C-reactive protein (CRP), a blood marker of inflammation and a hallmark of the acute-phase response, has been shown to be a powerful and specific predictor of cardiovascular event risk in populations of otherwise healthy persons. Here we review what is known about CRP gene polymorphisms, discuss how these might affect the epidemiology of CRP and our understanding of CRP’s contribution to cardiovascular disease, and examine their potential clinical usefulness. Evidence shows that certain subtle variations in the CRP gene sequence, mostly single nucleotide polymorphisms, predictably and strongly influence the blood level of CRP. Some of these variations are associated with clinical correlates of cardiovascular disease. If future studies can establish with certainty that CRP influences cardiovascular biology, then CRP gene profiling could have clinical utility. (J Am Coll Cardiol 2007;50:1115–22) © 2007 by the American College of Cardiology Foundation

C-reactive protein (CRP) was discovered in 1930 by Tillet and Francis during their studies of patients with acute pneumonia (1). They found that when serum from febrile patients was mixed with a cell-wall component of pneumococci that they called “Fraction C,” a precipitate formed. This property was subsequently found to be due to reactivity of CRP, present at high levels in “acute-phase” sera, with a polysaccharide in the pneumococcal cell wall (C-polysaccharide). C-reactive protein was the first of many acute-phase reactants subsequently discovered (2), but today elevation of CRP is still considered the hallmark of the acute-phase response.

C-reactive protein is a member of the phylogenetically ancient and conserved pentraxin family of proteins. Human CRP (Fig. 1) consists of 5 noncovalently bound subunits of 206 amino acids, each arranged symmetrically around a central pore. The molecule has a ligand recognition face that contains a Ca²⁺-dependent binding site, as well as an effector molecule binding face that is able to initiate fluid-phase pathways of host defense (by activating the complement system) and cell-mediated pathways (by activating complement and by binding to the Fc receptors of immunoglobulin G) (3) (Fig. 1). The rise in blood CRP after tissue insult or injury is rapid and robust, with levels increasing by as much as 1,000-fold above baseline within 24 h. This behavior makes blood CRP an ideal clinical marker of a patient’s general health status, and it has thus been used for decades (4). The recent introduction of “high-sensitivity CRP” assays, technologies that allow clinicians to detect the low levels of blood CRP in ostensibly healthy people, has resulted in accumulation of vast amounts of data linking blood CRP to various kinds of cardiovascular diseases (5). At the same time, new evidence from animal models indicates that CRP might actually have a pathogenic role in vascular disease (6–9).

It has long been recognized that environmental variables and patient behaviors and traits such as smoking, infections, age, gender, lipid levels, and blood pressure can contribute to variation in baseline CRP level1 (10). Indeed, obesity is a major determinant of CRP levels in humans, and elevated levels of CRP predict the development of type 2 diabetes mellitus and the metabolic syndrome (11,12). Elevated CRP levels in these conditions are related to insulin resistance (13), and weight loss can reduce CRP levels (14). Newer evidence indicates an additional and substantial genetic component (10,15,16). The realization that CRP genetic polymorphisms do exist and that certain of these directly and predictably influence steady-state blood CRP level could be of substantial clinical importance, because genetic predisposition to high baseline CRP might account for a significant proportion of people with a higher than average risk of heart disease (17). Herein we review the available evidence showing that a variety of CRP gene polymorphisms are statistically associated with blood CRP level, that certain of these are biologically functional in that they directly alter CRP blood levels, and that some CRP variants are linked to a risk of heart and blood-vessel disease.

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1Because of the highly skewed distribution of human CRP blood levels, the population statistic most often reported in the epidemiologic literature is the median. Mean CRP levels and transformed CRP levels are also reported.
Localization and Structure of the CRP Gene

On the basis of the amino acid sequence of the protein, Whitehead et al. (18) synthesized CRP-specific oligonucleotides and used these to screen a human liver c-deoxyribonucleic acid library. The clone pCRP1 was thus isolated, sequenced, and used as a probe to localize the human CRP gene to chromosome 1. This launched the era of CRP genetics. C-reactive protein was subsequently mapped to the proximal long arm of chromosome 1 in the 1q23.2 region (19, 20) (Fig. 2). The CRP gene sequence was simultaneously determined in 1985 by 2 different groups (21, 22), both reporting that it is composed of 1 intron separating 2 exons (Fig. 2). The first exon encodes a signal peptide and the first 2 amino acids of the mature protein. This is followed by a 278-nucleotide-long intron that includes a GT repeat sequence. The second exon encodes the remaining 204 amino acids, followed by a stop codon. Goldman et al. (23) were the first to show that the GT stretch in the intron is polymorphic in length.

