Interleukin-6 Receptor Gene, and Diabetes Risk in Women

Lu Qi,1,2 Nader Rifai,3 and Frank B. Hu1,2

OBJECTIVE—Recent genome-wide association studies (GWASs) related common variants in the interleukin-6 (IL-6) receptor (IL6R) gene to plasma C-reactive protein (CRP) concentrations. Because IL6R variants were previously associated with IL-6 levels, we tested whether the associations with CRP were independent of IL-6 and the interactions between IL6R variants and CRP in relation to diabetes risk.

RESEARCH DESIGN AND METHODS—Plasma CRP and IL-6 levels and 10 IL6R polymorphisms were determined in a nested case-control study of 633 diabetic and 692 healthy Caucasian women.

RESULTS—In both nondiabetic and diabetic women, IL6R polymorphisms were associated with plasma CRP levels, independent of IL-6 concentration. After adjustment of IL-6 levels, CRP concentrations in the genotype AA, AC, and CC of the GWAS polymorphism rs8192284 were 0.32, 0.26, and 0.24 pg/ml, respectively, among nondiabetic women (P for trend = 0.005; false discovery rate [FDR] = 0.01) and 0.63, 0.48, and 0.43 pg/ml among diabetic women (P for trend <0.0001; FDR = 0.0001). Haplotypes inferred from polymorphisms within a linkage disequilibrium block including rs8192284 were also significantly associated with CRP levels (P = 0.0002). In an exploratory analysis, rs8192284 showed significant interactions with CRP levels in relation to diabetes risk (P for interaction = 0.026). The odds ratios across increasing quartiles of CRP were 2.19 (95% CI 1.42–3.36), 2.03 (1.27–3.23), and 2.32 (1.77–4.82) in the carriers of allele-C and 2.21 (1.18–4.12), 3.77 (1.87–7.57), and 5.02 (2.4–10.5) in the noncarriers.

CONCLUSIONS—IL6R variants were significantly associated with plasma CRP, independent of IL-6 levels. IL6R variants may interact with CRP in predicting diabetes risk.

Plasma C-Reactive Protein, and Diabetes Risk in Women

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CONCLUSIONS—IL6R variants were significantly associated with plasma CRP, independent of IL-6 levels. IL6R variants may interact with CRP in predicting diabetes risk.
Haplotype analysis was conducted based on the Stochastic-EM algorithm statistical package was used for the analyses (SAS, version 8.2 for UNIX). The proportion of results declared positive that are actually false (18). The SAS and Hochberg (17) using SAS procedure PROC MULTTEST. FDR estimates rate (FDR) for the analyses on the polymorphisms by the method of Benjamini and Hochberg (17) to adjust for the multiple testing. The FDRs for all of these associations were <0.05. In most cases, similar associations between IL6R polymorphisms and CRP levels were observed in the diabetic patients.

We inferred the haplotypes from the polymorphisms within the linkage disequilibrium block (including SNPs rs6684439, rs4845622, rs8192284, rs4329505, rs4240872, rs2229238, and rs4845617). Because rs6684439, rs4845622, and rs8192284, which were in strong linkage disequilibrium, were significantly associated with low plasma CRP levels after adjusting for IL-6 and other covariates (Table 2). SNPs rs12083537 and rs4329505 were associated with high plasma CRP levels. We calculated FDR by the method of Benjamini and Hochberg (17) to adjust for the multiple testing. The FDRs for all of these associations were <0.05. In most cases, similar associations between IL6R polymorphisms and CRP levels were observed in the diabetic patients.

To account for multiple statistical testing, we calculated false discovery rate (FDR) for the analyses on the polymorphisms by the method of Benjamini and Hochberg (17) using SAS procedure PROC MULTTEST. FDR estimates the proportion of results declared positive that are actually false (18). The SAS statistical package was used for the analyses (SAS, version 8.2 for UNIX). Haplotypes were inferred using the Stochastic-EM algorithm using THESIAS program (19). All P values are two sided.

RESULTS
Table 1 shows age and age-adjusted baseline characteristics according to rs8192284 genotypes in nondiabetic women with both CRP and IL-6 measurements available. IL-6 levels were higher in women with AC and CC genotypes compared with those with AA genotype. The genotypes were not associated with any other characteristics.

In nondiabetic women, the three SNPs rs6684439, rs4845622, and rs8192284, which were in strong linkage disequilibrium, were significantly associated with low plasma CRP levels after adjusting for IL-6 and other covariates (Table 2). SNPs rs12083537 and rs4329505 were associated with high plasma CRP levels. We calculated FDR by the method of Benjamini and Hochberg (17) to adjust for the multiple testing. The FDRs for all of these associations were <0.05. In most cases, similar associations between IL6R polymorphisms and CRP levels were observed in the diabetic patients.

