Quantitative trait locus on chromosome 20q13 for plasma levels of C-reactive protein in healthy whites: the HERITAGE Family Study

Timo A. Lakka,1,2,3 Tuomo Rankinen, Treva Rice,4 Arthur S. Leon,5 D. C. Rao,4,6 James S. Skinner,7 and Claude Bouchard1

1Pennington Biomedical Research Center, Baton Rouge, Louisiana; 2Institute of Biomedicine, Department of Physiology, University of Kuopio; 3Kuopio Research Institute of Exercise Medicine, Kuopio, Finland; 4Division of Biostatistics, Washington University School of Medicine, St. Louis, Missouri; 5School of Kinesiology, University of Minnesota, Minneapolis, Minnesota; 6Departments of Genetics and Psychiatry, Washington University School of Medicine, St. Louis, Missouri; and 7Department of Kinesiology, Indiana University, Bloomington, Indiana

Submitted 1 March 2005; accepted in final form 5 June 2006

C-reactive protein (CRP) is a sensitive marker of systemic low-grade inflammation. Increased plasma levels of CRP predict the risk of cardiovascular and metabolic diseases, such as coronary heart disease (23, 40), ischemic stroke (7), peripheral artery disease (39), hypertension (42), and Type 2 diabetes (16), independently of traditional risk factors. Elevated plasma CRP levels have also been associated with features of the metabolic syndrome, including increased body adiposity, insulin resistance, increased plasma triglyceride levels, reduced plasma high-density lipoprotein (HDL) cholesterol levels, elevated blood pressure, and endothelial dysfunction (15, 50). Recent evidence suggests that CRP not only is a marker of systemic inflammation but also actively contributes to arterial wall inflammation, endothelial dysfunction, and atherosclerosis (45, 47). Moreover, CRP is an important component of the innate immune system, providing host defense by activating the classical complement pathway (49).

Cardiovascular and metabolic diseases and their risk factors have been associated with increased plasma CRP levels, whereas healthy lifestyle changes and cholesterol-lowering medications appear to reduce CRP levels (34). Genetic factors have been found to account for ~30–40% of individual differences in plasma CRP levels (32, 48). The Framingham Heart Study showed no evidence of linkage for CRP, whereas linkages were found for two other biomarkers of inflammation, monocyte chemoattractant protein-1 and soluble intercellular adhesion molecule-1 (13). However, some polymorphisms in genes involved in the inflammatory process have been associated with CRP levels (6, 46, 48). As genomic regions contributing to plasma CRP levels remain unknown, we performed a genome-wide linkage scan for plasma CRP levels in healthy whites from the HERITAGE Family Study. CRP was measured with a high-sensitivity assay. Multipoint linkage analyses were performed in 280 sibling pairs with 654 markers using regression and variance components-based methods. Data were adjusted for independent correlates of plasma CRP. We showed the strongest evidence of linkage for plasma CRP levels on chromosome 20q13. Markers which gave suggestive linkages in this region were D20S52 [logarithm of odds (LOD) score 3.18, \( P = 0.00006 \)], D20S857 (LOD score 2.87, \( P = 0.00014 \)), D20S869 (LOD score 2.75, \( P = 0.0002 \)), D20S840 (LOD score 2.59, \( P = 0.0003 \)), D20S501 (LOD score 2.55, \( P = 0.0003 \)), D20S840 (LOD score 2.18, \( P = 0.0008 \)), and D20S876 (LOD score 2.07, \( P = 0.001 \)). We also detected suggestive linkage on chromosome 13 for marker D5S5470 (LOD score 2.23, \( P = 0.0007 \)). Chromosome 20q13 may contribute to plasma CRP levels in healthy whites. This region contains genes that are important in the inflammatory process and may play a role in the development of chronic inflammatory diseases. The present findings may be useful in the ongoing effort to search for genes contributing to inflammation and to identify individuals at an increased risk of chronic inflammatory diseases.

genres: genomic scan; immune system; inflammation; health; risk factors; exercise training; and genetics

METHODS

Study design and subjects. The HERITAGE Family Study is a multicenter exercise intervention trial carried out by a consortium of five universities in the United States and Canada (4). The study was designed to investigate the contribution of exercise training to changes in risk factors for Type 2 diabetes and cardiovascular diseases and the role of genetic factors in cardiovascular, metabolic, and hormonal responses to exercise training in white and black families. The study protocol was approved by each of the Institutional Review Boards of the HERITAGE Family Study research consortium. Written informed consent was obtained from each participant.