Once the CRP gene was identified and its sequence reported, several groups investigated its proximal promoter region and how this regulates messenger ribonucleic acid production. Several reviews of that subject are available (3, 24, 25). Fundamentally, regulation of CRP expression occurs mostly at the transcriptional level, with interleukin (IL)-6 being the major inducer and IL-1 acting synergistically to enhance the effect (26). Accordingly, certain polymorphisms of the IL-1 (27, 28) and IL-6 (29) genes associate with differences in blood CRP level. Both IL-1 and IL-6 polymorphisms have been linked to myocardial infarctions and stroke (30, 31) and to mortality after acute coronary syndromes (32). Studies using mice expressing human CRP from the human CRP promoter have shown that although IL-6 is necessary for the induction of the CRP gene, it is not by itself sufficient (33, 34). In humans, CRP blood levels are generally higher in females than in males (35), whereas in mice, expression of the human CRP transgene exhibits the opposite pattern (33). It is important to note that in vitro studies of regulation of CRP gene expression have focused solely on primary hepatocytes, hepatocyte cell lines, or a variety of transfected cell lines (3, 24, 25). With the recent realization that CRP can be locally produced by the vasculature (7, 36), however, this area needs to be reinvestigated to determine whether the same regulatory mechanisms are operating in cells of non-hepatic origin.

In this review we will not discuss further IL-6, IL-1, gender hormones, or any other of the multitude of transacting factors and their genes that might themselves be polymorphic and might participate in CRP gene regulation. Rather, we will focus on the CRP gene per se and systematically discuss its many polymorphic cis-acting elements. We will in turn examine evidence that the CRP gene coding and promoter region is polymorphic, that this sequence variation affects how much blood CRP is made, and that this in turn affects CRP’s association to cardiovascular disease.
The CRP Gene Is Polymorphic

Pankow et al. (37) estimated that the interindividual variation in blood CRP level is 35% to 40% heritable. This has since been confirmed in twin studies (38–40). For example, MacGregor et al. (39) compared monozygotic versus dizygotic twin pairs and found that, although CRP was associated with body mass index, smoking status, and hormone replacement therapy, there was a greater correlation of CRP level among monozygotic than dizygotic twins, with an estimated heritability of 52%. Similarly, Retterstol et al. (40) found in monozygotic twins the within-pair correlation coefficient for CRP level was 0.40. Soon after it was established that differences in CRP level associate with genetic differences, efforts were made to identify different CRP gene polymorphisms to determine whether they directly affect the protein’s blood level. We have already mentioned that soon after the gene was sequenced, a polymorphism in the length of the GT repeat in the CRP intron was identified (23,41). Some time later, Cao and Hegele reported (42) the first coding region variation, i.e., a single nucleotide polymorphism (SNP) in exon II of CRP. That SNP, +1059 G/C (rs1800947) (Table 1), is silent at the amino acid level (CTG → CTC, Leu → Leu), and the rarer C allele was reportedly found in Caucasians but not Inuits (43). Pronounced ethnic and race effects are now known to be a general characteristic of the distribution of CRP gene polymorphs. Indeed, a recent systematic resequencing of the CRP gene revealed as many as 40 SNPs forming as many as 29 different haplotypes, with by far the highest nucleotide diversity observed in African Americans (44). It is indisputable that the CRP gene is polymorphic.

CRP Gene Polymorphisms Associate With Differences in CRP Blood Levels

Ours was the first group to report an association between CRP gene polymorphism and baseline blood CRP (45). We examined the relationship between GT repeat alleles and blood protein levels in patients with systemic lupus erythematosus (SLE) versus healthy control patients and therein identified 13 alleles ranging in length from 9 to 25 repeats (GT9 to GT25). Two of these, GT16 and GT21, accounted for more than 75% of all the alleles found. The statistical relationship between CRP genotype and CRP blood level was best described by a sine-wave model having a periodicity of 5 GT repeats, with the GT16 and GT21 variants associated with lowest CRP levels (Fig. 3). No particular GT allele was associated with SLE (45).

