Original Contribution

Variants in the CRP Gene as a Measure of Lifelong Differences in Average C-Reactive Protein Levels

The Cardiovascular Risk in Young Finns Study, 1980–2001

Mika Kivimäki1,2, Debbie A. Lawlor3, George Davey Smith3, Carita Eklund4, Mikko Hurme4,5, Terho Lehtimäki6, Jorma S. A. Viikari7, and Olli T. Raitakari8

1 Department of Epidemiology and Public Health, Faculty of Biomedical Sciences, University College London, London, United Kingdom.
2 Finnish Institute of Occupational Health, Helsinki, Finland.
3 Department of Social Medicine, Faculty of Medicine and Dentistry, University of Bristol, Bristol, United Kingdom.
4 Department of Microbiology and Immunology, Faculty of Medicine, University of Tampere, Tampere, Finland.
5 Laboratory Centre, Tampere University Hospital, Tampere, Finland.
6 Department of Clinical Chemistry, Tampere University Hospital and University of Tampere, Tampere, Finland.
7 Department of Medicine, Faculty of Medicine, University of Turku, Turku, Finland.
8 Department of Clinical Physiology, Faculty of Medicine, University of Turku, Turku, Finland.

Received for publication February 7, 2007; accepted for publication April 23, 2007.

Genetic association studies have used variants in the C-reactive protein (CRP) gene to estimate causal effects of lifelong circulating CRP levels on disease endpoints. However, the extent to which the genetic variants are actually associated with lifelong circulating CRP levels has not been demonstrated empirically. In a population-based prospective cohort study (1980–2001) of 1,609 young Finns (768 men and 841 women), the authors genotyped five single nucleotide polymorphisms in the CRP gene (−717A/G, −286C/T/A, +1059G/C, +1444T/C, and +1846G/A) and assessed circulating CRP levels at ages 3–18 years and 24–39 years. The haplotypes from the five single nucleotide polymorphisms were associated with circulating CRP levels in childhood and adulthood, with the strongest effect being found for average CRP level across these two measures taken at two time points in the life course. In combination, the haplotype pairs accounted for 3.9%, 3.3%, and 5.0% of the variation in circulating CRP levels in childhood, in adulthood, and for the mean of CRP levels at both time points, respectively. These findings support the assumption that the above genetic variants define groups with long-term differences in circulating CRP levels.

C-reactive protein; haplotypes; random allocation; variation (genetics)

Abbreviations: CRP, C-reactive protein; SNP, single nucleotide polymorphism.

There is debate as to whether C-reactive protein (CRP), a nonspecific marker of acute-phase inflammatory response, is a cause of coronary heart disease or just a marker of other causal factors and preclinical disease. Several genetic association studies have suggested that CRP may be noncausal, because variants in the CRP gene have not consistently been related to coronary outcomes (1–5). In the epidemiologic literature, this approach of studying causality using genetic variants is often called “Mendelian randomization” (6). The underlying assumption is that, because alleles are randomly allocated from parents to offspring at birth, persons with genetic variants that are related to higher CRP levels will...
have been, in effect, randomly allocated to somewhat higher levels of CRP across their life course than persons with genotypes related to lower CRP levels (6, 7). In theory, therefore, these lifelong differences in CRP level should translate into differences in coronary heart disease risk, if CRP were causally related to coronary heart disease and if population stratification did not confound associations of genotype with CRP levels or coronary heart disease risk (6–9).

A major limitation of genetic association evidence on CRP is that no previous studies are available to demonstrate empirically that the genetic variants are indeed a valid measure of lifelong differences in CRP levels. Previous studies have demonstrated relations of genotype with circulating CRP levels measured at one point in adulthood (most commonly in middle or older age) (1–5). Demonstrating an association with mean CRP levels across the life course would support the inference that the association of CRP genotype with coronary heart disease reflects lifetime differences in CRP levels (10, 11).

In this study, we determined the magnitude of the association between variants in the CRP gene and circulating CRP levels from childhood to adulthood in a population-based cohort of Finns. Since various nonstable environmental factors affect circulating CRP levels, we hypothesized that the genetic variants would be more robustly related to average CRP level across the life course than to CRP measured at a single point in time only.

