Original Article

Genetic Variation, C-Reactive Protein Levels, and Incidence of Diabetes

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C-reactive protein (CRP) has been shown to be associated with type 2 diabetes, but whether CRP has a causal role is not yet clear. We examined the association in the Rotterdam Study, a population-based prospective cohort study. The association of baseline serum CRP and incident diabetes during follow-up was investigated, and a meta-analysis was conducted on the BMI-adjusted relation of CRP and diabetes. Furthermore, the association of CRP haplotypes with serum CRP and risk of diabetes was assessed. The age- and sex-adjusted hazard ratio for diabetes was 1.41 (95% CI 1.29–1.54) per 1 SD increase in natural logarithm of CRP, and it was 1.88, 2.16, and 2.83 for the second, third, and fourth quartiles of CRP, respectively, compared with the first quartile. The risk estimates attenuated but remained statistically significant after additional adjustment for obesity indexes, which agreed with the results of the meta-analysis. The most common genetic haplotype was associated with a significantly lower CRP level compared with the three other haplotypes. The risk of diabetes was significantly higher in the haplotype with the highest serum CRP level compared with the most common haplotype (OR 1.45, 95% CI 1.08–1.96). These findings support the hypothesis that serum CRP enhances the development of diabetes. *Diabetes* 56:872–878, 2007

Prospective studies have shown that C-reactive protein (CRP), which is a general marker of systemic inflammation, is associated with the risk of diabetes (1–9). CRP is produced by hepatocytes, and its gene expression is regulated by tumor necrosis factor-α and interleukin-6, which are secreted by adipocytes (10). As a result, obese individuals who have more and larger adipocytes also have higher baseline serum CRP. Because diabetes is more common in obese individuals, an association is expected between serum CRP and diabetes. However, some studies found that obesity does not explain the association of CRP with diabetes completely, suggesting an independent role for CRP in the development of diabetes (1,5,9).

Twin and familial studies have shown a substantial hereditability for CRP level (11), and a recent study found a strong association of serum CRP with genetic variations in the CRP promoter region (12). Four haplotypes broadly represent the CRP gene variation in the European population (13). Therefore, an association of CRP haplotypes with serum CRP and the incidence of diabetes may point to a contribution of CRP in the development of diabetes.

We studied the association of serum CRP with the risk of diabetes in the Rotterdam Study, a prospective population-based cohort study among participants aged ≥55 years. Furthermore, we conducted a meta-analysis, which included our own study, to clarify whether CRP serum level is associated with diabetes, independent of obesity indexes. Finally, to investigate a potential role of CRP in the development of diabetes, we studied the association of genetic CRP haplotypes with the risk of diabetes.

RESEARCH DESIGN AND METHODS

This study was conducted within the framework of the Rotterdam Study, an ongoing prospective population-based cohort study on determinants of several chronic diseases. The Rotterdam Study has been described in detail elsewhere (14). In brief, all inhabitants of Ommoord, a district of Rotterdam in the Netherlands, who were aged ≥55 years were invited to participate in this study. Of all 10,275 eligible individuals, 7,983 agreed to participate (78%).

Participants were visited at home for an interview. Subsequently, they came to the research center for blood sampling and further examinations. Follow-up started at baseline, and examinations were carried out periodically. In addition, participants were continuously monitored for major events through automated linkage with files from general practitioners and pharmacies working in the study district of Ommoord. Information on vital status was obtained regularly from the municipal health authorities in Rotterdam. For the current study, follow-up data were present until 1 October 2005. Written informed consent was obtained from all participants, and the medical ethics committee of Erasmus University Rotterdam approved the study.

Diabetes. At baseline, prevalent cases of diabetes were diagnosed and excluded. Prevalent diabetes was defined as use of antidiabetes medication and/or abnormal nonfasting glucose and/or an abnormal oral glucose tolerance test. A nonfasting or postload glucose level ≥11.1 mmol/l was considered abnormal (15).