Soon after our study was published, Brull et al. (46) reported their own findings. They measured blood CRP in healthy men before and after 48 h of endurance exercise, and in patients before and after coronary artery bypass graft (CABG) surgery and screened each of their respective CRP genes. Two new polymorphisms were identified; a −717G/A SNP (rs2794521) in the CRP promoter and a +1444C/T SNP (rs1130864) in the 3′ untranslated region. The former did not associate with CRP, but +1444TT homozygotes had significantly higher CRP at baseline, after exercise training, and after CABG. The +1444C/T SNP by itself accounted for 3.7% of the total variance in baseline blood CRP. Later the same group reported that CRP was higher in periodontal patients carrying the +1444T versus the C allele (47), and another group showed that healthy young men with the +1444TT genotype had 64% higher blood CRP than those with the +1444CC genotype (48).

Many other CRP polymorphisms with association to blood CRP level have since been reported. Thus, Obisesan et al. (49) identified association between a −717A/G SNP and CRP levels before and after exercise training; blood CRP was higher in −717AA homozygotes. A similar
correlation was reported for a $219G/A$ SNP (rs3093066) in the 3' untranslated region: GG homozygotes had higher CRP levels at baseline and after exercise (49). Impressively, in a multivariate analysis, the $717A/G$ and $219G/A$ SNPs together accounted for almost 9% of the total variation in CRP blood level (49). Finally, a $29A/T$ SNP (rs1417938) was found to be significantly associated with baseline CRP level: individuals with the A allele had higher blood CRP, a relationship that persisted even after controlling for clinical characteristics (50). It is now clear that polymorphisms in the CRP gene associate with differences in CRP blood level.

Some CRP Gene Polymorphisms Are Functional

None of the polymorphisms so far discussed has been shown to be functional, that is, to directly contribute to differences in baseline CRP blood level among individuals. Few studies have actually pursued this question, and in the absence of evidence, the common assumption made is that CRP polymorphisms alter the structure of neighboring deoxyribonucleic acid and thus impede or bolster its translation to messenger ribonucleic acid, or that known polymorphisms are in linkage disequilibrium with yet-to-be identified functional polymorphisms. To investigate this issue, we sequenced the CRP promoter in 287 healthy individuals and identified 2 SNPs, a biallelic one at nucleotide $409G/A$ (rs3093062) and a triallelic one at $390C/T/A$ (rs3091244) (51). Blood CRP was lowest in people with the $409AA$ genotype, intermediate in $409GA$, and highest in $409GG$, and there was a tendency toward high CRP values in individuals carrying $-390TT$. Both SNPs reside within E-box elements we named E-box 1 and E-box 2, respectively. Using electrophoretic mobility shift assays, we proved that both E-boxes are functional and that transcription factors are capable of binding E-box 1. Importantly, the SNP in E-box 1 affects its ability to interact with transcription factors. We also showed that individuals with a $-409G/-390T$ haplotype (the only haplotype found that encoded a high transcription factor binding version of E-box 1 and a transcription factor binding version of E-box 2) had highest blood CRP, whereas individuals with $-409A/-390T$ (weak transcription factor binding versions of both E-boxes) had the lowest (Fig. 4A). Individuals with either of the 2 remaining haplotypes had intermediate CRP levels. Finally, using CRP promoter-firefly luciferase reporters, we confirmed that transcription at baseline and in response to IL-6 was different for the different E-box haplotypes (Fig. 4B). Impressively, carriers of the $-409G/-390T$ haplotype who never smoked still had higher CRP levels than $-409G/-390C$ carriers who currently did (51).

Kovacs et al. (52) also identified and studied the triallelic $390C/T/A$ SNP. They examined the correlation between CRP and the GT repeat polymorphism in the CRP gene. Each circle and whisker is the mean ± SEM for baseline (age-adjusted) CRP obtained from the indicated number of individuals. The correlation coefficient ($r$) for the sine-wave regressions is given. The arrows point to the common GT$^{16}$ and GT$^{21}$ variants having the lowest (approximately equal) CRP levels. CRP = C-reactive protein.
this SNP, a biallelic one at −717A/G, the +1059G/C SNP in exon 2, and the +1444C/T SNP in the 3′ flanking region. That study confirmed that the triallelic SNP correlated with baseline CRP. In the large Framingham Heart Study (16), 9 of 13 CRP SNPs were found to be associated with blood CRP after adjustment for clinical covariates. Of these 9, only the triallelic SNP remained associated with blood CRP after accounting for correlation among SNPs. Finally, because CRP level after an acute coronary syndrome is predictive of recurrent events (53, 54), Danik et al. (55) evaluated patients with acute coronary syndrome and found that the highest CRP blood level was associated with a −757T/C SNP (rs3093059) and again with the −390C/T/A SNP. The evidence from our initial study (51) and independent additional studies thus points to at least 1 CRP gene polymorphism that might truly be functional: −390C/T/A.