MATERIALS AND METHODS

Study population

The participants were from the Cardiovascular Risk in Young Finns Study, an ongoing multicenter follow-up study of cardiovascular disease risk factors in Finnish children and adolescents (12, 13). The original sample comprised 4,320 White children and adolescents aged 3, 6, 9, 12, 15, and 18 years in five areas of Finland who had been randomly chosen from a national register (the Finnish Population Information System). The baseline examination was conducted in 1980, with the participation rate being 83 percent (3,596 of those invited). At the latest follow-up in 2001, the participants had reached 24–39 years of age. A total of 1,609 persons (768 men and 841 women) with full information on five genetic variants of the CRP gene and circulating CRP levels in 1980 and 2001 formed the cohort for the present study (5). Their CRP values were less than 10 mg/liter in 1980 and 2001; they were free of a history of recent infection and chronic rheumatic disease; and they did not include women who were lactating or pregnant in 2001.

This study was conducted according to the guidelines of the Declaration of Helsinki, and the study protocol was approved by local ethics committees. All participants gave their informed consent.

CRP genotyping

We genotyped five single nucleotide polymorphisms (SNPs) in the CRP gene (CRP–717A/G (rs2794521), CRP–286C/T/A (rs3091244), CRP+1059G/C (rs1800947), CRP+1444T/C (rs1130864), and CRP+1846G/A (rs1205)) using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, California) for both polymerase chain reaction and allelic discrimination. For the SNP CRP+1059G/C, a commercial kit from Applied Biosystems was used (Assays-on-Demand; C_177490_10 CRP). The other SNPs were genotyped using Assays-by-Design from Applied Biosystems under standard conditions; the only exception was the triallelic tagSNP, which was genotyped as described previously (14), except for the genotype calling, which was conducted manually from the polymerase chain reaction run component tab.

Haplotypes were estimated from the five SNPs with the PHASE program, version 2.0.2 (www.stat.washington.edu/stephens), which uses a Bayesian statistical method for reconstructing haplotypes from population genotype data and lists the most probable haplotype pair for each individual (15). We used 100 iterations, one thinning interval, and 100 burn-ins—that is, settings that are compatible to the relatively small region analyzed (all studied SNPs were within 2,900 base pairs of each other), one of the most conserved regions in the human genome (16). Repeated independent runs varying the value of the seed of the pseudo-random number generator produced identical haplotype frequencies, supporting the consistency of the results. The SNPs were strongly linked, with D’ values of 0.98–0.99; such close physical proximity and strong linkage disequilibrium further reduce uncertainty in the determination of the most-like haplotype pairs (17). There were no genotype data missing in our study sample. From subsequent analyses of haplotype and CRP levels, we excluded 41 persons with rare haplotypes (frequency <1 percent) and eight persons with ambiguous haplotype estimation.

Measurement of CRP levels

We assessed serum CRP levels in 1980 (childhood) and 2001 (adulthood) using an automated analyzer (Olympus AU400; Olympus America Inc., Center Valley, Pennsylvania) and a highly sensitive turbidimetric immunoassay kit (CRP-UL assay; Wako Chemicals, Neuss, Germany) (18, 19). The detection limit of the assay was 0.06 mg/liter. The interassay coefficient of variation was 3.33 percent at the mean level of 1.52 mg/liter (n = 116) and 2.65 percent at the mean level of 2.51 mg/liter (n = 168). Childhood serum samples were taken in 1980, stored at −20°C, and analyzed in 2005 using the same method as that used in 2001. Samples were not thawed or refrozen during storage.

