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Abstract—Circulating C-reactive protein (CRP) level is associated with cardiovascular disease. Whether this relationship is causal has been subject of debate, especially as a potential therapeutic target. Previous studies have demonstrated an association between circulating CRP levels and arterial pulse wave velocity, an accepted measure of arterial stiffness. We investigated the association between circulating CRP levels, *CRP* genotype, and aortic pulse wave velocity by examining data on 790 healthy male participants of the Caerphilly study. Circulating CRP levels were associated with aortic pulse wave velocity after adjustment for cardiovascular risk factors and other potential confounders ($P=0.001$). Three single nucleotide polymorphisms in the *CRP* gene (rs1130864, rs1800947, and rs1205) were associated with differences in circulating CRP levels (ratio of geometric means: 1.12, 95% CI 1.03 to 1.21, $P=0.005$; 0.76, 95% CI 0.66 to 0.87, $P<0.001$; 0.88, 95% CI 0.81 to 0.95, $P=0.001$, respectively). However, there was no relationship between any of the genotypes and aortic pulse wave velocity (regression coefficient for C:G/G:G versus C:C genotypes for single nucleotide polymorphism rs1800947 $\beta=0.005$; 95% CI, −0.57 to 0.58; $P=0.99$). These results suggest that although circulating CRP levels are associated with aortic pulse wave velocity, CRP does not have a causal role in the development of arterial stiffness. CRP may simply act as a marker of vascular damage (ie, reverse causality), or the association reflects residual confounding. Further studies are needed to confirm these findings, particularly in view of the central role CRP has played in cardiovascular disease so far. **(*Hypertension*. 2009;53:150-157.)**

Key Words: C-reactive protein ■ vascular resistance ■ atherosclerosis ■ risk factors ■ genotype

Atherosclerosis is increasingly thought of as an inflammatory process[1] in which the acute phase reactant C-reactive protein (CRP) may be an important contributor. Elevated CRP levels have been positively associated with incident cardiovascular disease,[2] and some studies suggest that CRP could be used as a novel predictor in risk assessment.

However, compared with classical risk factors for ischemic heart disease, CRP does not add greatly to current risk prediction models.[3,4] Thus, it remains unclear whether CRP is solely a surrogate marker of cardiovascular disease[5] or causally related to it. In particular, a causal relation would raise the question of whether high-risk persons should have interventions to lower their CRP levels[2] and whether CRP antagonists merit evaluation in acute ischemia.[6] Two studies[7,8] even report that statin therapy reduces circulating CRP levels and thereby causes a reduction in atherosclerotic disease progression and improved cardiovascular outcomes.

Under the assumption of a CRP effect being causal, one potential mechanism linking inflammation and CRP to cardiovascular disease is arterial stiffening. The gold standard measure of stiffness is aortic pulse wave velocity (aPWV), which can be measured noninvasively and has been shown to be associated with the presence of atherosclerotic plaques.[9] Numerous studies report an association between CRP and PWV,[10–21] but, in some, the association disappears or becomes borderline after adjustment for classical cardiovascular disease risk factors, such as cholesterol, smoking, and features of the metabolic syndrome.[11,12,22] Several studies have reported no association between CRP and PWV.[23,24]

Observational studies experience residual problems of poorly measured or unmeasured confounding and reverse causality when the disease process has an incipient onset and long latency period. One approach to overcome these problems is the use of Mendelian randomization, whereby functional genotypic variants serve as markers of exposure unrelated to other covariates and, hence, overcome issues with confounding and reverse causality.[25] Hence, if elevated circulating CRP is causally related to increased stiffness, one would expect a higher aPWV in those persons with genotypes that generate higher circulating CRP.