The subjects came from families that included the biological mother and father (≥65 yr of age) and their offspring (≥17 yr of age). The white families had at least two offspring; the black families were smaller. The subjects were required to be sedentary, defined as not having participated in regular physical activity during the previous 6 mo. Moreover, we disallowed subjects who had chronic diseases that could have prevented their participation in a structured exercise training program or affected the inflammatory process. The diseases
included coronary heart disease, stroke, peripheral vascular disease, severe obesity, hypertension, hypercholesterolemia, hyperglycemia, Type 1 or Type 2 diabetes, other endocrinological diseases, autoimmune or collagen vascular diseases, respiratory diseases, gastrointestinal diseases, urinary tract or genital diseases, neurological or neuromuscular diseases, psychiatric diseases, hematological diseases, cancer in the past 5 yr except skin cancer therapeutically controlled, and musculoskeletal diseases. Also, the subjects were not allowed to take regularly medications, such as cholesterol-lowering drugs, that could have affected the inflammatory process.

We excluded eight subjects whose CRP value separated by ≥4 SD from the mean or by ≥1 SD from the next highest value. The final study sample with complete data on CRP and all other variables needed for the analyses included 444 whites (220 men, 224 women) from 96 nuclear families and 220 blacks (79 men, 141 women) from 106 nuclear families. The number of sibling pairs for the linkage analyses was 280 in whites and 79 in blacks.

**Measurement of CRP.** Plasma CRP was measured with a high-sensitivity solid-phase chemiluminescent immunometric assay (IM- MULITRAK High Sensitivity CRP; Diagnostic Products, Los Angeles, CA) implemented on an automated immunoassay instrument (Diagnostic Products). In 48 randomly selected subjects, the intraclass correlation between two CRP measurements during the same study visit was 0.98, and the coefficient of variation was 6.4%.

**Assessment of confounding factors.** The method for assessing smoking, alcohol intake, the use of hormone replacement therapy or oral contraceptives, body mass index, maximal oxygen uptake, plasma low-density lipoprotein (LDL) cholesterol, HDL cholesterol, triglycerides, glucose, and insulin, as well as systolic and diastolic blood pressure, has been presented previously (4).

**Data adjustment.** Because of skewed distributions, logarithmic transformation was used for CRP, insulin, HDL cholesterol, and triglycerides. CRP was regressed on its independent correlates by stepwise multiple regression analysis, as explained in detail previously (38). The analyses were performed separately within the race-by-sex-by-generation subgroups, and the variables included in the models were smoking status, the use of hormone replacement therapy or oral contraceptives, maximal oxygen uptake, body mass index, and up to a third-degree polynomial in age. Only significant (P < 0.05) terms were retained. The significant terms were body mass index and the use of hormone replacement therapy or oral contraceptives in white daughters and body mass index in white fathers, mothers, and sons and in black sons, daughters, and mothers. No terms were significant in black fathers. The residuals, or the raw scores if no terms were significant, were then standardized to zero mean and unit variance within each subgroup and constituted the final phenotypes.

**Molecular methods.** A total of 654 markers with an average spacing of 4.7 Mb were used. The marker density was higher on chromosome 20 because fine mapping was done in this region for another study (20q13) conducted in our laboratory 5 yr ago. The additional microsatellite markers on chromosome 20 were genotyped also on the HERITAGE markers (39% in whites. Heritability was not detectable in blacks, probably due to the limited number of sibling pairs. We therefore performed linkage analyses in whites only. We showed the strongest evidence of linkage for CRP on chromosome 20q13 between 49 and 58 Mb (Fig. 1). Markers that gave suggestive linkages in this region were D20S52 [logarithm of odds (LOD) score 3.18, P = 0.00006], D20S857 (LOD score 2.87, P = 0.00014), D20S869 (LOD score 2.75, P = 0.0002),...
DISCUSSION

The present study supports the previous finding that genetic factors account for ~30–40% of individual differences in plasma CRP levels in Caucasians (32, 48). The novel finding of the present study is the evidence of linkage for CRP levels on chromosome 20q13 in healthy whites. Chromosome 20q13 contains putative candidate genes for inflammation. CRP is principally produced by the liver, and proinflammatory cytokines induce the expression and release of CRP during the inflammatory process (48). CRP is a sensitive marker of systemic low-grade inflammation, and currently available assays allow the accurate measurement of very low plasma CRP levels in healthy individuals (34). Another advantage of CRP as a measure of low-grade inflammation is the small seasonal and diurnal variation in individuals with a stable lifestyle (29). Plasma CRP levels are increased in many proinflammatory conditions, such as smoking, overweight and obesity, diabetes, dyslipidemia, elevated blood pressure, the metabolic syndrome, the use of estrogen and progestogens, and chronic infections and inflammatory diseases (34). On the other hand, weight loss, regular physical activity, moderate alcohol consumption, and the use of lipid-lowering medications, including statins, fibrates, and niacin, appear to reduce CRP levels (34).

