CRP Gene Haplotypes, Serum CRP, and Cerebral Small-Vessel Disease

The Rotterdam Scan Study and the MEMO Study

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Background and Purpose—It remains unclear whether C-reactive protein (CRP) is a serum marker for atherothrombotic disease or a causal factor in the pathogenesis of atherosclerosis. We explored the association between CRP gene variations and cerebral small-vessel disease (SVD) in the Rotterdam Scan Study (N=1035) and the MEMO Study (N=268).

Methods—Common haplotypes within the CRP gene were determined by genotype-tagging single-nucleotide polymorphisms. Then their relation with periventricular and subcortical white matter lesions and the prevalence of lacunar brain infarcts was explored by regression analyses.

Results—There was no association between CRP haplotypes and measures of cerebral SVD in either study. There was no effect modification of the association between serum CRP levels and measures of SVD by CRP haplotypes.

Conclusions—Our observations suggest that CRP is not causally involved in the pathogenesis of SVD. (Stroke. 2007;38:2356-2359.)

Key Words: genetics ■ inflammation ■ lacunar infarcts ■ white matter disease

It remains unclear whether C-reactive protein (CRP) is only a severity marker of vascular disease or whether it plays a role in vascular disease development. The previously reported associations between CRP levels and vascular disease1–3 may have been caused by residual confounding.4 Study of the association between variation in the CRP gene and vascular disease may help to clarify this question.4

We examined whether haplotypes representing common variations in the CRP gene in European populations are associated with the risk of cerebral small-vessel disease (SVD), which had been associated with increased serum CRP levels in the Rotterdam Scan Study in previous analyses.5 We performed this analysis on data from the Rotterdam Scan Study and the Memory and Morbidity in Augsburg Elderly (MEMO) Study.

Subjects and Methods

Participants

The Rotterdam Scan Study is a prospective, population-based magnetic resonance imaging (MRI) study among 1077 participants, age 60 to 90 years.6 The MEMO Study is a population-based study among 385 participants, 65 years or older.7 In both studies, each participant underwent a neurologic examination, neuropsychological testing, and blood sampling. All participants in the Rotterdam Scan Study and 268 participants from the MEMO study7 underwent a brain MRI.

From the 1077 participants in the Rotterdam Scan Study, genotyping was unavailable for 39 (3.6%) subjects, and 3 persons (0.3%) were excluded because of genotyping errors, leaving 1035 participants in the final sample. Excluded participants in both studies were older and had a higher prevalence of vascular risk factors than did included subjects. There were no differences in the distribution of CRP genotypes or plasma CRP levels.

Magnetic Resonance Imaging

MRI scans were performed with 1.5-T machines and included proton density–, T1-, and T2-weighted images.7,8 White matter lesions (WMLs) were considered periventricular if they were abutting the lateral ventricle; otherwise they were considered subcortical. Periventricular WMLs were graded semiquantitatively on a severity scale (0 to 3) at the horns and the body of the lateral ventricle, with the total periventricular WML score being the sum of these 3 scores. For subcortical WMLs, the total volume (in mL) was approximated on the basis of the number and size of lesions. Lacunar infarct was defined as a focal hyperintensity on T2-weighted images of 3 to 15 mm in the subcortical white matter or basal ganglia.7,8

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Serum CRP Levels
Serum levels of CRP were determined by a high sensitivity–rate, near-infrared particle immunoassay method (Image high-sensitivity CRP, Beckman Coulter).

Genotyping
On the basis of data from 23 unrelated individuals of European descent, Seattle single-nucleotide polymorphisms (SNPs) (http://pga.gs.washington.edu/) identified 31 SNPs in the CRP gene. It subsequently identified 4 common haplotypes. We genotyped 3 tagging SNPs to infer these haplotypes.9 In the Rotterdam Scan Study, DNA was genotyped for 1184C>T, 2042C>T, and 4741C>G SNPs.9 In the MEMO Study, polymorphisms 1184C>T, 2042C>T, and 4363C>A were genotyped. SNPs 4363C>A and 4741C>G are in perfect linkage disequilibrium. DNA was processed according to standard techniques.9 Haplotypes were inferred with use of the program Haplo.Stats and coded as haplotypes 1 through 4 in order of decreasing frequency: coding from 1184C>T, 2042C>T, and 4741 C>G (4363C>A), haplotype 1 = CTC, 2 = CCC, and 3 = TCC. Haplotype 4 was in the CCG Rotterdam Scan Study and CCA in the MEMO Study.

Statistical Methods
We compared the mean serum CRP levels among the genotypes of each polymorphism by ANOVA. Then we compared the mean grades of WML severity among the genotypes of each polymorphism by ANOVA and estimated the association of each polymorphism with the prevalence of lacunar brain infarcts by logistic regression.

To explore the associations of CRP haplotypes with measures of cerebral SVD, we used the program Haplo.Stats.9

Results
The characteristics of the participants are shown in Table 1. All SNPs were in Hardy-Weinberg equilibrium. In both studies, we observed the expected associations of CRP polymorphisms and CRP serum levels.9 There were no differences in WML severity or the presence of lacunar infarcts among the genotypes of any polymorphism (Table 2).

In the Rotterdam Scan Study, haplotypes were present in the following frequencies: haplotype 1, 33.9%; haplotype 2, 30.4%; haplotype 3, 30.1%; and haplotype 4, 5.4%. In the MEMO Study, the frequencies were as follows: haplotype 1, 33.9%; haplotype 2, 31.6%; haplotype 3, 30.0%; and haplotype 4, 4.1%. In both studies, haplotype 1 was associated with the lowest CRP levels and served as the reference category.