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Table 1. Baseline Characteristics of Participants

	Participants With aPWV Reading			Participants Without aPWV Reading		
Subject Characteristics	n	Mean or Percentage	SD/IQR	n	Mean or Percentage	SD/IQR
Age, y	790	55.5	4.2	1608	57.6‡	4.5
BMI, kg/m^2	782	26.4	3.2	1580	26.5*	3.8
Waist–hip ratio	763	0.9	0.1	1228	0.9‡	0.07
SBP, mm Hg	784	142.7	21.8	1582	148.1‡	22.9
DBP, mm Hg	784	84.2	11.3	1582	85.1*	12.6
aPWV, m/s	790	11.5	2.8			
CRP,§ mg/L	662	1.4	0.8, 2.5	1304	2.0‡	1.1, 3.9
Triglycerides,† mmol/L	770	1.7	1.2, 2.2	1532	1.7†	1.2, 2.4
Total cholesterol, mmol/L	770	5.7	1.0	1532	5.6*	1.0
HDL cholesterol, mmol/L	770	1.1	0.3	1532	1.0‡	0.25
Liver function tests						
γ-Glutamyltransferase,§ IU/L	762	24.7	17, 35	1507	27.1‡	18, 38
Total alkaline phosphatase,§ U/L	758	157.8	128, 200	1494	175.2‡	139, 220
Liver alkaline phosphatase,§ U/L	755	86.9	68, 107	1487	98.4‡	74, 129
Alcohol consumption,§ U/wk	749	7.7	2.3, 21.3	1478	7.0*	1.4, 18.2
Smoking status, %	787					
Nonsmokers	196	24.9%		235	14.6%	
Ex-smokers	341	43.3%		566	35.3%	
Cigar, pipe, or <15 cigarettes/day	151	19.2%		455	28.4%	
≥15 cigarettes/day	99	12.6%		349	21.7%	
Diabetic status	790					
No	775	98.1%		1532	95.3%	
Yes/uncertain	15	1.9%		75	4.7%	
Activity scores	789			790		
Leisure						
Mild	220	27.9		575	36.1	
Moderate	299	37.9		496	31.1	
Heavy	270	34.2		524	32.9	
Work						
Mild	262	33.2		557	33.4	
Moderate	371	47		747	44.8	
Heavy	157	19.9		362	21.7	
Social class	789					
Manual	471	59.7		1139	71.1	
Nonmanual	318	40.3		462	28.9	
Presence of IHD	790					
No	603	76.3		1059	65.9	
Probable/yes	187	23.7		549	34.1	

IQR indicates interquartile range; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; IHD, ischemic heart disease.
P values indicate significance of difference between participants in each group; **P*>0.05; †*P* between 0.01 to 0.05; ‡*P*<0.001.
§Geometric mean.

To our knowledge, 2 cross-sectional studies have examined the relationship between CRP gene polymorphisms (single nucleotide polymorphisms [SNPs]) with circulating CRP levels and aPWV. Morita et al[26] describe in their study an association between CRP genotype and aPWV among a sample of 315 elderly Japanese men and women. However, a recent community-based cross-sectional study by Schnabel et al[27] of participants of the Framingham Heart Study (n=2409) has not found such an association.

Therefore, the aim of this study was to confirm observational relationships between circulating CRP and PWV and then compare these to the association between polymorphisms in the *CRP* gene known to be associated with circulating CRP and PWV in a prospective cohort. These data

Table 2. Association Between CRP Genotypes and Circulating CRP Levels (mg/L)

Genotype (n) SNP	Mean CRP (mg/L)	Geometric Mean CRP	GM/Ratio*	95% CI	*P* Value
rs1130864					
G:G (532)	2.48	1.49			
G:A (496)	2.67	1.69	1.13	1.01, 1.27	0.03
A:A (135)	3.02	1.85	1.24	1.04, 1.47	0.02
Per allele			1.12	1.03, 1.21	0.005
rs1800947					
C:C (998)	2.76	1.69			
C:G/G:G† (166)	1.96	1.28	0.76	0.66, 0.87	<0.001
rs1205					
C:C (538)	2.80	1.77			
C:T (490)	2.60	1.54	0.87	0.78, 0.98	0.02
T:T (134)	2.11	1.37	0.78	0.66, 0.92	0.004
Per allele			0.88	0.81, 0.95	0.001

*GM/ratio is the relative difference in geometric means between genotypes and per allele.
†Combined heterozygote and minor homozygote allele because of small numbers.

allow us to comment on whether the existing association between circulating CRP and PWV is likely to be causal or explained by residual confounding, reverse causality, or both.

