

Interleukin-6 Receptor Gene Variations, Plasma Interleukin-6 Levels, and Type 2 Diabetes in U.S. Women

Lu Qi,^{1,2} Nader Rifai,³ and Frank B. Hu^{1,2,3}

OBJECTIVE—To examine the associations between common variations in the *IL6R* gene and circulating interleukin (IL)-6 levels and diabetes risk.

RESEARCH DESIGN AND METHODS—We determined 10 linkage disequilibrium (LD)-tagging single nucleotide polymorphisms (SNPs) (SNP1 to SNP10) for the *IL6R* gene in a nested case-control study of 672 diabetic and 1,058 healthy European Caucasian women (IL-6 levels were measured in a subgroup of 1,348 women).

RESULTS—In both control and diabetic patients, polymorphisms within an LD block spanning ~42 kb were significantly associated with plasma IL-6 levels. A missense variant SNP7 in exon 9 (rs8192284, Asp358Ala) showed the strongest association ($P = 0.0005$ in control and $P = 0.004$ in case subjects). The corresponding false-discovery rates, which accounts for multiple testing, were 0.008 and 0.02, respectively. We inferred five common haplotypes to capture 94% allele variance of the LD block using SNP5, -7, -8, -9, and -10. Compared with the most common haplotype 12111 (one codes the common and two codes the minor alleles), haplotypes 11211 [difference in $\log(\text{IL-6}) = -0.11$ (95% CI -0.23 to -0.01); $P = 0.01$] and 21122 (-0.15 [-0.27 to -0.03]; $P = 0.01$) were associated with significantly lower IL-6 levels (global test, $P = 0.01$). However, *IL6R* genotypes were not significantly associated with the risk of type 2 diabetes.

CONCLUSIONS—*IL6R* genetic variations, especially SNP7 (rs8192284, Asp358Ala), were significantly associated with plasma IL-6 levels but not with diabetes risk in women. The strong associations between *IL6R* genetic variability and IL-6 concentrations deserve further investigation. *Diabetes* 56: 3075–3081, 2007

From the ¹Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts; the ²Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts; the ³Department of Laboratory Medicine, Children's Hospital and Harvard Medical School, Boston, Massachusetts; and the ⁴Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts.

Address correspondence and reprint requests to Dr. Lu Qi, Department of Nutrition, Harvard School of Public Health, 665 Huntington Ave., Boston, MA 02115. E-mail: nhlqi@channing.harvard.edu; or Dr. Frank Hu, frank.hu@channing.harvard.edu.

Received for publication 11 April 2007 and accepted in revised form 18 September 2007.

Published ahead of print at <http://diabetes.diabetesjournals.org> on 19 September 2007. DOI: 10.2337/db07-0505.

FDR, false-discovery rate; IL, interleukin; LD, linkage disequilibrium; SNP, single nucleotide polymorphism.

© 2007 by the American Diabetes Association.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Interleukin (IL)-6 is a pleiotropic cytokine that performs as the chief stimulator of most acute-phase proteins (1) and has both proinflammatory and anti-inflammatory effects (2). There is compelling evidence that augmented levels of IL-6 are associated with several metabolic diseases, including type 2 diabetes and cardiovascular disease (3–6). IL-6 acts via a receptor complex consisting of two functional membrane proteins: an 80-kDa ligand-binding IL-6 receptor and a 130-kDa signal transducer (gp130) (7,8). Soluble and active forms of the extracellular domain of the IL-6 receptor, which is produced both by differential splicing of the IL-6 receptor transcript and by proteolytic cleavage of the protein in a process termed shedding, are also found in normal serum and synovial fluids. Most IL-6 action may be mediated through membrane-bound IL-6 receptors.

Several studies (9,10) have documented that *IL6R* gene variants were associated with obesity, insulin sensitivity (11), metabolic syndrome (12), and diabetes risk (13). However, few studies have used genetic markers that represent the overall variability of the gene, and the results are not consistent across populations. Moreover, little is known about whether *IL6R* variations affect the levels of circulating IL-6.

In this study, we selected linkage disequilibrium (LD)-tagging single nucleotide polymorphisms (SNPs) for the *IL6R* gene and also included a polymorphism (rs8192284) previously associated with functional changes of the *IL6R* gene and diabetes risk (13,14). We examined the associations between *IL6R* variations, plasma IL-6 levels, and diabetes risk in a nested case-control study of women from a prospective cohort, the Nurses' Health Study.

RESEARCH DESIGN AND METHODS

The Nurses' Health Study was established in 1976, when 121,700 female registered nurses aged 30–55 years and residing in 11 large U.S. states completed a mailed questionnaire on their medical history and lifestyle (15). The lifestyle factors, including smoking, menopausal status and postmenopausal hormone therapy, and body weight, have been updated by validated questionnaires every 2 years. Samples for the present case-control study were selected from a subcohort of 32,826 women who provided a blood sample between 1989 and 1990 and were free from diabetes, cardiovascular disease, stroke, or cancer at the time of blood collection. Incident cases were defined as self-reported diabetes confirmed by a validated supplementary questionnaire and diagnosed at least 1 year after blood collection through 2000. The supplementary questionnaire obtained information on symptoms, diagnostic tests, and hypoglycemic therapy used to define type 2 diabetic cases. Medical record review confirmed the diagnosis of type 2 diabetes using this questionnaire for 98% of cases using the National Diabetes Data Group criteria (16). We used the American Diabetes Association diagnostic criteria for diagnosis of diabetes cases during the 1998 and 2000 cycles (17).