CRP Gene Polymorphism Associates With Disease Status

Some of the most compelling reports of a strong association between CRP polymorphism and disease status did not include measurement of blood CRP level as a variable. For example, Roy et al. (56) linked one poly(GT) allele (likely GT16) (Fig. 3) to increased susceptibility to infection with Streptococcus pneumonia, and Wolford et al. (57) found that −717TT was associated with an increased prevalence of type 2 diabetes mellitus. More recently, it was reported (58) that having the C allele at the −717T/C locus was significantly associated with training-induced improvements in the insulin-sensitivity index.

Not all such studies revealed a gene–disease association. Thus, although baseline CRP is a good predictor of restenosis (59), investigation of the +1059G/C and +29T/A SNPs found no association with restenosis risk after angioplasty (60). These data consistently point toward an association of CRP polymorphisms with blood CRP levels. The strength and direction of this association appear to depend on the type of disease, however, and the polymorphisms may or may not associate with disease risk.

The Big Question: Does CRP Gene Polymorphism Alter CRP Blood Levels and Thus Disease Status?

Zee et al. (61) examined the relationship of +1059G/C with blood CRP and risk of arterial thrombosis. In a large cohort of healthy American men (726 case-control pairs), 12% were found to carry the C allele, and those turned out to have lower median CRP values compared with individuals having the GG genotype. No correlation with arterial thrombosis was found (61). Likewise, in the British Women’s Heart and Health Study (62), carriers of +1059C had lower blood CRP, but this did not affect blood pressure, pulse pressure, or hypertension. In contrast, Balistreri et al. (63) found that CRP blood level was higher in subjects with the C allele, and there was a higher frequency of +1059C in patients with a history of acute myocardial infarction compared with healthy control patients. In a nested-case control study of SLE (64), certain alleles of the intronic GT repeat were found to be associated with vascular events (myocardial infarction, stroke, peripheral arterial thrombosis, CABG, angina or claudication). Systemic lupus erythematosus patients with these vascular events were more likely than SLE patients without them to have blood CRP in the highest quintile of measured values, and these patients had a higher prevalence of the GT20 variant (64). In another study of SLE patients, Russell et al. (65) directly sequenced the CRP gene in British families and found a correlation between baseline blood CRP, the +1059G/C SNP, and a +1846G/A SNP (rs1205) in the 3′ flanking region. The latter was associated with SLE and the presence of antinuclear antibodies.

Other efforts to associate CRP gene polymorphism with blood CRP level and disease status are noteworthy. For example, in Han Chinese subjects, although neither −717A/G nor +1846 A/G was found to correlate with blood CRP, the frequency of the −717A allele was significantly higher among patients with coronary heart disease than control patients. Impressively, in that study, individuals carrying the A allele had an odds ratio for coronary heart disease of 6.8 compared with those not carrying that allele (66). This finding has since been confirmed, as the same SNP was found to correlate with occurrence of myocardial infarction or thromboembolic stroke in the Physician’s Health Study (67). In a very recent and thorough examination of 7,159 individuals from the Third National Health and Nutrition Examination Survey, several CRP SNPs were found to be associated with CRP levels, but only the functional −390C/T/A SNP was linked to both CRP levels and to increased prevalence of coronary heart disease (68). One interpretation of the findings of these studies, which were performed independently using diverse and unrelated populations, is that there are some CRP sequence polymorphisms that influence expression of the protein that might also affect cardiovascular well being.