Methods

Subjects

The Caerphilly Prospective Study is a population-based male cohort study of all men 45 to 59 years of age who were resident in the small South Wales town of Caerphilly. The initial examination (phase 1) took place between 1979 and 1983. Of the 2818 eligible men, 2512 (89%) were recruited. An additional 447 patients were recruited at phase 2 so that some men have no phase 1 data. Men were reinvited for additional follow-up approximately every 5 years. The last follow-up (phase 5) occurred between 2002 and 2004.

Subjects gave written informed consent, and the study had the approval of the local research ethics committee. At commencement, a detailed questionnaire with a review of personal and medical history was collected. Items included occupational social class (grouped as an ordinal variable: I, II, IIINM; IIIM; IV, V), work and leisure time activity, vasoactive medication use, alcohol intake, and smoking habit.

Clinic Measures at Phase 2

Height in bare feet was measured using a Holtain stadiometer and weight in light clothes using standardized scales, from which we derived body mass index. Peripheral blood pressure was measured in duplicate using a Hawksley random zero sphygmomanometer at phases 1 and 2. Waist and hip circumference were measured by standard methods and expressed as waist–hip ratio.

Venous Blood Samples

At phases 1 and 2, subjects attended an early morning clinic, and a fasting blood sample was taken with minimal venous stasis. This was assayed for CRP, triglycerides, liver function tests (liver and total alkaline phosphatase, γ-glutamyltransferase), insulin, and glucose.

In phase 1, CRP was measured using an in-house ELISA method (details have been described previously[5]). In phase 2, CRP was measured in a citrated sample, which had been centrifuged within 1 hour and stored at −70°C and analyzed using a high-sensitivity nephelometric assay (Dade-Behring). A direct comparison of the 2 assays has not been performed.

aPWV at Phase 5

aPWV was measured in duplicate in the supine position after 10 minutes of rest. aPWV was measured by sequentially recording ECG-gated carotid and femoral artery waveforms. Wave transit time was calculated by the system software, using the R wave of a simultaneously recorded ECG as a reference frame (Sphygmocor; AtCor Medical, Sydney, Australia). The distance from the sternal notch to femoral and carotid probe recording sites was obtained with a tape measure. aPWV was determined by dividing the distance between the 2 recording sites by the wave transit time. Mean arterial pressure in phase 5 was derived from duplicate peripheral blood pressure measurements using an Omron 711 automatic.

Genotyping

Genetic variants of the *CRP* gene on chromosome 1q21-q23 were identified on blood samples taken at phase 4. Three SNPs were used in the analysis; rs1130864 (G-A base change in the 3′ untranslated region, AF449713 position 3014), rs1800947 (C-G base change at codon 188, AF449713 position 2667), and rs1205 (C-T base change in the 3′ flanking/untranslated region, AF449713 position 3872). These SNPs were chosen on the basis of available evidence showing their replicable and robust association with circulating CRP levels.[28–30]

Genotyping was undertaken by Kbiosciences (Hertfordshire, UK), which designed and used assays based on its proprietary competitive allele-specific polymerase chain reaction system method (www.kbioscience.co.uk). All assays were validated before use with a standard 96-well validation plate used by Kbiosciences. All variants were checked for adherence to Hardy–Weinberg equilibrium to avoid gross genotyping error, and a small number of internal validations and non-DNA test controls were used to validate genotyping after assay. Hardy–Weinberg equilibrium was tested at each SNP locus on a contingency table of observed-versus-predicted genotypic frequencies using an exact test. Our allelic frequencies at rs1800947 represent the base on the antisense strand, and C and G frequencies are therefore reversed in our analyses. The G allele is the predominant allele in worldwide SNP frequency studies (www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=1800947); this equates to our C allele on the antisense strand.