The present study included 1,730 European Caucasian subjects, 672 incident case subjects, and 1,058 healthy control subjects, matched on age, month and year of blood draw, and fasting status. For the case subjects diagnosed in 1996 or earlier, two control subjects were matched to each case subject. One of two control subjects was also matched according to BMI (± 1

TABLE 1
Age and age-adjusted clinical characteristics of healthy control and diabetic case subjects with IL-6 measurement (1989–1990)

	Healthy control subjects	Diabetic patients	<i>P</i>
<i>n</i>	706	642	
Age (years)	56 ± 8	56 ± 8	0.90
BMI (kg/m ²)	26.5 ± 6.2	30.6 ± 5.6	<0.001
Physical activity (MET h/week)	14.9 ± 17.8	12.2 ± 15.0	0.003
Alcohol consumption (g/day)	5.80 ± 9.87	2.83 ± 6.57	<0.001
Current smoker (%)	11.7	12.9	0.48
Postmenopausal status (%)	78.9	81.3	0.27
Log(IL-6) (ng/ml)	0.64 ± 0.70	0.90 ± 0.69	<0.001

Data are means ± SD or percent.

kg/m²). For the case subjects diagnosed after 1996, one control subject was matched to each case subject. To improve statistical control for obesity at the upper extreme of the distribution, control women were also matched on BMI to case subjects in the top 10% of the BMI distribution (18).

Assessment of plasma IL-6 levels and covariates. Blood sample collection and processing were previously described (6,19). Plasma concentrations of IL-6 were measured in a subset of women (642 diabetic case and 706 control subjects) using a quantitative sandwich enzyme immunoassay technique (Quantikine HS Immunoassay kit). The coefficient of variation was 5.9%. BMI was calculated as weight in kilograms divided by the square of height in meters. Physical activity was expressed as metabolic equivalent task (MET) hours based on self-reported types and durations of activities over the previous year.

Tagging SNP selection and genotype determination. DNA was extracted from the buffy coat fraction of centrifuged blood using the QIAmp Blood Kit

(Qiagen, Chatsworth, CA). Tagging SNPs for *IL6R* were selected from HapMap (HapMap Phase II, public release no. 19) using the pairwise tagging mode (20). We defined the common variants as those with minor allele frequency >5% and set the threshold of 0.8 for LD measure *r*², at which all alleles are to be captured. Ten polymorphisms (rs4845618, rs12083537, rs4075015, rs6684439, rs4845622, rs8192284, rs4329505, rs4240872, rs2229238, and rs4845617) were genotyped using Taqman SNP allelic discrimination by means of an ABI 7900HT (Applied Biosystems, Foster City, CA). The genotyped polymorphisms are presented in Table 1. Replicate quality-control samples (10%) were included and genotyped with >99% concordance. For convenience, we named the polymorphisms SNP1 to SNP10 (Fig. 1).

Statistical analyses. A χ^2 test was used to assess whether the genotypes were in Hardy-Weinberg equilibrium and to compare the genotype and allele frequencies between case and control subjects. Odds ratios were calculated using the unconditional logistic regression model. General linear models were used to compare geometric mean values of quantitative traits across groups. Plasma IL-6 was not normally distributed and was logarithmically transformed to improve normality. We adjusted for covariates including age (continuous), BMI (<23, 23–24.9, 25–29.9, 30–34.9, or >35 kg/m²), physical activity (<1.5, 1.5–5.9, 6.0–11.9, 12–20.9, and > 21.0 MET h/week), smoking (never, past, and current), alcohol intake (nondrinker and drinker [0.1–4.9, 5–10, or >10 g/day]), family history of diabetes, and menopausal status (pre- or postmenopausal [never, past, or current hormone use]).

To account for multiple statistical testing, we calculated the false-discovery rate (FDR) for the analyses on the polymorphisms by the method of Benjamini and Hochberg (21) using SAS procedure PROC MULTTEST. FDR estimates the proportion of results declared positive that are actually false (22). 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 the THESIAS program (23). Mendelian randomization instrumental variable analysis has been used to estimate the unconfounded causal effects in epidemiological studies (24,25). This approach bypasses the need to adjust for the confounders by estimating the average effect of biomarker levels on disease from two effects of the instrumental variable: 1) the average effect of the instrumental variable on disease and 2) the average effect of the instrumental variable on biomarker levels. We conducted Mendelian randomization analysis to estimate the association between IL-6 levels and diabetes