Statistical analysis

Using an exact test, we evaluated Hardy-Weinberg equilibrium at each SNP locus on a contingency table of observed-versus-predicted genotype frequencies. The distribution of circulating CRP levels was highly skewed, and the level of circulating CRP was dependent on age and sex (20, 21). Thus, the three variables for circulating CRP used in the analyses were age- and sex-standardized z scores for log CRP in childhood, in adulthood, and across the life course (i.e., the average of age- and sex-standardized z scores for
log CRP in childhood and adulthood). In agreement with previous studies (1, 5), we chose to use a model for the haplotype-CRP association that assumed that each of a participant’s two haplotypes contributed additively to his/her CRP value. We used age- and sex-adjusted least-squares regression analysis to assess the association of haplotypes with circulating CRP indicators. All haplotypes were simultaneously entered into the regression model to obtain the total variance in circulating CRP that was explained by the haplotypes. We repeated this regression analysis with a single independent variable of 14 haplotype pairs instead of five separate haplotypes. All analyses were performed with SAS statistical software, version 9.1 (SAS Institute Inc., Cary, North Carolina).

RESULTS

Of the five CRP gene SNPs selected for analysis, two are promoter-region polymorphisms (−717 and −286), one is exonic (+1059), and two are in the 3′-untranslated region (+1444 and +1846). The genotype frequencies for all five SNPs were in Hardy-Weinberg equilibrium. Median CRP levels were 0.21 mg/liter (interquartile range, 0.11–0.53) in childhood and 0.65 mg/liter (interquartile range, 0.30–1.52) in adulthood (5). Because of skewness, mean CRP levels were higher: 0.65 mg/liter (standard deviation, 1.29) in childhood and 1.25 mg/liter (standard deviation, 1.60) in adulthood.

After exclusion of rare haplotypes (frequency < 1 percent), the remaining five haplotypes were associated with circulating CRP levels. In all cases, haplotype had a stronger effect on the mean level of CRP across the life course than on the level of CRP in childhood or adulthood alone (table 1). All five haplotypes together explained 3.8 percent of the variation in childhood CRP, 3.0 percent of the variation in adulthood CRP, and 4.9 percent of the variation in life-course CRP. The corresponding figures for 14 haplotype pairs were 3.9 percent, 3.3 percent, and 5.0 percent, respectively.

### TABLE 1. Median C-reactive protein (CRP) levels and mean age- and sex-standardized z scores for CRP level in childhood and adulthood and across the life course, according to five CRP gene haplotypes* (n = 1,560), Cardiovascular Risk in Young Finns Study, 1980–2001

<table>
<thead>
<tr>
<th>No. of haplotypes</th>
<th>No. of subjects</th>
<th>CRP level in childhood</th>
<th>CRP level in adulthood</th>
<th>CRP level across life course</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median (mg/liter)</td>
<td>Mean z score</td>
<td>Median (mg/liter)</td>
</tr>
<tr>
<td>ATGTG</td>
<td></td>
<td>0.16 (0.09–0.44)†</td>
<td>−0.16</td>
<td>0.57 (0.27–1.43)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.23 (0.12–0.55)</td>
<td>0.06</td>
<td>0.66 (0.30–1.51)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.32 (0.15–0.84)</td>
<td>0.29</td>
<td>0.76 (0.42–1.79)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;0.0001</td>
<td></td>
<td>0.002</td>
</tr>
<tr>
<td>ACGCA</td>
<td></td>
<td>0.23 (0.12–0.63)</td>
<td>0.09</td>
<td>0.69 (0.31–1.58)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.20 (0.10–0.53)</td>
<td>−0.03</td>
<td>0.64 (0.29–1.42)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.13 (0.08–0.30)</td>
<td>−0.35</td>
<td>0.49 (0.24–1.25)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;0.0001</td>
<td></td>
<td>0.14</td>
</tr>
<tr>
<td>GCCG</td>
<td></td>
<td>0.23 (0.11–0.54)</td>
<td>0.04</td>
<td>0.68 (0.32–1.59)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.18 (0.10–0.52)</td>
<td>−0.05</td>
<td>0.56 (0.27–1.42)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.17 (0.10–0.55)</td>
<td>−0.32</td>
<td>0.72 (0.32–1.39)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.08</td>
<td></td>
<td>0.08</td>
</tr>
<tr>
<td>ACCCA</td>
<td></td>
<td>0.22 (0.11–0.55)</td>
<td>0.03</td>
<td>0.66 (0.31–1.53)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.18 (0.10–0.42)</td>
<td>−0.18</td>
<td>0.54 (0.22–1.29)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.09 (0.06–0.25)</td>
<td>−0.30</td>
<td>0.11 (0.09–0.18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.09</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AAGCG</td>
<td></td>
<td>0.20 (0.11–0.52)</td>
<td>−0.03</td>
<td>0.61 (0.29–1.42)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.28 (0.12–0.67)</td>
<td>0.19</td>
<td>1.02 (0.39–2.03)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.01</td>
<td></td>
<td>0.0001</td>
</tr>
</tbody>
</table>