Statistics

Statistical analysis was conducted in Stata version 10. The average aPWV value was derived from 2 measures unless only 1 was available. We log-transformed variables that were positively skewed (eg, CRP, triglycerides, and alkaline phosphatases). After transformation of CRP, we standardized the values and expressed them as *z* scores because the assays were done in different laboratories. We then took the average value for the *z* scores for phases 1 and 2, unless there was only 1 value, in which case we took this instead. This removes problems associated with the different laboratory assays

Table 3. Mean Values and Coefficients for Each Genotype of SNP rs1800947

Subject Characteristics	C:C	C:G/G:G	Difference (β-coefficient or %)	95% CI	*P* Value
Age, y	56.8	56.5	−0.29	−0.96, 0.38	0.40
BMI, kg/m^2	26.6	26.5	−0.11	−0.67, 0.44	0.69
Waist–hip ratio	0.93	0.93	0.0001	−0.01, 0.01	0.98
SBP, mm Hg	145.5	146.5	0.98	−2.45, 4.41	0.57
DBP, mm Hg	84.4	84.3	−0.07	−1.84, 1.70	0.93
Triglycerides,* mmol/L	1.69	1.65	0.98†	0.9, 1.06	0.59
Total cholesterol, mmol/L	5.62	5.48	−0.15	−0.3, −0.003	0.05
HDL cholesterol, mmol/L	1.03	1.02	−0.004	−0.04, 0.03	0.81
Liver function tests					
γ-Glutamyltransferase,* IU/L	25.53	24.85	0.97†	0.89, 1.06	0.55
Total alkaline phosphatase,* U/L	165.6	164.37	0.99†	0.95, 1.04	0.75
Liver alkaline phosphatase,* U/L	91.46	88.93	0.97†	0.92, 1.03	0.36
Alcohol consumption,* U/week	7.43	7.34	0.99†	0.77, 1.26	0.92
Smoking status, %					
Nonsmokers	18.3	24.1			
Ex-smokers	40.1	34.4			
Cigar, pipe or <15 cigarettes/day	25.1	25.1			0.22‡
≥15 cigarettes/day	16.6	16.4	−0.2	−5.9, 5.4	0.94
Diabetic status, %					
No	96.7	98.0			
Yes/uncertain	3.3	2.1	−1.2	3.8, −1.4	0.37
Activity scores, %					
Leisure	31.7		26.2		
Mild					
Moderate	33.8	34.4			0.26‡
Heavy	34.7	39.5	4.8	−2.4, 12.1	0.19
Work					
Mild	32.0	39.1			
Moderate	47.0	41.1			0.13‡
Heavy	21.1	19.8	−1.3	−7.4, 4.8	0.68

*Geometric mean; †geometric mean ratio between 2 alleles; ‡*P* value for heterogeneity.

because it essentially rescales the raw data along comparable ranges. Where CRP was the outcome variable, we simply used the log–CRP values because then the back-transformed regression coefficients represent the ratio of geometric means in CRP (eg, 1.20 implies a 20% relative increase in geometric mean value of CRP for a unit change in exposure status). In all analyses, PWV was adjusted for current age, heart rate, mean arterial pressure, and vasoactive medication use (measured at phase 5) because these are all strong predictors of PWV.

We calculated the strength of the association between CRP and aPWV by using linear regression. Multivariable regression was used to examine whether simple associations were changed after adjustment for potential confounders or intermediaries. We considered the following as potential confounders as a measure of socioeconomic status: body mass index, waist–hip ratio, triglycerides, high-density lipoprotein (HDL) and total cholesterol, systolic blood pressure, liver function tests (which may influence CRP synthesis), smoking status (never, ex-smoker, or current smoker), physical activity, diabetic status, prevalent ischemic heart disease, and social class (manual versus nonmanual).

Haplotypes were constructed using the genetic data analysis program SIMHAP (http://www.genepi.com.au). SIMHAP uses current estimation–maximization-based methods for the estimation of haplotypes from unphased genotype data.[31] The current implementation of SIMHAP uses the statistical computing package R (www.r-project.org) to resolve haplotypes and provide their posterior probabilities.