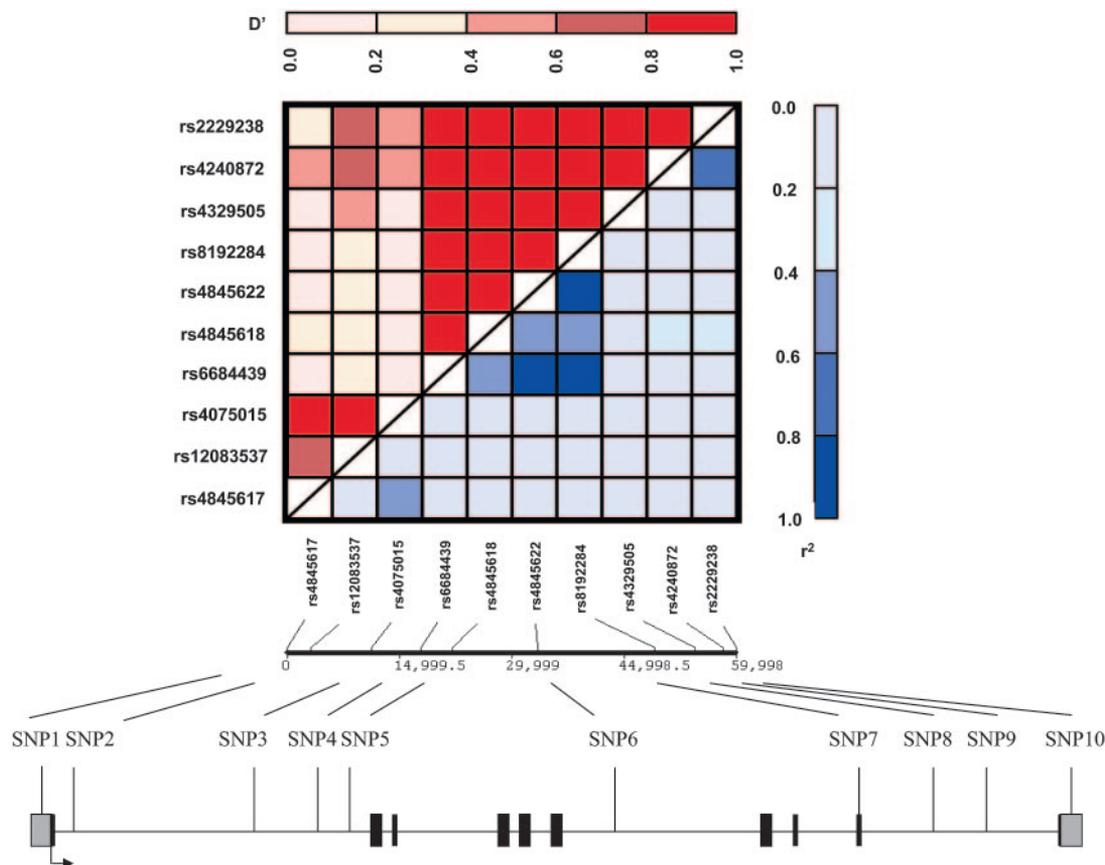


FIG. 1. Pairwise LD matrix for *IL6R* genes. *D'* is presented above the diagonal and *r*² is presented below the diagonal. (Please see <http://dx.doi.org/10.2337/db07-0505> for a high-quality digital representation of this figure.)

TABLE 2
Log(IL-6) by *IL6R* genotypes in healthy control and diabetes case subjects

	Healthy women			<i>P</i> *	FDR	Diabetic women			<i>P</i> *	FDR
	11	12	22			11	12	22		
SNP1	246 0.66 ± 0.04	317 0.61 ± 0.04	114 0.68 ± 0.06	0.51	0.60	223 0.85 ± 0.04	299 0.92 ± 0.04	99 0.86 ± 0.07	0.34	0.45
SNP2	409 0.64 ± 0.03	243 0.62 ± 0.04	37 0.76 ± 0.11	0.94	0.94	394 0.92 ± 0.03	208 0.85 ± 0.05	25 0.99 ± 0.14	0.29	0.41
SNP3	222 0.61 ± 0.05	357 0.66 ± 0.04	112 0.68 ± 0.07	0.38	0.47	215 0.88 ± 0.04	303 0.91 ± 0.04	110 0.87 ± 0.06	0.62	0.69
SNP4	238 0.54 ± 0.04	341 0.67 ± 0.04	107 0.76 ± 0.07	0.005	0.02	235 0.81 ± 0.04	309 0.90 ± 0.04	87 1.10 ± 0.07	0.018	0.05
SNP5	215 0.72 ± 0.05	345 0.63 ± 0.04	114 0.50 ± 0.06	0.039	0.07	176 0.99 ± 0.05	325 0.90 ± 0.04	119 0.77 ± 0.06	0.035	0.07
SNP6	225 0.51 ± 0.05	355 0.68 ± 0.04	105 0.75 ± 0.07	0.0008	0.008	225 0.80 ± 0.04	312 0.91 ± 0.04	96 1.08 ± 0.07	0.01	0.03
SNP7	239 0.51 ± 0.04	347 0.69 ± 0.04	105 0.76 ± 0.07	0.0005	0.008	227 0.79 ± 0.04	308 0.91 ± 0.04	88 1.11 ± 0.07	0.004	0.02
SNP8	476 0.65 ± 0.03	197 0.64 ± 0.05	17 0.45 ± 0.17	0.66	0.69	448 0.92 ± 0.03	171 0.80 ± 0.05	12 1.19 ± 0.18	0.12	0.20
SNP9	405 0.69 ± 0.03	253 0.57 ± 0.04	27 0.53 ± 0.13	0.02	0.05	362 0.96 ± 0.03	237 0.82 ± 0.04	32 0.74 ± 0.11	0.008	0.03
SNP10	462 0.68 ± 0.03	218 0.55 ± 0.05	17 0.64 ± 0.17	0.026	0.06	412 0.92 ± 0.03	198 0.86 ± 0.05	19 0.65 ± 0.15	0.23	0.35

Data are *n* or means ± SE. 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, and menopausal status.

risk, accounting for variances in both the *IL6R* genotype-IL-6 levels and genotype-diabetes associations using the Murphy-Topel method (26). We treated *IL6R* SNP7 as an instrumental variable for IL-6 levels. The QVF command in STATA 9.2 (STATA, College Station, TX) was used for instrumental variable analysis. All *P* values are two sided.

