The role of inflammation in cardiovascular disease has gained enormous interest in recent years, to a significant extent as a consequence of the ease with which the acute phase protein C-reactive protein (CRP) can be assessed as a marker of inflammation. More recently, CRP has not only to be considered a marker, but also a potential participant in the pathogenesis of cardiovascular disease, and various roles in cellular activation and in inflammatory processes have been proposed.\(^1\)

In twin studies, MacGregor et al.\(^4\) observed 26% heritability in elderly twins; Hengstenberg et al.\(^5\) found in families with observed heritability of 37% in male and 19% in female middle-aged twins; de Maat et al. (unpublished data, 2003) the genetic approach and the consequences of the results. Now to discuss important possible lessons we can learn from mechanisms of involvement of CRP in disease processes and to genetic variability in the CRP gene.

In the case of a positive outcome of CRP as a participating factor in one or more disease phenotypes, we can add CRP-lowering to the list of desired therapeutic targets and establish it as a surrogate endpoint for the specific phenotypes.\(^8\) At present, targeting CRP is already supported quite well for its role in activating complement during the acute phase of myocardial infarction by both animal studies\(^9\) and recent interventions in complement activation with c1-esterase inhibitor.\(^10\) The elucidation of the exact role of CRP in the determination of infarct size might be a first target for a genetic study and would concur with the theory that genetics play a role in the CRP response as documented by Brull et al.\(^6\)

Preferably, such evaluations of relationships between phenotypes and genetics is carried out with a functional polymorphism or haplotypes of functional polymorphisms and according to proposed guidelines.\(^11\)

For the presently known polymorphisms in the CRP gene, we have no experimental data as yet about functionality, but many polymorphisms have already been reported (for a summary, please see the data supplement at http://atvb.ahajournals.org). The comprehensive evaluation of Wolford et al.\(^12\) adds to our abilities here and points, as do Brull et al.\(^6\) to a marker function or role of the 1444 C/T polymorphism. At present, for three polymorphisms in the CRP gene, an association with CRP levels has been documented. Zee and Ridker\(^13\) showed in a large, male study population of mean age 60 years, a median CRP concentration was associated with the 1059 C allele in exon 2 of CRP that was 36% lower than the 1059 G allele. Szalai et al.\(^14\) showed a sine-wave relation of baseline CRP levels with the length of an intron GT repeat with differences in CRP level amounting to around a factor of two between two low and two high alleles among the 13 identified alleles of the microsatellite. Brull et al.\(^6\) showed an approximately two-fold difference in level between homozygotes for a +1444 TT homozygotes and carriers of the 1444C allele in Army recruits. Brull et al.\(^6\) did not find an association of the 1059 C/G polymorphism and the CRP level. The associations of polymorphisms with levels is suggestive of the existence of a functional regulatory polymorphism marked by or possibly being one of the ones mentioned. On the basis of present information, the rare 1059 C/G is the least likely candidate.\(^6\)

The suggestion about the functionality of the intron GT repeat is quite appealing and may possibly involve a hormone response element.\(^7,14\)

At present, the only polymorphism tested against one clinical endpoint (acute coronary syndromes) is the 1059 C/G in exon 2 with a low frequency of $\sim 6\%$ of the rare allele, showing a negative result.\(^11\)

---

**Editorial**

**Genetics of C-Reactive Protein**

**New Possibilities and Complications**

Cornelis Kluft, Moniek P.M. de Maat

---

CRP as a Factor in Pathogenesis

A polymorphism in a gene, which affects the function of the gene (mutation in the regulatory sequences) or the gene product (mutation in the protein coding part), can teach us about the role of the gene product(s) in disease.

When we have identified a genetic variation in the CRP gene associated with functional consequences, we can start the endeavor of linking it with pathophysiologial consequences. For CRP, we have now clear evidence for a genetic association with variation in production at baseline and after stimulation. No common variation in the protein has been found.\(^7\) Thus, we can concentrate on CRP baseline or stimulated levels.

---

From the Gauhuis Laboratory, TNO-PG, The Netherlands.

Corresponding to Cornelis Kluft, Gauhuis Laboratory, TNO-PG, Zernikedreef 9, 2333 CK Leiden, The Netherlands. E-mail c.kluft@pg.tno.nl


© 2003 American Heart Association, Inc.