In the MEMO Study, haplotype 3 was associated with more severe subcortical WMLs than haplotype 1 (Table 3). After adjusting for multiple comparisons, this association became nonsignificant. There was no association between any other haplotype and subcortical WMLs, or between haplotypes and periventricular WMLs or lacunar brain infarcts. The association between serum CRP levels and

<table>
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<tr>
<th>TABLE 1. Genotype Distributions and Demographic and Clinical Characteristics in the Rotterdam Scan Study and the MEMO Study</th>
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<tbody>
<tr>
<td>Rotterdam Scan Study (n=1035)</td>
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<tr>
<td>-------------------------------------------------------------</td>
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<tr>
<td>Women, n (%)</td>
</tr>
<tr>
<td>Mean age, y (SD)</td>
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<tr>
<td>APOE 4−/4 or 4/4 genotype, n (%)</td>
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<td>Mean CRP serum level, mg/L (SD)</td>
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<tr>
<td>Mean grade of periventricular WMLs (SD)*</td>
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<tr>
<td>Mean volume of subcortical WMLs, mL (SD)†</td>
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<tr>
<td>Lacunar infarcts, n (%)</td>
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<tr>
<td>1184C&gt;T polymorphism genotype, n (%)</td>
</tr>
<tr>
<td>CC</td>
</tr>
<tr>
<td>CT</td>
</tr>
<tr>
<td>TT</td>
</tr>
<tr>
<td>2042C&gt;T polymorphism genotype, n (%)</td>
</tr>
<tr>
<td>GG</td>
</tr>
<tr>
<td>GA</td>
</tr>
<tr>
<td>AA</td>
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<tr>
<td>4741C&gt;G (4363C&gt;A) polymorphism genotype, n (%)‡</td>
</tr>
<tr>
<td>CC (CC)</td>
</tr>
<tr>
<td>CG (CA)</td>
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<tr>
<td>GG (AA)</td>
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</table>

*Periventricular WMLs were graded semiquantitatively on a severity scale (0–3) at the frontal and occipital horns and the body of the lateral ventricle. The total periventricular WML score is the sum of these 3 scores.†The total volume of subcortical WMLs was approximated on the basis of the number and size of lesions (range, 0 to 30.0 mL).‡4741C>G and 4363C>A polymorphisms are in perfect linkage disequilibrium. The 4741C>G polymorphism was genotyped in the Rotterdam Scan Study; the 4363C>A polymorphism was genotyped in the MEMO study.
Discussion

Although we found in both studies the expected associations between CRP polymorphisms/haplotypes and CRP serum levels, there was no relation between any polymorphism/haplotype and WMLs or lacunar infarcts. There was no effect modification of the association between serum CRP levels and measures of cerebral SVD by CRP gene variation.

In both studies, exclusion from the analyses was associated with older age and a higher prevalence of vascular risk factors. This may have led to an overestimation of the associations between polymorphisms and SVD, because the genetic contribution to disease is less in older age. However, because we observed no association, this seems unlikely.

We did not find an association between CRP polymorphisms or haplotypes and measures of SVD, although we observed associations with plasma CRP levels, which were associated with vascular disease in observational studies\(^1\)–\(^3\) and with SVD in the same sample of the Rotterdam Scan Study.\(^2\) Because CRP is associated with several risk factors, previously observed relations between CRP and vascular disease may have been caused by residual confounding.\(^4\) The approach used in the present study overcomes this problem.\(^4\)

It remains possible that our study lacked power to detect a small effect. In reference to haplotype 1, we were able to demonstrate relative risks for lacunar brain infarcts of 1.45, for periventricular WMIs of 1.31, and for subcortical WMIs of 1.32 (for haplotype 2, frequency of 30.4%, 80% power, and \(\alpha=0.05\)). In the MEMO Study, we had power to detect relative risks for lacunar brain infarcts of 2.98, for periventricular WMIs of 2.76, and for subcortical WMIs of 2.75 (for haplotype 2, frequency of 31.6%). If there is an association between variation in the CRP gene and SVD, it must be small. Thus, the results of this study do not suggest that CRP is causally involved in the pathogenesis of SVD.

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<th>Rotterdam Scan Study</th>
<th>MEMO Study</th>
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<tr>
<td></td>
<td>CC or CT (TT)</td>
<td>CC or CT (TT)</td>
</tr>
<tr>
<td>Haplotype 1 (CTC)</td>
<td>CC (0.09)</td>
<td>CC or CT (TT)</td>
</tr>
<tr>
<td>Haplotype 2 (CCC)</td>
<td>0.09 (0.11)</td>
<td>0.11 (0.09)</td>
</tr>
<tr>
<td>Haplotype 3 (TCC)</td>
<td>0.11 (0.11)</td>
<td>0.09 (0.16)</td>
</tr>
<tr>
<td>Haplotype 4 (CCG/CCA)‡</td>
<td>0.27 (0.19)</td>
<td>0.08 (0.28)</td>
</tr>
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</table>

*All models were adjusted for age and sex.

*\(\beta\) coefficients and SEs.

†Odds ratios (OR) and 95% confidence intervals.

‡Haplotype 4 was coded CCG in the Rotterdam Scan Study and CCA in the MEMO Study.
Disclosures

None.

References


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