RESULTS

In healthy control subjects, the allele frequency of *IL6R* polymorphisms ranged from 0.17 to 0.43. All genotypes fit Hardy-Weinberg equilibrium. SNP4 to SNP10 were in strong LD ($D' > 0.8$), with pairwise r^2 ranging from 0.05 to 0.95 (Fig. 1). This is consistent with the earlier-reported LD structure of the *IL6R* gene (11). The characteristics of the diabetes case and control subjects in the present study have been previously described (18,19). Plasma IL-6 levels were measured in a subgroup of women (642 diabetic patients and 706 control subjects). There was no significant difference in the basic characteristics between women with and without IL-6 measurement. Table 1 shows the age-adjusted baseline characteristics for women with IL-6 measurement available. Diabetic women had significantly higher IL-6 levels (log transformed) than the control subjects.

In control subjects, carriers of the minor allele at SNP4, SNP6, and SNP7 had significantly higher plasma IL-6 levels, whereas carriers of the minor allele at SNP5, SNP9, and SNP10 had significantly lower plasma IL-6 levels (Table 2). Adjustment for age, BMI, smoking, alcohol consumption, physical activity, family history of diabetes, and menopausal status did not appreciably change the associations. Similar but less significant associations between *IL6R* polymorphisms and IL-6 levels were also observed in the diabetic patients. We calculated FDR by the method of Benjamini and Hochberg (21) to adjust for the multiple testing. SNP7 (Asp358Ala) showed the strongest association with IL-6 levels with an FDR of 0.008 and 0.02 in the control women and diabetic patients, respectively. In nondiabetic women, the SNP7-associated differ-

ence in IL-6 levels was consistently significant across strata by obesity (30 kg/m² as a cutoff), smoking (never versus current or past smoking), alcohol consumption (0 g/day as a cutoff), and physical activity (6.0 MET h/week as a cutoff) (Fig. 2). Tests for the interactions with these lifestyle factors were not significant.

Because haplotype analysis conserves joint LD structure and incorporates information from multiple adjacent genetic markers, it can be useful in narrowing down the culprit of the association. We inferred the haplotypes from the polymorphisms within the LD block (SNP4 to SNP10, ~42 kb). Because SNP4, SNP6, and SNP7 are nearly completely correlated ($r^2 > 0.9$; Fig. 1), only SNP7 was kept in haplotype inference, together with SNP5, SNP8, SNP9, and SNP10. Five common haplotypes accounted for ~94% allele variance of the LD block. We treated the most common haplotype 12111 as the reference. All other common haplotypes, differed by SNP7 from haplotype 12111, were associated with lower IL-6 levels compared with haplotype 12111 in nondiabetic women (Table 3). This indicated that SNP7 might be in strong LD with a SNP that could be the causal variant contributing to the elevated IL-6 levels.

We further examined the associations between *IL6R* polymorphisms and diabetes risk. The distributions of *IL6R* genotypes were not significantly different in diabetic patients and the control subjects (Table 4). Also, the *IL6R* haplotypes were not significantly associated with the risk of diabetes (data not shown). Adjustment for the IL-6 levels and other covariates did not appreciably change the results. Logistic regression analyses indicated that IL-6 levels were significantly associated with diabetes risk, with an odds ratio of 1.78 (95% CI 1.49–2.10) per unit change of log(IL-6). In the Mendelian randomization instrumental variable analysis, using SNP7 as an instrument for IL-6 concentration, the estimated causal effect was an