To minimize confounding, we adjusted data for independent correlates of CRP within each race, sex, and generation subgroup. Moreover, due to the design of the HERITAGE Family Study, we only included healthy sedentary individuals who did not have chronic diseases or were not taking regular medications, such as lipid-lowering drugs, that could have affected the inflammatory process. However, we cannot exclude the possibility that some of the subjects used aspirin or other anti-inflammatory analgesics, although this is an unlikely explanation for the results. We therefore believe that the observed linkage for CRP on chromosome 20q13 is a true finding and not due to confounding.

The maximal heritability for plasma CRP levels in whites was comparable to that observed in previous studies (32, 48), and the number of sibling pairs was large enough for reliable linkage analyses. However, we did not detect a heritable component for CRP levels in blacks. A possible explanation for this is the limited statistical power in blacks due to the smaller number of sibling pairs. Therefore, we performed linkage analyses in whites only.

To our knowledge, no previous genome-wide linkage scan reports are available for plasma CRP levels. Interestingly, however, there is accumulating evidence of linkage on chromosome 20q13 for cardiovascular and metabolic diseases and their risk factors that have been associated with inflammation, such as obesity (11, 12, 25, 26, 31), Type 2 diabetes (5, 18, 21, 22, 27, 30, 35, 44, 51), fasting plasma insulin levels (26), plasma HDL cholesterol levels (33, 43), and systolic blood pressure (20). A quantitative trait locus (QTL) for body fatness in the Pennsylvania study (25) spanned across five markers (D20869, D20857, D20839, D20840, D20876), all of which showed linkage with CRP in the present study. A QTL for Type 2 diabetes in the Finland United States Investigation of Noninsulin-Dependent Diabetes Mellitus Genetics (FUSSION) study (18) also included three markers (D20840, D20840, D20857), which were linked with CRP in our study. Elevated CRP levels have been associated with obesity (15, 50), an increased risk of developing Type 2 diabetes (16), elevated insulin levels (15, 50), reduced HDL cholesterol levels (15), and increased blood pressure (42). These associations may be partly due to the effect of genes located on chromosome 20q13 that are involved in the inflammatory process. Other genomic scans in healthy individuals and in those who already have diseases that are associated with

Table 2. Baseline CRP (mg/l) by race, sex, and generation

<table>
<thead>
<tr>
<th>Race</th>
<th>Sex</th>
<th>n</th>
<th>Median (Interquartile Range)</th>
<th>n</th>
<th>Median (Interquartile Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whites</td>
<td>Fathers</td>
<td>90</td>
<td>1.44 (0.86–2.51)</td>
<td>23</td>
<td>1.92 (1.29–4.25)</td>
</tr>
<tr>
<td></td>
<td>Mothers</td>
<td>84</td>
<td>2.04 (0.99–4.54)</td>
<td>42</td>
<td>2.69 (0.73–5.49)</td>
</tr>
<tr>
<td></td>
<td>Sons</td>
<td>130</td>
<td>0.78 (0.36–1.56)</td>
<td>56</td>
<td>1.05 (0.46–2.11)</td>
</tr>
<tr>
<td></td>
<td>Daughters</td>
<td>140</td>
<td>1.15 (0.53–2.82)</td>
<td>99</td>
<td>1.58 (0.65–4.25)</td>
</tr>
<tr>
<td>Blacks</td>
<td>Fathers</td>
<td>90</td>
<td>1.44 (0.86–2.51)</td>
<td>23</td>
<td>1.92 (1.29–4.25)</td>
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</tr>
</tbody>
</table>

CRP, C-reactive protein.

D20S480 (LOD score 2.59, P = 0.0003), D20S501 (LOD score 2.55, P = 0.0003), D20S840 (LOD score 2.23, P = 0.0007), and D20S876 (LOD score 2.07, P = 0.001). We also detected suggestive linkage on chromosome 5p13 for marker D5S1470 (LOD score 2.23, P = 0.0007).