(Art)erioscler Thromb Vasc Biol. is available at http://www.atvbaha.org

DOI: 10.1161/01.ATV.0000100113.47260.EB

1956
The article by Brull et al also reveals that the contribution of genetic variation of 1444 C/T in the CRP gene itself to CRP phenotypes (baseline levels and increases) is modest, \( \approx 4\% \). This contrasts with the heritability of baseline levels of \( \approx 30\% \). Either the right functional polymorphism may increase this estimate eventually or the heritability of CRP resides for the major part in genetic variability in upstream stimulating or inhibiting factors. For the latter, candidates are receptors such as toll-like receptor 4 and CD14 and biological response modifiers in pro-inflammatory pathways such as chemokines, interleukin-1 and 6, and TNF-\( \alpha \), and in anti-inflammatory pathways, for instance, interleukin 1RA, interleukin-10, and TGF-\( \beta \). In this latter case, CRP is important as a marker (see below). For myocardial infarction families, the contribution of genes other than CRP (chromosome 1) is supported by a genome scan showing loci on chromosomes 5, 10, and possibly 2.5

**CRP as a Marker With Genetic Variation of Response**

The presently available evidence of a genetic variation influencing CRP levels and response to stimulation may have consequences for the use of CRP as a marker.

- Brull et al also showed a genetic determinant in both baseline levels and response to stimulation, which may involve separate mechanisms. It can also be reasoned that this in fact may be one basic principle and one genetic aspect where the baseline level is a response to chronic and acute increases to temporary stimulation. This reasoning is supported by the fact that there is a significant age dependence of baseline CRP levels starting in neonates and in cord serum at levels around 0.03 to 0.2 mg/L,15 and increasing gradually to very old age to \( \approx 3 \) mg/L.16 We do not know all the chronic or long-lasting stimulation factors, except for some determinants, such as obesity and low levels of physical activity, dental health, stress and depression, sleep disturbances, arthritis, and possibly general factors of ageing,1,17 resulting in a different stimulation package for every individual.

As illustrated in Figure 1 for two hypothetical homozygotes with a different response allele for CRP, the response to the putative stimulation package is different. When two individuals with different genetics have an elevated baseline CRP 3 mg/L, it represents a different degree of stimulation (A and B in Figure 1). When the risk marked by baseline CRP in fact is marking the burden of chronic or long-lasting stimulation, the use of CRP as a marker for this burden will be improved by using genotype-specific risk categories. This can be experimentally tested and will answer the question whether or not risk assessment with CRP should include genetic analysis. The burden of chronic stimulation may be a disease-promoting factor by predisposing to increased pro-inflammatory reactivity and by contributing to autoimmunity and T helper cell 1 dominance.

- Another option is that the known risk marking of CRP is in fact marking the hyperresponse genetics of CRP. The group with high levels of CRP may include relatively larger numbers of individuals with the hyperresponse allele (I). It is possible that this is relevant in the acute phase of myocardial infarction and determines the posttraumatic timing and peak in CRP as shown for CABG and exercise by Brull et al.6 The observations of Liuzzo et al18 that pretreatment CRP is a determinant of traumatic responses after PTCA, and CABG may also involve a similar genetic background. This induced peak in CRP might determine the degree of complement activation and size of the myocardial infarction.9,10 It should be noted that most clinical endpoints for studies on CRP involve myocardial infarction and effects on the size may result in increase of numbers of clinically recognized infarctions and increase of early and late consequences. In this option, the use of CRP levels for risk assessment can in theory be replaced by assessment of CRP genetics alone.

**CRP as a Marker of Upstream Processes**

A different option is that the observed heritability of baseline CRP stems to a significant extent, from heritability in upstream inflammatory pathways. As illustrated in Figure 2, this may involve pro- and anti-inflammatory factors. The possibility that genetic variation in such factors is associated with CRP levels is sparsely reported and requires confirmation and extension to all potentially relevant factors. The study groups should be rigorously selected for comparability with the groups studied for CRP heritability, and preferably, studies should be done in the same groups to assure the same health status. In this respect, it has been

---

**Figure 1.** Response of two homozygotes for the hypothetical CRP-alleles I or II coding for a different response of CRP to stimulation.

**Figure 2.** Stimulation pathway of liver synthesis of CRP: stimulation (infections, autoantibodies lipid factors, other inflammatory processes) through receptor pathways (eg, toll-like receptor 4 and CD14); modulated by biological response modifiers (pro-inflammatory factors including chemokines; IL-1\( \alpha \), -1\( \beta \), and -6 and TNF-\( \alpha \); anti-inflammatory factors including interleukin 1RA, interleukin-10, TGF-\( \beta \), haptoglobin, COX-2); gene activation through nuclear factors (eg, PPAR, NF\( \kappa \)B).
reported that genetic variations in IL-1α and IL-1β show 100% and 50% CRP differences, respectively, in patients referred for coronary angiography.19 This may agree with a possible locus on chromosome 2 in myocardial infarction families.5 But there is also a negative report about CRP levels and the important pro-inflammatory IL-1 locus (including IL-1α, IL-1β, IL-1RA) in the NHLBI family study2 that used the dinucleotide repeat in intron 5 of IL-1α.