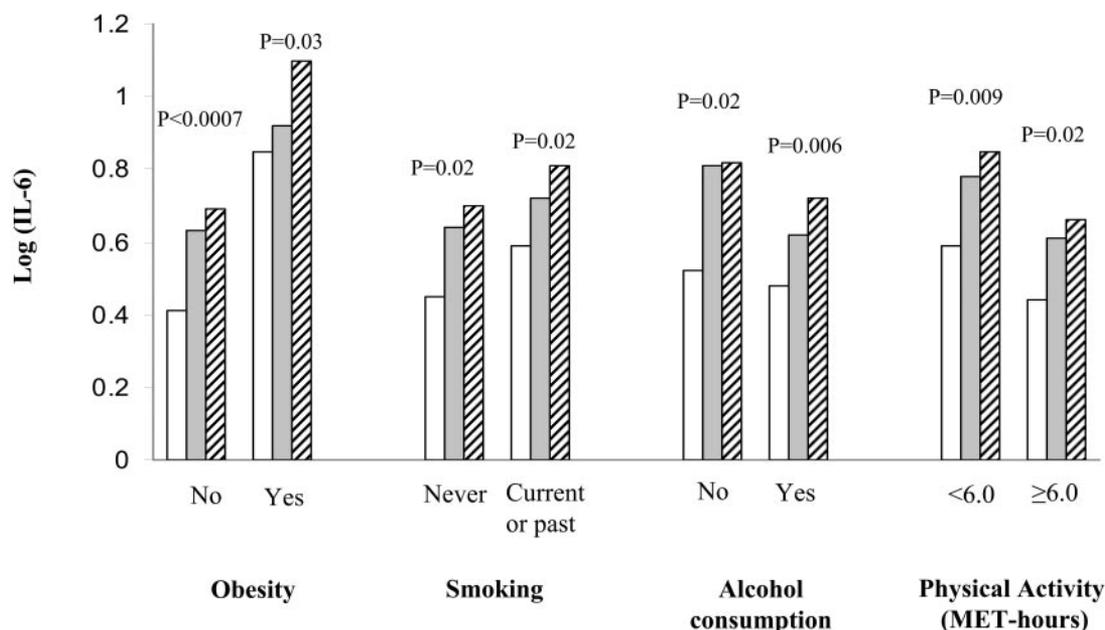


FIG. 2. The geometric means of log(IL-6) (plasma IL-6, in ng/ml) by the genotypes of SNP7 (rs8192284) stratified by obese status (30 kg/m² as a cutoff), smoking (never versus current or past smoking), alcohol consumption (0 g/day as a cutoff), and physical activity (6.0 MET h/week as a cutoff). The analyses were conducted in nondiabetic women with IL-6 measurement. Covariates, including age, BMI, smoking, alcohol consumption, physical activity, family history of diabetes, and menopausal status, were adjusted when they were not used as the strata variables. Genotypes: □, AA; ▣, AC; ▨, CC.

odds ratio of 1.59 (0.45–5.66) for diabetic risk per unit change of log(IL-6).

DISCUSSION

We found significant associations between *IL6R* variations, especially SNP7 (rs8192284) and plasma IL-6 levels. Our results are highly consistent with a recent study (27). Reich et al. (27) conducted an admixture mapping in 1,184 African Americans and a replication association study in 1,674 European Americans from the Health ABC study, in which *IL6R* variant rs8192284 (SNP7 in our study) was found to be strongly and significantly associated with higher IL-6 levels, in addition to elevated IL-6 soluble receptor levels.

The independent findings from our study and Reich et al.'s study (27) and the close functional relatedness between the *IL6R* gene and IL-6 activity strongly support a causal relation between *IL6R* genetic variability and IL-6 homeostasis. Moreover, following several lines of evidence indicate that our results are less likely due to chance: 1) The observed associations were robust and survived the adjustment for multiple testing. The FDR method controls the expected proportion of false-positives among all positive results over multiple testing (28,29). This method is thought to be an efficient approach for

multiple-comparison adjustment and has been widely used in the genetic association studies (30,31). The low value of FDR (e.g., <0.05 or 0.10) usually suggests that the observed associations is less likely a false signal. 2) The genotype-associated differences in IL-6 were consistently observed in both healthy and diabetic women and were independent of adiposity and lifestyle confounders. Several polymorphisms in the *IL6R* gene, which are in strong LD, were associated with IL-6 levels. Results from the haplotype analyses suggested that SNP7 was the principal polymorphism contributing to the elevation of IL-6 levels.

There is some evidence that IL-6 production is genetically influenced (32). However, little is known about the genes regulating IL-6 homeostasis. Data on the relation between variations in the *IL6* gene, which encodes IL-6, and IL-6 levels are conflicting (33–35). In an earlier analysis of U.S. women, we did not find significant associations between *IL6* genetic variability and IL-6 levels (19). This suggests that other chromosome loci may account for the interindividual variance. Our results from the present study and the findings from Reich et al.'s study (27) together suggest that *IL6R* may be a potential candidate gene for IL-6 homeostasis.

The association between *IL6R* variants and IL-6 levels appeared less evident in the diabetic than in the healthy

TABLE 3
Plasma levels (log transformed) of IL-6 according to *IL6R* haplotypes in nondiabetic women

Haplotypes					Frequency	Difference in log(IL-6) (95% CI)	P	Global P
SNP5	SNP7	SNP8	SNP9	SNP10				
1	2	1	1	1	37.6	0.0		
1	1	2	1	1	15.8	-0.11 (-0.23 to -0.01)	0.05	0.01
2	1	1	1	1	18.2	-0.12 (-0.22 to -0.03)	0.01	
2	1	1	2	1	4.6	-0.17 (-0.38 to 0.04)	0.12	
2	1	1	2	2	17.5	-0.15 (-0.27 to -0.03)	0.01	

Haplotype coding: 1 represents the common allele and 2 represents the minor allele; analyses were adjusted for age and BMI.

TABLE 4
Distribution of *IL6R* polymorphisms in diabetic and control subjects

SNPs	Gene region	Genotypes	Frequency		P value	FDR
			Case subjects	Control subjects		
SNP1	Exon 1	GG	238 (36.6)	347 (34.0)	0.43	0.84
		GA	311 (47.8)	493 (48.3)		
		AA	102 (15.6)	180 (17.7)		
SNP2	Intron 1	TT	414 (63.0)	625 (60.3)	0.42	0.84
		TC	215 (32.7)	357 (34.4)		
		CC	28 (4.3)	55 (5.3)		
SNP3	Intron 1	TT	223 (33.9)	353 (34.1)	0.59	0.84
		TA	315 (47.9)	513 (49.6)		
		AA	119 (18.1)	168 (16.3)		
SNP4	Intron 1	CC	241 (36.5)	372 (36.0)	0.55	0.84
		CT	327 (49.5)	498 (48.2)		
		TT	92 (14.0)	164 (15.8)		
SNP5	Intron 1	AA	185 (28.6)	323 (31.7)	0.34	0.84
		AC	338 (52.3)	521 (51.2)		
		CC	123 (19.1)	174 (17.1)		
SNP6	Intron 6	TT	231 (34.9)	354 (34.3)	0.97	0.97
		TG	330 (49.8)	518 (50.2)		
		GG	101 (15.3)	160 (15.5)		
SNP7	Exon 9	AA	233 (35.7)	372 (35.9)	0.96	0.97
		AC	327 (50.1)	510 (49.1)		
		CC	93 (14.2)	156 (15.0)		
SNP8	Intron 9	TT	471 (71.3)	715 (68.8)	0.16	0.84
		TC	178 (26.9)	290 (27.9)		
		CC	12 (1.8)	34 (3.3)		
SNP9	Intron 9	AA	375 (56.8)	614 (59.4)	0.58	0.84
		AG	252 (38.2)	372 (36.0)		
		GG	33 (5.0)	48 (4.6)		
SNP10	Exon 10 (3' untranslated region)	GG	426 (64.6)	696 (66.5)	0.88	0.97
		GA	213 (32.3)	321 (30.7)		
		AA	20 (3.1)	29 (2.8)		