After the construction of haplotypes, SIMHAP is also able to examine relationships between common CRP haplotypes and circulating CRP concentration. All possible haplotype configurations are resolved for each individual within the program itself, and the posterior probability of each configuration is calculated. Association analyses within the generalized linear model framework then uses simulation to correctly deal with the uncertainty around imputed haplotypes. In the case of plasma CRP concentration, mean values by common *CRP* haplotype were derived from the regression coefficients and CIs estimated by SIMHAP. For these analyses, all common haplotypes were run in the model assuming additive effect of each haplotype and setting a baseline haplotype as the most common in this sample (1000 iterations were used). This was then repeated with log-transformed aPWV for ease of comparison.

Results

Of the 2398 members from phase 2, 906 men (37.8%) died before we could undertake measurement of their aPWV. A total of 103 men were known to have moved out of the area,

so they were not invited, leaving 1389 potential men. A total of 124 men could not be found at their last known addresses, and 438 men either refused to take part or felt unable to attend the clinic, resulting in 827 phase 2 men, of whom 790 had valid measures of aPWV (62.5% of men contacted).

Table 1 shows the baseline characteristics of participants with aPWV and subjects who were lost to follow-up. Subjects who were in the initial phases but who had no aPWV at phase 5 were more likely to have higher values for waist–hip ratio, systolic blood pressure, and levels of circulating CRP, triglycerides, HDL cholesterol, γ-glutamyltransferase, total and liver alkaline phosphatase ($P<0.05$). This is consistent with the healthy survivor effect: participants who had died by phase 5 (ie, the majority of the group without aPWV reading) were more likely to have had more advanced cardiovascular damage, reflected by higher systolic blood pressure and circulating levels of cholesterol and CRP. When we compared *CRP* genotypes of men in phase 5 with the group without aPWV reading, we noted that although there was a 3% difference in allele distribution, the *P* value was 0.1; hence, this finding is consistent with chance.

The genotypes of the 3 SNPs were in Hardy–Weinberg equilibrium (rs1130864 $P=0.08$; rs1800947 $P=0.99$; rs1205 $P=0.1$). Because of low numbers in the minor homozygote for SNP rs1800947 (G:G n=8), we combined this group with the heterozygote as C:G/G:G (n=166).

We looked at the association between *CRP* genotypes and circulating levels of CRP, and for all 3 SNPs, there was a significant allelic difference in CRP levels for each genotype (rs1130864 $P=0.005$; rs1800947 $P<0.001$; rs1205 $P=0.001$; Table 2). The difference was greatest for rs1800947, with a ratio of geometric means of 0.76.

We then checked the Mendelian randomization assumption that our *CRP* genotype would be unrelated to other potential confounders (Table 3). Only total cholesterol levels showed nominal difference by genotype ($\beta=-0.15$; $P=0.05$), but given that there are 18 tests of statistical significance, this would not survive correction for multiple testing ($P=\approx 0.9$ after Bonferroni correction).

Table 4 shows the results for regression analyses relating circulating CRP to aPWV (models 1 through 4) and for *CRP* genotype (rs1800947) to aPWV (models 5 and 6). For the sake of simplicity, we only presented this genotype in the table because of all SNPs, it explained the greatest proportion of variance in circulating CRP (ratio of geometric means comparing C:C to C:G/G:G 0.77; $P<0.001$). In model 1, the linear regression analysis between circulating CRP (log-transformed) and aPWV shows an association with a β-coefficient of 0.46 ($P<0.001$) for a 1 log-unit change in CRP levels. In models 2 through 4, we added various potential confounders in a sequential order, such that model 2 includes diabetes mellitus, body mass index, systolic blood pressure, waist–hip ratio, smoking status, and social class. Model 3 additionally includes triglycerides, cholesterol, high-density cholesterol, γ-glutamyltransferase, liver and total alkaline phosphatase, and alcohol consumption, and in model 4, we added presence of ischemic heart disease and work/leisure activity. Some variables were not measured on the whole cohort; hence, the total number of subjects incorporated in the multivariable regression models decreased to 549. Despite this attenuation of numbers, results show only a modest reduction of effect ($\beta=0.39$ after adjustment for a range of confounders) with strong evidence against the null hypothesis ($P=0.001$).

