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Accessibility
Interleukin-6 Receptor Gene, Plasma C-Reactive Protein, and Diabetes Risk in Women

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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 2.92 (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. Diabetes 58: 275–278, 2009

Chronic systemic inflammation can induce insulin resistance and is a key mechanism linking obesity and diabetes (1). As a nonspecific marker of systemic inflammation, C-reactive protein (CRP) is an acute-phase reactant synthesized in the liver in response to cytokines (2), especially interleukin-6 (IL-6) (3). In epidemiological studies, circulating CRP levels significantly predict the risk of type 2 diabetes (4–6).

In an earlier analysis (7), we found that the common variants in the IL-6 receptor (IL6R) gene, especially a single nucleotide polymorphism (SNP) rs8192284 were significantly related to high-plasma IL-6 concentration. Similar associations between IL6R SNPs and IL-6 concentration were also observed in an admixture study (8). In a recent genome-wide association study (GWAS) on plasma CRP levels among 6,345 apparently healthy women, common SNPs in the IL6R gene including rs8192284 were associated with CRP concentration at genome-wide significance level (P < 5 × 10⁻⁸) (9). Because of the close relationship between IL-6 and CRP, we hypothesized that the IL6R gene–CRP associations observed in the GWAS might be secondary to the changes in circulating IL-6 levels.

To test this hypothesis, we examined the associations between IL6R gene variants and CRP concentrations in healthy and diabetic women, controlling for IL-6 levels. We also assessed the interactions between IL6R SNPs and plasma CRP levels in predicting the risk of type 2 diabetes.
Participants (n) 234 342 101
Age (years) 55 ± 9 57 ± 7 56 ± 7 0.56
BMI (kg/m²) 26.2 ± 6.6 26.3 ± 5.6 27.6 ± 6.6 0.15
Physical activity (MET h/week) 12.9 ± 15.4 16.3 ± 19.4 14.8 ± 13.2 0.07
Alcohol consumption (g/day) 6.34 ± 11.0 5.54 ± 9.62 5.31 ± 7.87 0.54
Family history of diabetes 20.9 21 22.8 0.92
Current smoker 14.7 10.8 8 0.37
Postmenopausal status 76.1 81.3 78.2 0.31
IL-6 (ng/ml) 1.84 ± 1.97 1.99 ± 2.07 2.14 ± 1.82 0.003

Data are percent or means ± SD.

rs12083537, rs4075015, rs6684439, rs4845622, rs1922824, rs4329505, rs4240872, rs2229238, and rs4845617 for IL6R were selected as previously described (7). The tagging SNPs cover ~94% allele variance. The polymorphisms were genotyped using Taqman SNP allelic discrimination by means of an ABI 7900HT (Applied Biosystems, Foster City, CA). Replicate quality-control samples (10%) were included and genotyped with >99% concordance. All genotypes fit Hardy-Weinberg equilibrium (HWE).

**Statistical analyses.** A χ² test was used to assess whether the genotypes were in HWE. General linear models were used to compare geometric mean values of quantitative traits across groups. Plasma CRP and IL-6 were logarithmically transformed to improve the normality. We adjusted for covariates, including age (continuous), BMI (kg/m²), physical activity (<1.5, 1.5–5.0, 6.0–11.9, 12–20.9, or >20.9 MET h/week), smoking (never, past, or current), alcohol intake (nondrinker or drinker [0.1–4.9, 5–10, or >10 g/day]), family history of diabetes, menopausal status (pre- or postmenopausal [never, past, or current hormone use]), and IL-6 levels (in quartiles). Because of the missing data in biomarkers and genotyping, some matching pairs in the case-control design were broken. Therefore, we used unconditional logistic regression as the primary analysis 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). Haplotype analysis was conducted based on 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 for 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.

We inferred the haplotypes from the polymorphisms within the linkage disequilibrium block (including SNPs rs6684439, rs4845622, rs1922824, rs4329505, rs4240872, rs2229238, and rs4845617). Because rs6684439, rs4845622, and rs1922824 are in nearly perfect linkage disequilibrium (r² > 0.9), only rs8192284 was kept in haplotype inference, together with the other four SNPs in this linkage disequilibrium block (rs4845618, rs4329505, rs4240872, and rs2229238). Five common haplotypes accounted for ~90% allele variance of the linkage disequilibrium block. Haplotypes 21122 (P = 0.033; 1, the common allele; 2, the minor allele) and 11211 (P = 0.0006) were associated with 0.13 (0.01–0.26) and 0.23 (0.10–0.37) pg/ml higher CRP levels, respectively, compared with the most common haplotype 12111, adjusting for IL-6 and other covariates (Fig. 1). The
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 IL6R 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 r^2 = 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.

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