Data are n (%), unless otherwise indicated. The missing genotyping is not counted.

women. Diabetes represents a constellation of many metabolic abnormalities, such as hyperglycemia and insulin resistance, as well as elevated inflammation response. We suspect that these metabolic and inflammation changes may affect the expression of IL-6 and thus dilute the genetic effects of *IL6R* variants.

Hamid et al. (13) reported that the amino acid change variant Asp358Ala (SNP7) was associated with risk of type 2 diabetes in Danish whites under a recessive inheritance model. However, *IL6R* variations were not associated with diabetes in Pima Indians and North European whites (9,11), as well as in our study sample of U.S. women. Given the sample size of the present study, we had >80% power to identify an odds ratio of ≥ 1.25 , but our study may be underpowered to identify the weaker genetic effects. In addition, we did not find significant associations between *IL6R* variations and adiposity (data not shown). It was reported that variant Asp358Ala (SNP7) was associated with BMI in Pima Indians (9). However, the relations between *IL6R* variations and adiposity were not found in some other studies (11,13).

The discrepancy between various studies may be partly attributed to the population heterogeneity, different confounding structure, and varying study power. It is believed that many susceptibility genes may predispose to diabetes, while the contribution of individual genes is likely to be moderate. We suspect that the metabolic changes solely related to *IL6R* variations may not be sufficient to lead to

transition from subclinical to clinical disease. This is very similar to the recent observations that *CRP* genotypes were strongly associated with plasma C-reactive protein concentration but were weakly related to cardiovascular risk (36,37).

According to the theory of Mendelian randomization (38), the *IL6R* genotype may represent an instrument for IL-6 levels that is free from reverse causation bias and confounding. Therefore, if the association between IL-6 levels and diabetes is causal, then *IL6R* variants should be related to diabetes risk. The lack of associations between *IL6R* variants and diabetes risk suggests that the observed associations between circulating IL-6 and diabetes (3,4,6,39) may be due to reverse association or confounding rather than causality. Confounding may bias the relation between IL-6 levels and diabetes risk. The results from the Mendelian randomization instrumental variable analysis, however, were inconclusive. The point estimate for the instrumental variable (albeit nonsignificant) did not substantially differ from that obtained from the primary analyses. Thus, based on the instrumental variable analyses, we could not determine whether the positive association between IL-6 concentration and diabetes risk is causal or not. A larger sample size is needed to elucidate the causal effects. Moreover, it has yet to be demonstrated that the *IL6R* genotype a good instrumental variable for IL-6 levels (i.e., IL-6 is the only mediator for *IL6R* genetic effect on diabetes). Little is known about whether *IL6R*

variants affect other metabolic changes (pleiotropy) related to the development of diabetes. Therefore, we cannot exclude the possibility that other mechanisms may play a role, which may distort the association between *IL6R* genotype and diabetes risk. Finally, a significant association between IL-6 level and diabetes risk has been observed in several large prospective studies (3,4), which lowers the likelihood that the changes of IL-6 levels are the consequence of diabetes.

More evidence, from both epidemiological and experimental studies, is warranted to elucidate whether the changes in IL-6 levels are causally involved in the development of diabetes. Diabetes, as a disorder of hyperglycemia, can induce inflammatory response, especially through overproduction of reactive oxygen species (40,41). Experiments assessing the effects of hyperglycemia on the expression of IL-6 may help to clarify the mechanisms. It is also informative to compare plasma IL-6 concentrations before and after the development of diabetes in a prospective setting.

Several limitations need to be considered. The population stratification may bias the observed associations. However, our study population is highly homogeneous by including only European whites, lessening concerns about this source of bias as a potential cause of spurious associations. In addition, our analyses were restricted to women and therefore may not be generalized to men.

Low-grade systemic inflammation is involved in the pathogenetic processes causing type 2 diabetes. IL-6 may affect insulin secretion (42) and induce insulin resistance (43,44). IL-6 levels predicted diabetes in several prospective studies (3,4). These observations may reflect a pathogenic role of IL-6 in diabetes. Although our data suggest that *IL6R* variants are not directly associated with diabetes risk, our findings are important in clarifying the complex relationships between *IL6R* variants, plasma IL-6 concentrations, and diabetes risk.

In summary, we found that common variations in the *IL6R* gene, especially SNP7 (rs8192284, Asp358Ala), were significantly associated with plasma IL-6 levels. However, the *IL6R* variations were not significantly related to diabetes risk in U.S. women. Further research is warranted to replicate the associations in other populations and to elucidate the potential mechanisms.