Table 4. Association Among CRP Genotype, Circulating CRP, and aPWV*

CRP Serum/ Genotype	n (Frequency)	β-Coefficient	95% CI	*P* Value
Circulating CRP				
Model 1	646	0.46	0.26, 0.67	<0.001
Model 2	608	0.39	0.18, 0.61	<0.001
Model 3	551	0.40	0.17, 0.63	0.001
Model 4	549	0.39	0.15, 0.62	0.001
SNP rs1800947 C:G/G:G vs C:C				
Model 5	565	0.005	−0.57, 0.58	0.99
Model 6	551	0.03	−0.56, 0.62	0.93

Model 1 encompasses linear regression analysis circulating CRP–aPWV; model 2, adjusted for diabetes, body mass index (SD), systolic blood pressure (SD), waist–hip ratio, smoking, and social class; model 3, as model 2 plus triglycerides, cholesterol, HDL, γ-glutamyltransferase, liver and total alkaline phosphatase, and alcohol consumption; model 4, as model 3 plus work and leisure activity and presence of ischemic heart disease; model 5, linear regression analysis CRP genotype–aPWV; model 6, as model 5, adjusting for total cholesterol.

*aPWV adjusted for age, heart rate, mean arterial pressure, and vasoactive medication use.

Model 5 shows that there is no difference in aPWV between the C:C and C:G/G:G genotypes ($\beta=0.005$, 95% CI, −0.57 to 0.58, $P=0.99$). This remained essentially unchanged when cholesterol levels were added in model 6 ($\beta=0.03$, 95% CI, −0.56 to 0.62, $P=0.93$). The results for aPWV with the other 2 genotypes were as follows; for rs1130864 $\beta=0.003$, 95% CI, −0.28 to 0.28, $P=0.98$; for rs1205 $\beta=-0.104$, 95% CI, −0.39 to 0.18, $P=0.47$.

We repeated the analysis with common (>5%) constructed haplotypes and found largely the same patterns of association. Table 5 shows common haplotypes, their frequency and their relationship with both circulating CRP and aPWV. Specifically, haplotype 212/GGT for the SNPs rs1800947, rs1130864 and rs1205 (taken as that which was observed to have the greatest effect on circulating CRP) was associated with a reduction in circulating CRP when compared with the ancestral haplotype 111 (geometric mean for circulating CRP for haplotype 111=1.75 (1.55, 2.00), ratio of geometric means for haplotype 212=0.79 (0.67, 0.93), $P=0.004$. For the same haplotype, there was no observed association with aPWV (geometric mean for the baseline haplotype 111=11.16 (10.75, 11.58), ratio of geometric means for haplotype 212=1.01 (0.96, 1.07), $P=0.7$).

Discussion

Our results confirm the established association between circulating CRP and aPWV, and show a robust association

Table 5. Haplotype Analysis of CRP Polymorphisms With aPWV

Haplotype	Allelic Complement	Frequency	SE	Mean CRP/Ratio of Geometric Means (95% CI)	*P* Value	Mean aPWV/Ratio of Geometric Means (95% CI)	*P* Value
111	**CGC**	**0.34**	**0.009**	**1.75 (1.55, 2.00)**	. . .	**11.16 (10.75, 11.58)**	. . .
121	CAC	0.33	0.009	1.05 (0.96, 1.15)	0.3	1.00 (0.97, 1.03)	0.9
112	CGT	0.26	0.009	0.94 (0.85, 1.03)	0.2	0.99 (0.96, 1.02)	0.6
212	GGT	0.07	0.005	0.79 (0.67, 0.93)	0.004	1.01 (0.96, 1.07)	0.7

Overall *P* for the whole model $P=0.004$ (log-rank test for null model compared with that including all haplotypes). Haplotype 111 (bold) is set at baseline for regression of circulating CRP on haplotype, as performed by SIMHAP (taking into account the posterior probability of haplotype reconstruction). All other figures represent ratios of geometric means for difference by haplotype. Common haplotypes together account for >99% of the population and account for ≈1% of the variance in circulating CRP.

between *CRP* genotype and circulating CRP levels. However, there was no evidence of an association between CRP genotype and aPWV. This challenges the proposition that circulating CRP has a causal effect on aPWV and supports the notion that observational relationships between the two may be the result of either reverse causation or confounding.