Table 3. Linkages for plasma CRP levels in whites

<table>
<thead>
<tr>
<th>Chromosome Marker</th>
<th>Map Position, Mb</th>
<th>SIBPAL Empirical P Value</th>
<th>LOD Score (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5p13</td>
<td>D5S1470</td>
<td>32.53779</td>
<td>0.0007</td>
</tr>
<tr>
<td>20q13</td>
<td>D20S869</td>
<td>50.24202</td>
<td>0.0008</td>
</tr>
<tr>
<td></td>
<td>D20S857</td>
<td>50.79369</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>D20S839</td>
<td>51.70721</td>
<td>0.0075</td>
</tr>
<tr>
<td></td>
<td>D20S857</td>
<td>52.54275</td>
<td>0.0025</td>
</tr>
<tr>
<td></td>
<td>D20S840</td>
<td>52.83278</td>
<td>0.0026</td>
</tr>
<tr>
<td></td>
<td>D20S876</td>
<td>53.03317</td>
<td>0.0030</td>
</tr>
<tr>
<td></td>
<td>D20S851</td>
<td>54.32661</td>
<td>0.0026</td>
</tr>
<tr>
<td></td>
<td>D20S852</td>
<td>54.94566</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

LOD, logarithm of odds.
inflammation are needed to confirm the linkage results on chromosome 20q13.

Putative candidate genes for inflammation on chromosome 20q13 include NFAT1, C/EBPβ, and ADA. NFAT1 is a member of the nuclear factor of activated T cells (NFAT) family of transcription factors (28), is expressed in most immune system cells, and is important in the transcription of many genes regulating the production of proinflammatory cytokines and the activation of immune system (10, 37). We found evidence of linkage with the marker D20S857 located in the NFAT1 gene at 50.8 Mb. NFAT1 also regulates the transcription of insulin and glucagon genes in the pancreatic β-cells (17, 24), which may partly explain the linkage for Type 2 diabetes on chromosome 20q13 (5, 18, 21, 22, 27, 30, 35, 44, 51). Moreover, NFAT1 regulates the differentiation and development of vascular endothelial cells, skeletal muscle cells, adipocytes, and chondrocytes (19) and could thereby contribute to chronic inflammatory diseases, such as atherosclerosis, diabetes, obesity, and arthritis. C/EBPβ is a member of the CCAAT/enhancer binding protein (C/EBP) family and is one of the key transcription factors in the induction of genes involved in the acute phase response, innate immunity, and inflammation (35). The C/EBPβ gene is located 1.3 Mb from the NFAT1 gene at 49.5 Mb. C/EBPβ is important in the induction of CRP expression by interleukin (IL)-6 (3). C/EBPβ is also involved in the differentiation of adipocytes (36), which could partly explain the linkage for obesity on chromosome 20q13 (11, 12, 25, 26, 31). The inherited deficiency of adenosine deaminase (ADA) causes one form of severe combined immunodeficiency disease, in which there is a dysfunction of both B and T lymphocytes, with an impaired cellular immunity and a decreased production of immunoglobulins (9). A milder dysfunction of the ADA gene, which is located at 43.9 Mb, may contribute to the linkage for plasma CRP levels in healthy whites of the present study. However, chromosome 20q13 does not contain CRP. IL-6, or TNF-α genes, the variants of which have been previously reported to be associated with plasma CRP levels (6, 46, 48).

The present genome-wide linkage scan suggests that chromosome 20q13 may contribute to plasma CRP levels in healthy whites. This region contains genes that are important in the inflammatory process and may play a role in the development of chronic inflammatory diseases. The present findings may be useful in the ongoing effort to search for genes contributing to inflammation and to identify individuals at an increased risk of chronic inflammatory diseases.

ACKNOWLEDGMENTS

Gratitude is expressed to Dr. Andre Nadeau and the staff of the Diabetes Research Unit and to the staff of the Lipid Research Center, Laval University Medical Center, Ste-Foy, Québec, Canada, for their contribution to the HERITAGE Family Study, and to Dr. Jacob Brand for his contribution to the statistical analyses. Some of the results were obtained by using the SAGE statistical package, which is supported by a US Public Health Service Resource Grant (1 P41 RR-03655) from the National Center for Research Resources.

REFERENCES