ACKNOWLEDGMENTS

This study was supported by National Institutes of Health Grants DK58845 and CA87969. L.Q. is supported by an American Heart Association Scientist Development Grant Award (0730094N).

REFERENCES

- Gauldie J, Richards C, Harnish D, Lansdorp P, Baumann H: Interferon beta 2/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. *Proc Natl Acad Sci U S A* 84:7251-7255, 1987
- Gabay C: Interleukin-6 and chronic inflammation. *Arthritis Res Ther* 8 (Suppl. 2):S3, 2006
- Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM: C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA* 286:327-334, 2001
- Spranger J, Kroke A, Mohlig M, Hoffmann K, Bergmann MM, Ristow M, Boeing H, Pfeiffer AF: Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation Into Cancer and Nutrition (EPIC)-Potsdam Study. *Diabetes* 52:812-817, 2003
- Ridker PM, Rifai N, Stampfer MJ, Hennekens CH: Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation* 101:1767-1772, 2000
- Hu FB, Meigs JB, Li TY, Rifai N, Manson JE: Inflammatory markers and risk of developing type 2 diabetes in women. *Diabetes* 53:693-700, 2004
- Jones SA, Horiuchi S, Topley N, Yamamoto N, Fuller GM: The soluble interleukin 6 receptor: mechanisms of production and implications in disease. *FASEB J* 15:43-58, 2001
- Yawata H, Yasukawa K, Natsuka S, Murakami M, Yamasaki K, Hibi M, Taga T, Kishimoto T: Structure-function analysis of human IL-6 receptor: dissociation of amino acid residues required for IL-6-binding and for IL-6 signal transduction through gp130. *EMBO J* 12:1705-1712, 1993
- Wolford JK, Colligan PB, Gruber JD, Bogardus C: Variants in the interleukin 6 receptor gene are associated with obesity in Pima Indians. *Mol Genet Metab* 80:338-343, 2003
- Escobar-Morreale HF, Calvo RM, Villuendas G, Sancho J, San Millan JL: Association of polymorphisms in the interleukin 6 receptor complex with obesity and hyperandrogenism. *Obes Res* 11:987-996, 2003
- Wang H, Zhang Z, Chu W, Hale T, Cooper JJ, Elbein SC: Molecular screening and association analyses of the interleukin 6 receptor gene variants with type 2 diabetes, diabetic nephropathy, and insulin sensitivity. *J Clin Endocrinol Metab* 90:1123-1129, 2005
- Hamid YH, Rose CS, Urhammer SA, Glumer C, Nolsoe R, Kristiansen OP, Mandrup-Poulsen T, Borch-Johnsen K, Jorgensen T, Hansen T, Pedersen O: Variations of the interleukin-6 promoter are associated with features of the metabolic syndrome in Caucasian Danes. *Diabetologia* 48:251-260, 2005
- Hamid YH, Urhammer SA, Jensen DP, Glumer C, Borch-Johnsen K, Jorgensen T, Hansen T, Pedersen O: Variation in the interleukin-6 receptor gene associates with type 2 diabetes in Danish whites. *Diabetes* 53:3342-3345, 2004
- Mullberg J, Oberthur W, Lottspeich F, Mehl E, Dittrich E, Graeve L, Heinrich PC, Rose-John S: The soluble human IL-6 receptor: mutational characterization of the proteolytic cleavage site. *J Immunol* 152:4958-4968, 1994
- Colditz GA, Manson JE, Hankinson SE: The Nurses' Health Study: 20-year contribution to the understanding of health among women. *J Womens Health* 6:49-62, 1997
- Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance: National Diabetes Data Group. *Diabetes* 28:1039-1057, 1979
- Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 20:1183-1197, 1997
- Qi L, Meigs J, Manson JE, Ma J, Hunter D, Rifai N, Hu FB: HFE genetic variability, body iron stores, and the risk of type 2 diabetes in U.S. women. *Diabetes* 54:3567-3572, 2005
- Qi L, van Dam RM, Meigs JB, Manson JE, Hunter D, Hu FB: Genetic variation in IL6 gene and type 2 diabetes: tagging-SNP haplotype analysis in large-scale case-control study and meta-analysis. *Hum Mol Genet* 15:1914-1920, 2006
- de Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D: Efficiency and power in genetic association studies. *Nat Genet* 37:1217-1223, 2005
- Benjamini Y, Hochberg Y: Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc B* 57:289-300, 1995
- Benjamini Y, Yekutieli D: Quantitative trait loci analysis using the false discovery rate. *Genetics* 171:783-790, 2005
- Tregouet DA, Escolano S, Tiret L, Mallet A, Golmard JL: A new algorithm for haplotype-based association analysis: the Stochastic-EM algorithm. *Ann Intern Med* 68:165-177, 2004
- Davey Smith G, Lawlor DA, Harbord R, Timpson N, Rumley A, Lowe GD, Day IN, Ebrahim S: Association of C-reactive protein with blood pressure and hypertension: life course confounding and mendelian randomization tests of causality. *Arterioscler Thromb Vasc Biol* 25:1051-1056, 2005
- Timpson NJ, Lawlor DA, Harbord RM, Gaunt TR, Day IN, Palmer LJ, Hattersley AT, Ebrahim S, Lowe GD, Rumley A, Davey Smith G: C-reactive protein and its role in metabolic syndrome: mendelian randomisation study. *Lancet* 366:1954-1959, 2005
- Hardin JW: The robust variance estimator for two-stage models. *Stata J* 2:253-266, 2002
- Reich D, Patterson N, Ramesh V, De Jager PL, McDonald GJ, Tandon A, Choy E, Hu D, Tamraz B, Pawlikowska L, Wassel-Fyr C, Huntsman S, Waliszewska A, Rossin E, Li R, Garcia M, Reiner A, Ferrell R, Cummings S, Kwok PY, Harris T, Zmuda JM, Ziv E: Admixture mapping of an allele affecting interleukin 6 soluble receptor and interleukin 6 levels. *Am J Hum Genet* 80:716-726, 2007
- Weller JI, Song JZ, Heyen DW, Lewin HA, Ron M: A new approach to the

