | 2042 | 2911 | *n* | CRP Level (mg/L) | 95% CI |
|---|---|---|---|---|---|---|
| 1[a] | C | T | C | 1873 | 0.67 | 0.63–0.72 |
| 2 | T | C | C | 1809 | 0.87* | 0.83–0.91 |
| 3 | C | C | C | 1716 | 0.79* | 0.74–0.83 |
| 4 | C | C | G | 334 | 0.92* | 0.81–1.02 |

Based on ln-transformed CRP (C-reactive protein) levels. Age- and sex-adjusted.
*Significant difference in comparison with reference haplotype 1, all $p < 0.001$.
[a] Haplotype 1 is reference.

the total common genetic variation across the CRP gene. By genotyping these three haplotype tagging SNPs we were able to infer all four haplotypes, that describe the common variation across the CRP gene. Few studies have used this approach.[29–31] Miller et al. and Carlson et al. used more tagging SNPs than in our study to describe common variation in the CRP gene.[29,31] In these studies, however, ethnic diverse populations were used. For example, the population used in the study by Carlson et al. consisted of participants partly of European descent and partly of African descent. Therefore, more and other haplotypes were found and compared with the population of European descent used in our study. Both Miller et al. and Carlson et al. report associations between haplotypes and CRP levels that are in agreement with the associations between haplotypes and CRP levels in our study.[30]

While the studied genotypes and haplotypes were associated with CRP level, we found no consistent relation of the geno- and haplotypes of the CRP gene with arterial stiffness. Only 2911-GG homozygotes had a higher pulse wave velocity in comparison to heterozygotes. Although this is in line with the association of the polymorphism with an increased CRP level, one has to take into account that the number of homozygous subjects was limited; this finding may therefore have been a chance finding.

The absence of a relation between variation in the CRP gene with measures of arterial stiffness might suggest that the relation between the CRP level and arterial stiffness is not causal. The effect of variation in the CRP gene on CRP level, however, was only modest. Haplotypes 2, 3 and 4 were associated with a higher CRP level compared with haplotype 1. The differences, however, varied only from 0.12 to 0.25 mg/L. In general, effects of genetic variation on a trait with a complex and multifactorial pathogenesis, such as arterial stiffness, are generally modest. In that respect, our study is in line with current views on this topic.[32] Therefore, a small effect of genetic variation in CRP on arterial stiffness may have gone undetected and a judgment about causality cannot be given with certainty.

The association between the CRP polymorphisms/haplotypes and level of CRP was described earlier in the Rotterdam Study.[30] The same applies for the association between CRP level ($n = 866$) and pulse wave velocity.[14] These studies, however, were based on CRP levels obtained at baseline. In the current study, we have used CRP levels obtained during the third examination phase of the Rotterdam Study. Our results are in concordance with these earlier results.

Although we found no clear association of genetic variation in the CRP gene and arterial stiffness, an association between the CRP level and arterial stiffness is nonetheless biologically plausible and may be explained in several ways.[14] Increased CRP levels and impaired endothelial dysfunction have been found to be related before.[33–37] Fichtlscherer et al. described CRP to be associated with decreased endothelial vasodilator function.[37] CRP level is also described to be associated with various markers of endothelial dysfunction.[35,36] Inflammatory processes inhibit endothelium-dependent vasodilatation.[14,38] The

**Table 4** Measures of arterial stiffness by CRP haplotype.

| Model | Haplotype | Pulse wave velocity | | | | Distensibility coefficient | | | | Pulse pressure | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *n* | m/s | CI | *p* | *n* | 1/MPa | CI | *p* | *n* | mmHg | CI | *p* |
| 1 | 1 | 1961 | 13.57 | 13.46–13.68 | – | 1732 | 10.38 | 10.22–10.54 | – | 2346 | 68.3 | 67.6–69.0 | – |
| | 2 | 1903 | 13.67 | 13.56–13.78 | 0.22 | 1703 | 10.41 | 10.25–10.57 | 0.77 | 2285 | 68.5 | 67.8–69.2 | 0.71 |
| | 3 | 1817 | 13.55 | 13.44–13.67 | 0.82 | 1597 | 10.42 | 10.26–10.58 | 0.72 | 2142 | 67.9 | 67.2–68.6 | 0.46 |
| | 4 | 359 | 13.54 | 13.28–13.80 | 0.80 | 307 | 10.50 | 10.13–10.87 | 0.56 | 423 | 68.7 | 67.1–70.3 | 0.64 |
| 2 | 1 | 1961 | 13.57 | 13.46–13.68 | – | 1732 | 10.38 | 10.23–10.54 | – | 2346 | 68.3 | 67.6–68.9 | – |
| | 2 | 1903 | 13.66 | 13.55–13.77 | 0.25 | 1703 | 10.41 | 10.26–10.57 | 0.77 | 2285 | 68.5 | 67.8–69.1 | 0.68 |
| | 3 | 1817 | 13.56 | 13.45–13.67 | 0.87 | 1597 | 10.42 | 10.26–10.59 | 0.71 | 2142 | 67.9 | 67.2–68.6 | 0.47 |
| | 4 | 359 | 13.53 | 13.27–13.78 | 0.74 | 307 | 10.45 | 10.08–10.81 | 0.75 | 423 | 68.7 | 67.1–70.3 | 0.58 |
| 3 | 1 | 1961 | 13.57 | 13.47–13.68 | – | 1732 | 10.39 | 10.23–10.54 | – | 2346 | 68.2 | 67.6–68.9 | – |
| | 2 | 1903 | 13.67 | 13.56–13.78 | 0.21 | 1703 | 10.42 | 10.26–10.57 | 0.78 | 2285 | 68.5 | 67.8–69.2 | 0.57 |
| | 3 | 1817 | 13.55 | 13.44–13.66 | 0.76 | 1597 | 10.41 | 10.25–10.57 | 0.81 | 2142 | 67.9 | 67.2–68.6 | 0.49 |
| | 4 | 359 | 13.52 | 13.27–13.77 | 0.69 | 307 | 10.45 | 10.08–10.81 | 0.76 | 423 | 68.7 | 67.2–70.3 | 0.57 |