Variation in the IL-6 gene is a likely candidate. Two reports show an association between IL-6 polymorphisms and CRP levels in a family study3 and in a study on healthy postmenopausal women.20 The differences in CRP levels between genotypes amount to 79%21 and 91%.3 The IL-6 genotype explained 14% of the observed variation in log plasma CRP in the family study.3 It indicates that CRP can indeed mark genetic variability in upstream inflammatory pathways. It should be noted, however, that these associations are illustrations of the principle. The associations are presently inconsistent in the attribution to a specific genotype showing in one study higher CRP with the −174C and in others with the −174G allele.19,20 A similar inconsistency has been noted for IL-6 levels showing higher values in various situations with either the −174C24 or −174G allele.21–24 Analysis of multiple mutations in the interleukin-6 gene have shown that effects are not simply additive but show complex interactions determined by haplotypes.25 In addition, sex and age of the studied groups may be relevant in view of the effects of 17-β estradiol on −174 G/C.26 It can be concluded that definitive studies with haplotypes are required to define the exact role of genetic variation in the IL-6 gene for baseline and stimulated levels of CRP in various groups.

Also some associations between CRP levels and genetic variability in further upstream factors such as haptoglobin,27–28 COX-2,29 toll-like receptor 4 (trend)30 and CD 1431 are reported and require further attention. The analysis in families ascertainment for myocardial infarction points to loci on chromosomes 2, 5, and 10,3 which are, except for the IL-1 cluster and CD14, not among the examples in Figure 2.

Comments

It is clear that the evidence for the existence of a functional genetic variation in the CRP gene should be followed-up by experimental studies to identify this variation. The use of this putative functional variability is expected to result in a further increase in knowledge of the role of CRP in the pathogenesis of cardiovascular disease. The report of Brull et al8 focused our attention specifically on the variability in the response of CRP to stimuli and consequently to the acute phase of coronary syndromes.

Heritability and genetic variation in the CRP gene and upstream processes result on the other hand in significant questions regarding the role of CRP in risk assessment. Various options need evaluation, and the results might influence the use of CRP in risk assessment and the selection of patients for treatment.

Acknowledgment

We are indebted to B. Hoegee-de Nobel for the generation of the data for the figure in the online supplement, which can be accessed at http://atvb.ahajournals.org.

References


Genetics of C-Reactive Protein: New Possibilities and Complications
Cornelis Kluft and Moniek P.M. de Maat

doi: 10.1161/01.ATV.0000100113.47260.EB

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/23/11/1956

Data Supplement (unedited) at:
http://atvb.ahajournals.org/content/suppl/2003/11/17/23.11.1956.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/
Legends to figures

Figure I:

Reported genetic variability and heterozygosity in and around the CRP-gene and its single intron (not drawn to scale). Top 5’ UTR to bottom 3’ UTR; E1,2 are exon 1 and 2; I = intragenic and introgenic sequences. Base numbers according to http://www.ncbi.nlm.gov/entrez/viewer.fcgi, rs = SNP number (NCBI SNP database), including notations used in Wolford et al (1) and Brull et al (2) for clarification and mutual reference. The microsatellite at 6136487> is not given in detail: see for details Szalai et al (3) and Weber et al (4). In the Wolford notation * and # refer to 2 clusters based on equal allelic frequencies and near-complete linkage disequilibrium. Brull et al (2) noted allelic association between –717 and +1444 of \( \Delta = -0.37 \) - -0.39 (##). The T allele of 133552 (**) is associated with an increased prevalence of type 2 diabetes mellitus (1).