- problem of multiple comparisons in the genetic dissection of complex traits. *Genetics* 150:1699–1706, 1998
29. Benjamini Y, Drai D, Elmer G, Kafkafi N, Golani I: Controlling the false discovery rate in behavior genetics research. *Behav Brain Res* 125:279–284, 2001
 30. Zee RY, Cook NR, Cheng S, Erlich HA, Lindpaintner K, Ridker PM: Polymorphism in the beta2-adrenergic receptor and lipoprotein lipase genes as risk determinants for idiopathic venous thromboembolism: a multilocus, population-based, prospective genetic analysis. *Circulation* 113:2193–2200, 2006
 31. Smith NL, Hindorff LA, Heckbert SR, Lemaitre RN, Marcianti KD, Rice K, Lumley T, Bis JC, Wiggins KL, Rosendaal FR, Psaty BM: Association of genetic variations with nonfatal venous thrombosis in postmenopausal women. *JAMA* 297:489–498, 2007
 32. Patsulaia I, Trofimov S, Kobylansky E, Livshits G: Genetic and environmental influences on IL-6 and TNF-alpha plasma levels in apparently healthy general population. *Cytokine* 19:138–146, 2002
 33. Ravaglia G, Forti P, Maioli F, Chiappelli M, Dolzani P, Martelli M, Bianchin M, Mariani E, Bolondi L, Licastro F: Associations of the -174 G/C interleukin-6 gene promoter polymorphism with serum interleukin 6 and mortality in the elderly. *Biogerontology* 6:415–423, 2005
 34. Brull DJ, Montgomery HE, Sanders J, Dhamrait S, Luong L, Rumley A, Lowe GD, Humphries SE: Interleukin-6 gene -174g>c and -572g>c promoter polymorphisms are strong predictors of plasma interleukin-6 levels after coronary artery bypass surgery. *Arterioscler Thromb Vasc Biol* 21:1458–1463, 2001
 35. Fernandez-Real JM, Broch M, Vendrell J, Gutierrez C, Casamitjana R, Pugeat M, Richart C, Ricart W: Interleukin-6 gene polymorphism and insulin sensitivity. *Diabetes* 49:517–520, 2000
 36. Kathiresan S, Larson MG, Vasani RS, Guo CY, Gona P, Keaney JF, Jr, Wilson PW, Newton-Cheh C, Musone SL, Camargo AL, Drake JA, Levy D, O'Donnell CJ, Hirschhorn JN, Benjamin EJ: Contribution of clinical correlates and 13 C-reactive protein gene polymorphisms to interindividual variability in serum C-reactive protein level. *Circulation* 113:1415–1423, 2006
 37. Kardys I, de Maat MP, Uitterlinden AG, Hofman A, Witteman JC: C-reactive protein gene haplotypes and risk of coronary heart disease: the Rotterdam Study. *Eur Heart J* 27:1331–1337, 2006
 38. Davey Smith G, Ebrahim S: “Mendelian randomization:” can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol* 32:1–22, 2003
 39. Krakoff J, Funahashi T, Stehouwer CD, Schalkwijk CG, Tanaka S, Matsuzawa Y, Kobes S, Tataranni PA, Hanson RL, Knowler WC, Lindsay RS: Inflammatory markers, adiponectin, and risk of type 2 diabetes in the Pima Indian. *Diabetes Care* 26:1745–1751, 2003
 40. Esposito K, Nappo F, Marfella R, Giugliano G, Giugliano F, Ciotola M, Quagliaro L, Ceriello A, Giugliano D: Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. *Circulation* 106:2067–2072, 2002
 41. Brownlee M: Biochemistry and molecular cell biology of diabetic complications. *Nature* 414:813–820, 2001
 42. Eizirik DL, Sandler S, Welsh N, Cetkovic-Cvrlje M, Nieman A, Geller DA, Pipeleers DG, Bendtzen K, Hellerstrom C: Cytokines suppress human islet function irrespective of their effects on nitric oxide generation. *J Clin Invest* 93:1968–1974, 1994
 43. Lagathu C, Bastard JP, Auclair M, Maachi M, Capeau J, Caron M: Chronic interleukin-6 (IL-6) treatment increased IL-6 secretion and induced insulin resistance in adipocyte: prevention by rosiglitazone. *Biochem Biophys Res Commun* 311:372–379, 2003
 44. Cardellini M, Perego L, D'Adamo M, Marini MA, Procopio C, Hribal ML, Andreozzi F, Frontoni S, Giacomelli M, Paganelli M, Pontiroli AE, Lauro R, Folli F, Sesti G: C-174G polymorphism in the promoter of the interleukin-6 gene is associated with insulin resistance. *Diabetes Care* 28:2007–2012, 2005