Genotypic effects can be thought of as lifetime exposures that cannot be altered (although there may be interactions with other genes or environmental factors), and their random allocation at conception can be thought of as a paralogue to the design of a randomized controlled trial, hence providing good evidence for causal inferences.[32] Therefore, if CRP was causally related to aPWV and, hence, arterial stiffness, then we would have expected a difference in PWV according to the *CRP* genotypes that was proportionate to the effect of the same *CRP* genotypes on circulating levels of CRP.

Two previous publications investigated the relationship between CRP genotype and PWV to date.[26,27] Morita et al based their findings on 315 elderly (mean age 77.9 years) Japanese men and women recruited from the New Elder Citizen Movement. They analyzed 5 SNPs in the CRP gene and found a significant association with the C-allele of rs1800947 compared with the G-allele ($P=0.04$). However, their haplotypic analysis showed a paradoxical effect because although the H4 haplotype (TTG haplotype of rs1341665, rs3091244, and rs1800947) was associated with a greater proportion of subjects with elevated CRP levels (16.2% versus 3.2%; $P=0.002$), it was also associated with a reduced risk of having increased PWV, defined as ≥14m/s (89% versus 97%; $P=0.038$ using Fisher's exact test).

Schnabel et al investigated the association between multiple inflammatory biomarker genotypes and arterial stiffness measures. In their study, they analyzed 2409 participants of the Framingham Heart Study with diverse ethnic backgrounds and a mean age of 60 years. Their findings do not support an association between CRP genotype (specifically rs1800947) and aPWV.

Our study supports the evidence against a causal relationship between *CRP* genotype and PWV. As such, this does suggest a possible confounding effect in the relationship between circulating CRP and PWV for which previous studies have not adjusted. Alternatively, given that arterial stiffness begins much earlier in life, this may in turn induce an inflammatory response with secondary elevation of CRP levels. A recent review[33] reports several studies that linked certain CRP polymorphisms to circulating CRP levels, and in some, there is an association with cardiovascular outcomes. However, one study presenting such findings[34] also reported no association between CRP polymorphisms and carotid intima-media thickness.

Timpson et al[35] found a relationship between circulating CRP and factors of the metabolic syndrome; however, this was abolished if *CRP* genotypes were analyzed instead, which suggests CRP is a marker of the metabolic syndrome that plays no active role in its development. Similarly, Davey Smith et al[36] report an association among CRP levels and blood pressure, pulse pressure, and hypertension, which is not supported by a Mendelian randomization approach examining the predicted effects of *CRP* genotype on these outcomes using an instrumental variable analysis.

There are several strengths to our study. First, it is population based and recruited men living in a well-defined geographic population. Our measure of serum CRP is based on 2 blood samples taken over 4 to 5 years and, hence, is less prone to regression to the mean bias.[37] CRP levels were measured ≈15 years before the aPWV assessment and so should be less influenced by reverse causality. We have also been able to adjust for a wide range of other covariates that may have confounded the association.

There are several important limitations to our study. First, our cohort consisted of Welsh male participants, hence its generalizability may be limited, although it is not obvious why the biological effects of CRP should differ by gender or ethnicity. In addition, our sample size cannot rule out moderate size effects given the width of our 95% CI. Therefore, larger population-based studies are still needed to confirm or refute our findings. Finally, we were only able to examine the survivors of the cohort, which could have introduced a healthy survivor effect. This bias would result in an underestimation of the association, but despite this, we still found a strong association between circulating CRP and aPWV, consistent with other studies.[10,13–16,18–21] These studies largely report an association between CRP or log–CRP and aPWV in middle-aged or elderly healthy populations, with widely ranging participant numbers. We do not feel that this is a major bias because our results are identical to the report from the Framingham Offspring Study,[27] in which the mean age of participants was >10 years younger and, hence, would have less selective mortality.

Perspectives

The findings of this study imply that CRP may have no causal role in arterial stiffness, although future larger

studies are required to confirm our observations. This does not exclude the value of circulating CRP as a biochemical marker for the prediction of atherosclerotic disease or a prognostic indicator for patients with established cardiovascular disease.

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Disclosures

None.

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