References
(3) Szalai AJ, McCrory MA, Cooper GS, Wu J, Kimberly RP. Association between baseline levels of C-reactive protein (CRP) and a dinucleotide repeat polymorphism in the intron of the CRP gene. Genes and Immunity 2002; 3: 14-9.
<table>
<thead>
<tr>
<th>Contig position</th>
<th>Bases involved</th>
<th>E/I</th>
<th>Wolford</th>
<th>SNP</th>
<th>Brull</th>
<th>aminoacid</th>
<th>heterozygosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>6134634</td>
<td>T &amp; C</td>
<td></td>
<td></td>
<td>rs1205</td>
<td></td>
<td></td>
<td>0.453</td>
</tr>
<tr>
<td>6134715</td>
<td>T &amp; C</td>
<td></td>
<td></td>
<td>rs3116640</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6134807</td>
<td>T &amp; C</td>
<td></td>
<td></td>
<td>rs3116639</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6134823</td>
<td>T &amp; C</td>
<td></td>
<td></td>
<td>rs3116638</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6134908</td>
<td>T &amp; C</td>
<td></td>
<td></td>
<td>rs3116637</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6134929</td>
<td>C &amp; T</td>
<td>I</td>
<td></td>
<td>rs3093067</td>
<td></td>
<td></td>
<td>0.080</td>
</tr>
<tr>
<td>6135071</td>
<td>A &amp; G</td>
<td></td>
<td></td>
<td>131126#</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6135250</td>
<td>C &amp; A</td>
<td></td>
<td></td>
<td>rs1050031</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6135492</td>
<td>G &amp; A</td>
<td></td>
<td></td>
<td>rs1130864</td>
<td>1444##</td>
<td></td>
<td>0.366</td>
</tr>
<tr>
<td>6135500</td>
<td>A &amp; C</td>
<td></td>
<td></td>
<td>rs3093066</td>
<td></td>
<td></td>
<td>0.434</td>
</tr>
<tr>
<td>6135614</td>
<td>A &amp; G</td>
<td></td>
<td></td>
<td>rs3093065</td>
<td></td>
<td></td>
<td>0.043</td>
</tr>
<tr>
<td>6135715</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6135839</td>
<td>C &amp; G</td>
<td>E2</td>
<td></td>
<td>rs1800947</td>
<td>1059</td>
<td>leucine/leucine</td>
<td>0.058</td>
</tr>
<tr>
<td>6136329</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6136487&gt;</td>
<td>(GT)n</td>
<td>I</td>
<td></td>
<td></td>
<td>13 alleles</td>
<td>Weber/Szalai</td>
<td></td>
</tr>
<tr>
<td>6136587</td>
<td>T &amp; A</td>
<td></td>
<td></td>
<td>rs1417938</td>
<td></td>
<td></td>
<td>0.339</td>
</tr>
<tr>
<td>6136616</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6136676</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6136782</td>
<td>T &amp; C</td>
<td></td>
<td></td>
<td>rs3093064</td>
<td></td>
<td></td>
<td>0.083</td>
</tr>
<tr>
<td>6136874</td>
<td>A &amp; C</td>
<td></td>
<td></td>
<td>rs3093063</td>
<td></td>
<td></td>
<td>0.043</td>
</tr>
<tr>
<td>6136881</td>
<td>G &amp; C</td>
<td></td>
<td></td>
<td>rs3122011</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6137066</td>
<td>A &amp; G &amp; T</td>
<td></td>
<td></td>
<td>rs3091244</td>
<td></td>
<td></td>
<td>0.638</td>
</tr>
<tr>
<td>6137085</td>
<td>C &amp; T</td>
<td>I</td>
<td></td>
<td>rs3093062</td>
<td></td>
<td></td>
<td>0.364</td>
</tr>
<tr>
<td>6137383</td>
<td>C &amp; T</td>
<td></td>
<td></td>
<td>rs3093061</td>
<td></td>
<td></td>
<td>0.349</td>
</tr>
<tr>
<td>6137497</td>
<td>T &amp; C</td>
<td>I</td>
<td></td>
<td>rs2794521</td>
<td></td>
<td>&quot;-717##&quot;</td>
<td>0.350</td>
</tr>
<tr>
<td>6137536</td>
<td>C &amp; T</td>
<td></td>
<td></td>
<td>rs3093060</td>
<td></td>
<td></td>
<td>0.043</td>
</tr>
<tr>
<td>6137537</td>
<td>A &amp; G</td>
<td></td>
<td></td>
<td>rs3093059</td>
<td></td>
<td></td>
<td>0.350</td>
</tr>
<tr>
<td>6137716</td>
<td>A &amp; T</td>
<td></td>
<td></td>
<td>rs3093058</td>
<td></td>
<td></td>
<td>0.363</td>
</tr>
<tr>
<td>6137729</td>
<td>T &amp; A</td>
<td></td>
<td></td>
<td>133783#</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6137928</td>
<td>T &amp; C</td>
<td></td>
<td></td>
<td>133982#</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6138884</td>
<td>T &amp; C</td>
<td></td>
<td></td>
<td>rs3116636</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6138894</td>
<td>C &amp; T</td>
<td></td>
<td></td>
<td>rs3116635</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6139088</td>
<td>C &amp; T</td>
<td></td>
<td></td>
<td>rs3116634</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>