Model 1: age, sex (and for PWV and DC also for mean arterial pressure and heart rate).
Model 2: model 1 + body mass index, total + HDL-cholesterol, smoking, diabetes mellitus.
Model 3: model 2 + measures of atherosclerosis.

endothelium itself produces vasoactive substances, such as nitric oxide, which have been demonstrated to play a significant role in peripheral resistance, blood pressure and vascular reactivity.[39,40] Furthermore, agonists that stimulate endothelial nitric oxide release, such as acetylcholine, reduce stiffness of muscular arteries in vivo.[41,42] Basal nitric oxide production has been demonstrated to influence muscular arteries distensibility in vivo positively and the effect of acetylcholine on large arteries is also mainly nitric oxide-mediated.[43] Increased CRP levels decrease nitric oxide production.[34] Therefore, as endothelium obviously plays an important role in the regulation of arterial stiffness, CRP-related impairment of endothelial function may very well lead to changes in arterial stiffness.[14]

Our study is based on a large ongoing population-based study, in a relatively homogeneous population, as 98% of the participants in our study are Caucasian and are all living in the same area, a suburb of Rotterdam. We used both genotype- and haplotype-based analyses. We adjusted all analyses to establish risk factors and measures of atherosclerosis.

To interpret the findings correctly, several methodological aspects of the measures of arterial stiffness need to be discussed. First, pulse waves in the carotid artery and the femoral artery travel in opposite directions, while measurement of carotid—femoral pulse wave velocity is based on the assumption that the pulse wave travels from the carotid artery to the femoral artery. In this way, measuring the distance between the carotid and the femoral artery leads to an overestimation of the distance between the sites of the pulse waves, resulting in overestimation of the velocity of the pulse waves. However, variations in anatomy are limited and this error may be considered similar for all subjects examined, therefore we do not think it has seriously biased our results. Second, the distance between the carotid and the femoral artery may be overestimated in (especially obese) subjects when this distance is measured by tape. To avoid this error we adjusted the analyses for body mass index. Third, in computing the carotid distensibility coefficient, we used the brachial pulse pressure rather than the carotid pulse pressure. Information on comparisons between the carotid and the brachial pulse pressure indicates that the carotid pulse pressure is lower than the brachial pulse pressure, but differences are relatively small.[44]

Data on measures of stiffness were not available for all subjects who visited the research center. Missing information was primarily due to logistic reasons, which are likely to be random and thus will not have biased our results. Furthermore, the advantage of the allele-based haplotype analysis is the increase in power in comparison with genotype-based analyses. However, the allele-based haplotype analyses offer no information on possible dominance or recessiveness. In recent years several studies have highlighted that potential effects of commercially obtained CRP may be due to azide contamination of the CRP instead of CRP itself.[45,46] However, as we only use CRP from our participants and do not use commercially obtained CRP, we feel that this does not necessarily apply in full to our study. Finally, because our study was performed in a population of predominantly elderly Caucasian subjects, the generalizability of our findings to younger individuals or other ethnicities remains uncertain.

In conclusion, genetic variation in the CRP gene, as expressed by polymorphisms 1184 C/T, 2042 C/T and 2911 C/G and concurrent haplotypes, is not consistently and significantly associated with arterial stiffness. However, we cannot exclude a small effect, which was not detected in our study. Further and larger studies are needed for confirmation and to further elucidate the role of genetic variation in the CRP gene in arterial stiffness.

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