All of the known CRP gene SNPs are in high-linkage disequilibrium, so tag SNPs have been used to define extended CRP haplotypes. Using this approach, Kardys et al. (69) found that although CRP blood level was associated with incident coronary heart disease and varied with CRP haplotype, the different CRP haplotypes themselves were not associated with coronary heart disease. In another tag SNP investigation of subjects from the NHLBI Family Heart Study, a trend toward a significant association of CRP haplotypes with blood CRP was reported. In this case, the −757T/C and −390C/T/A SNPs showed the strongest association. Again, however, none of the CRP haplotypes or tag SNPs showed an association with intima-media thickness as measured by ultrasound (70). Another study examining the correlation of various CRP SNPs to CRP blood level and arterial pulse wave velocity (71) found...
statistically significant association between CRP levels and 
$-390C/T/A$ and $+1444C/T$, and there was association of 
$+1059G/C$ with pulse-wave velocity. Finally, in the 
Cardiovascular Health Study, $+1846C/T$, $+29A/T$, 
$+1059G/C$, and $-757T/C$ were variably associated with 
blood CRP. Not surprisingly, given that the distribution of 
CRP alleles differed among the races, the disease risk also 
differed among the races. Thus, in Caucasians $+29A/T$ was 
associated with increased risk of stroke and cardiovascular 
mortality and $+1059G/C$ and $+1846C/T$ were associated 
with cardiovascular mortality, whereas in African Americans 
$-757T/C$ was associated with myocardial infarctions 
(72). At least some of the large-scale investigations using tag 
SNP approaches have provided some evidence that sequence 
differences in the CRP gene alter both CRP blood 
levels and disease risk.

Summary

We have attempted here to bring together for comparison 
and contrast all of the published reports showing (or not 
showing) that CRP gene polymorphisms associate with 
CRP blood levels or/and that CRP gene polymorphisms 
associate with cardiovascular disease risk. Some noteworthy 
patterns emerged. There is now ample direct evidence that 
some CRP gene polymorphisms affect the amount of CRP 
produced and indirect evidence that this in turn might have 
an impact on vascular disease. In the CRP gene, intron 
variations in the GT repeat have been linked to changes in 
blood CRP and the risk of vascular events, and the $+29A/T$ 
SNP was linked to blood CRP level but not to restenosis. In 
exon II, the often-studied $+1059G/C$ SNP has been shown to 
correlate well with blood CRP and in at least 1 study to 
associate with pulse-wave velocity. It does not correlate, 
however, with arterial thrombosis, blood pressure, or 
restenosis. In the promoter region, the $-390C/T/A$ triallelic 
SNP, the only functional CRP polymorphism so far 
described, has been shown to correlate with blood CRP and 
recently has been directly linked to risk of cardiovascular 
disease. The other SNPs in the promoter region have 
variable association with CRP level but good correlation 
with cardiac disease, diabetes mellitus, and insulin resistance. 
In the 3’ untranslated and 3’ flanking regions there are other 
SNPs that also correlate with blood CRP, but none that associate with cardiovascular disease (although 
$+1846C/T$ does associate with SLE). Importantly, nearly 
10% of the total variation in CRP blood level can be 
accounted for by the CRP gene SNPs. This is remarkable 
given that CRP blood level is as much as 40% heritable. The 
fact that most studies have not been able to link CRP 
polymorphisms to events is not that surprising, despite their 
clear relationship to blood CRP level and of the latter to 
hard clinical end points. Given the modest proportion of 
variation in blood CRP level that CRP polymorphisms 
explain, it would take a very large study (several orders of 
magnitude larger than those so far undertaken) to see a 
direct relationship between a particular SNP or CRP 
haplotype and actual clinical events.

Coronary artery disease is considered by many to be a 
disease of inflammation (73), and blood CRP has been 
shown to be a powerful predictor of cardiovascular events 
(74,75). The effects of lifestyle and trans-acting factors not 
withstanding, it is now increasingly apparent that certain 
subtle variations in the CRP gene sequence predictably and 
strongly influence blood CRP level. Furthermore, some of 
these variations map to cis-acting elements that have already 
been associated with clinical correlates of cardiovascular 
disease; notable in this respect is the triallelic SNP at 
position $-390$. New CRP SNPs and novel gene-protein-
disease associations will likely be reported at an increasing 
pace, so the exact contribution of CRP gene polymorphisms 
to the protein’s level and to disease status will continue to 
define itself. However, reports that individual SNPs in CRP 
might explain up to 10% of the variance in blood CRP level, 
and that the heritability of blood CRP is 30% to 40%, 
suggest that identifying an individual’s CRP SNPs might 
one day be a practical and useful clinical approach for 
prediction of cardiovascular event risk or for selecting 
patients for treatment with CRP targeting drugs. Of course, 
moving CRP genotyping to the clinical arena depends 
entirely on obtaining proof that CRP participates in disease 
processes in humans, and that proof is still not available. If 
ultimately CRP is found to not contribute to cardiovascular 
biology, its utility as a marker of inflammation will not be 
diminished. Ongoing and future studies will no doubt settle 
the issue, but until then, there will always be critics and 
supporters of the position that CRP blood levels and CRP 
gene polymorphisms really matter.

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