* CRP−717A/G (rs2794521), CRP−286C/T/A (rs3091244), CRP+1059G/C (rs1800947), CRP+1444T/C (rs1130864), and CRP+1846G/A (rs1205).
† Numbers in parentheses, interquartile range.
DISCUSSION

In this study, we empirically demonstrated that haplotypes in the CRP gene were associated with serum CRP levels in childhood and adulthood, with the strongest effects being found for average serum CRP level across the life course. This supports the underlying assumption in previous genetic association studies that the genetic variants define groups with lifelong differences in circulating CRP levels. Previously, genetic variation in the CRP gene has shown to be associated with circulating CRP levels in adulthood, but not from childhood to adulthood, as in the present study.

Variants in the CRP gene have not generally been associated with coronary heart disease or blood pressure, components of the metabolic syndrome, or carotid intima-media thickness, a measure of subclinical atherosclerotic disease, except in one study of older people, where an association was found with coronary heart disease but not with carotid intima-media thickness. In our data, as previously reported, CRP haplotypes were associated with carotid arterial compliance, but only in men, and there were no associations between the haplotypes and carotid intima-media thickness in men or women. To date, evidence from genetic epidemiologic studies provides no consistent support for the status of circulating CRP level as a causal risk factor for coronary heart disease. According to the present findings, the underlying assumption in these studies that the genetic variants define groups with long-term differences in circulating CRP level seems to be correct.

The variance in childhood and adulthood CRP levels accounted for by haplotype pairs ranged from 3.3 percent to 3.9 percent and was 5.0 percent for average CRP level across the life course. In the Cardiovascular Health Study, a US study that also assessed five CRP SNPs, the variance explained by genetic determinants ranged from 2.6 percent to 6.4 percent, depending on population, but in the Framingham Heart Study, available genetic determinants accounted for less than 2 percent of the variance in circulating CRP levels. These robust associations suggest that several haplotypes and SNPs could be used to clarify whether lifelong CRP levels are causally related to clinical disease, such as coronary heart disease. However, the magnitude of the associations between genetic variants and CRP is small, and so are the absolute differences in median CRP levels between genotypes (≤0.61 mg/liter in this study) that would mean that limited funds for drug development and trials for testing drugs’ effectiveness would be directed towards what would probably be the most effective targets. Since the phenotype of cardiovascular disease risk is complex and is related to clustering of cardiovascular risk factors, future studies should also examine whether CRP exerts biologically independent effects on coronary heart disease risk only in the presence of other risk factors observable in subsets of individuals.

ACKNOWLEDGMENTS

The Cardiovascular Risk in Young Finns Study has been supported by the Academy of Finland (grants 77841, 34316, and 210283), the Social Insurance Institution of Finland, the Finnish Work Environment Foundation, the Turku University Foundation, the Juho Vainio Foundation, the Finnish Foundation of Cardiovascular Research, and the Finnish Cultural Foundation. M. K. was supported by the Academy of Finland (grants 105195 and 117604), D. A. L. by a United Kingdom Department of Health Career Scientist Award, M. H. by the Research Foundation of Tampere University Hospital, T. L. by the Emil Aaltonen Foundation and the Medical Research Fund of Tampere University Hospital, and J. S. A. V. and O. T. R. by research grants from Turku University Central Hospital.

Conflict of interest: none declared.

REFERENCES