Table 2 Plasma CRP levels by IL6R genotypes in nondiabetic and diabetic women

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Nondiabetic (pg/ml)</th>
<th>Diabetic (pg/ml)</th>
<th>P*</th>
<th>FDR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(major/minor)</td>
<td>11</td>
<td>12</td>
<td>22</td>
</tr>
<tr>
<td>rs4845617</td>
<td>G/A</td>
<td>0.27 (0.02)</td>
<td>0.26 (0.02)</td>
<td>0.30 (0.03)</td>
</tr>
<tr>
<td>rs12083537</td>
<td>T/C</td>
<td>0.27 (0.01)</td>
<td>0.28 (0.02)</td>
<td>0.25 (0.05)</td>
</tr>
<tr>
<td>rs4075015</td>
<td>A/C</td>
<td>0.30 (0.02)</td>
<td>0.27 (0.02)</td>
<td>0.24 (0.03)</td>
</tr>
<tr>
<td>rs6684439</td>
<td>C/T</td>
<td>0.27 (0.02)</td>
<td>0.27 (0.02)</td>
<td>0.24 (0.03)</td>
</tr>
<tr>
<td>rs4845618</td>
<td>A/C</td>
<td>0.32 (0.02)</td>
<td>0.27 (0.02)</td>
<td>0.24 (0.03)</td>
</tr>
<tr>
<td>rs4845622</td>
<td>T/G</td>
<td>0.27 (0.02)</td>
<td>0.27 (0.02)</td>
<td>0.24 (0.03)</td>
</tr>
<tr>
<td>rs8192284</td>
<td>A/C</td>
<td>0.30 (0.02)</td>
<td>0.26 (0.02)</td>
<td>0.24 (0.03)</td>
</tr>
<tr>
<td>rs3329505</td>
<td>T/C</td>
<td>0.27 (0.01)</td>
<td>0.27 (0.02)</td>
<td>0.27 (0.03)</td>
</tr>
<tr>
<td>rs4240872</td>
<td>A/G</td>
<td>0.27 (0.01)</td>
<td>0.29 (0.02)</td>
<td>0.27 (0.03)</td>
</tr>
<tr>
<td>rs2229238</td>
<td>G/A</td>
<td>0.27 (0.01)</td>
<td>0.30 (0.02)</td>
<td>0.32 (0.08)</td>
</tr>
</tbody>
</table>

Data are means (SE) unless otherwise indicated. For each polymorphism, 11 represents the major allele homozygotes, 12 represents the heterozygotes, and 22 represents the minor allele homozygotes; missing genotyping is not included. *Comparisons between carriers and noncarriers adjusted for age, BMI, alcohol consumption, smoking, physical activity, family history of diabetes, menopausal status, and IL-6.
are significantly correlated (22). In our earlier analyses, we found that IL6R variants are significantly related to IL-6 levels (7). The same association was also observed in the Health ABC study (8). Our data from the present study suggest that the association between IL6R variants and CRP levels is unlikely due to the changes in IL-6 levels, because the IL6R gene–CRP association was independent of IL-6 levels.

Because CRP and IL-6 are not the direct products of the IL6R gene, the associations between IL6R variants and these biomarkers are likely mediated by other metabolic changes. SNP rs8192284 in the IL6R gene has been associated with soluble IL6R levels (8). These data suggest that IL6R variant may primarily affect IL6 levels and that the changes in CRP and IL-6 are likely secondary.

The precise mechanisms underlying the opposite associations of IL6R variant (rs8192284) with IL-6 and CRP are not clear. The differing associations are particularly puzzling considering that IL-6 may stimulate the product of CRP in the liver. However, the data from our study are highly consistent with the GWAS and previous studies (8,9,20). We suspect that the genotype-related changes in CRP and IL-6 levels may be parallel changes, rather than sequential events, both induced by the alterations in IL6R products.

Subclinical systemic inflammation is now considered an important mechanism leading to insulin resistance and type 2 diabetes (23). Epidemiological studies have documented that circulating inflammatory markers, including CRP, significantly predict diabetes risk (4–6). Previous studies indicate that polymorphisms affecting CRP levels may also influence the risk of type 2 diabetes (24). Although IL6R SNPs were not significantly associated with the incidence of diabetes in our study sample (7), the exploratory analysis indicated that the genetic variant might modify the association between CRP levels and diabetes risk. The associations between CRP and increased risk of type 2 diabetes are more evident in women carrying genotype AA of rs8192284 compared with those carrying the minor allele C. This observation reflects a synergic effect of IL6R genotype and CRP levels on the development of diabetes. The observed interaction needs to be confirmed in future studies.

Several limitations need to be considered. SNP rs4129267 reported by the GWAS (9) was not typed in the present study. However, this SNP is in near perfect linkage disequilibrium with rs8192284 (D’ = 1 and r² = 0.96; HapMap, CEU). Population stratification arising from ethnic admixture may cause spurious associations. However, the present study was less likely to be influenced by population stratification because the study populations were highly homogeneous, including only European Caucasians. In addition, our analyses were restricted to women and therefore may not be generalized to men.

In summary, we demonstrated that the IL6R variants are significantly associated with plasma CRP levels, independent of IL-6 levels. In addition, IL6R variant interacts with CRP in relation to diabetes risk. Further research is warranted to elucidate the potential mechanisms underlying the associations between IL6R variants and the opposite changes in CRP and IL-6 levels.

DISCUSSION

We found significant associations between IL6R variants and plasma CRP levels. Our results are consistent with the findings from recent GWASs (9,20). We demonstrated that the associations between IL6R variants and CRP levels are independent of IL-6 concentration. In an exploratory analysis, we found IL6R variant rs8192284 significantly interacts with CRP levels in relation to diabetes risk. The associations between CRP levels and increased diabetes risk are more evident in women carrying the wild-type genotype than in those with the minor allele C.

IL-6 is a pleiotropic cytokine that performs as the chief stimulator of the production of CRP from the liver (21). In epidemiological studies, circulating levels of IL-6 and CRP are significantly correlated (22). In our earlier analyses, we

**FIG. 1.** Haplotype (inferred from SNPs rs4845618, rs8192284, rs4329505, rs4240872, and rs2229238) associations with plasma CRP concentration. Haplotype coding: 1, the common allele; 2, the minor allele. Analyses were adjusted for age, BMI, smoking, alcohol consumption, physical activity, family history of diabetes, menopausal status, and IL-6.

**FIG. 2.** The ORs of diabetes risk associated with the plasma CRP levels (in quartiles) by the strata of the genotypes of IL6R variant rs8192284 (AA and AC+CC). Error bars represent 95% CIs.

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