During follow-up, incident cases of diabetes were diagnosed by use of information from the general practitioners, the pharmacies' databases, and our follow-up examinations. Based on guidelines of the American Diabetes Association (16) and the World Health Organization (17), we defined incident diabetes as follows: fasting plasma glucose level ≥7.0 mmol/l and/or random (nonfasting) plasma glucose level ≥11.1 mmol/l and/or use of oral antidiabetes medication and/or use of insulin and/or treatment by diet and registered by a general practitioner as having diabetes.

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Measurement of CRP serum levels. At baseline, serum levels of CRP were measured in 6,658 of 7,129 participants who visited the research center. Nonfasting serum samples were collected. The samples were immediately put on ice and were processed within 30 min, after which they were kept frozen at −20°C until measurement of CRP in 2003–2004. High-sensitivity CRP was measured using a rate near-infrared particle immunoassay (Immage Immunochemistry System, Beckman Coulter, Fullerton, CA). This system measures concentrations from 0.2 to 1,440 mg/l, with a within-run precision <5.0%, a total precision <7.5%, and a reliability coefficient of 0.995.

Genotyping. The Seattle SNPs Program for Genomic Applications has identified four haplotypes covering the CRP gene based on 18 SNPs that had a frequency of >5% in 23 unrelated individuals of European descent from the CEPH (Centre d'Etude du Polymorphisme Humain) pedigrees (13). Results in these 23 individuals showed that each of these four haplotypes could be identified by a single tagging SNP. Hence, by determining three nonoverlapping tagging SNPs, we were able to infer all four haplotypes.

Genotyping for the three tagging SNPs (1184C/T, 2042C/T, and 2911C/G) was performed for 5,460 of 7,059 participants, whose blood was sampled during the baseline visit. The polymorphisms have been described in relation to the start of the coding sequence of exon 1, using the human May 2004 (hg 17) assembly (http://genome.ucsc.edu). These polymorphisms have also been previously described (http://www.ncbi.nlm.nih.gov/projects/SNP) under identification numbers rs1130864 (1184C/T), rs1205 (2042C/T), and rs3093068 (2911C/G). CRP genotypes were determined in our study population in 2-ng genomic DNA with a TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA). We used the SNP assay-by-design service of Applied Biosystems to optimize the primer and probe sequences (for details, see http://store.appliedbiosystems.com). Reactions were performed with a TaqMan Prism 7900HT 384-wells format.

Statistical analysis. A Cox regression analysis was used to assess the association of CRP with incident diabetes. We tested the proportional hazards assumption by using "log-minus-log" plots. A log transformation of serum CRP, ln(CRP), was used because serum CRP had a right-skewed distribution. To examine the relation between CRP and incident diabetes, we computed the increase in hazard ratio (HR) per 1 SD increase in ln(CRP) level. We defined four quartiles of CRP level based on the population distribution (<0.88, 0.88–1.80, 1.81–3.5, and >3.5 mg/l). In addition, a sensitivity analysis was performed to assess the effect of adjustment for fasting blood glucose. Fasting blood glucose was measured in the third periodical examination of the Rotterdam Study. For this analysis, we used the third examination as the baseline measurement.

For the meta-analysis, previously published data were obtained by Medline searches and scanning the reference lists until December 2005. Nine studies were found to be relevant, and we added our results of the Rotterdam Study. No scoring system was used to qualify the studies. The effect estimates extracted were mostly HRs and incidence rate ratios. In one study, we used the odds ratio (OR) as an acceptable estimate of the risk ratio (4). The effect estimates extracted were based on CRP tertiles, quartiles, or quintiles, each stating the risk of diabetes in a specific range of serum CRP levels, compared with a reference group. To group the most relevant risk ratios for a corresponding serum CRP range, we defined three CRP level intervals. An effect estimate was allocated to an interval when its accredited CRP range was completely covered by that interval (0.5–1.8, 1–3.7, and >2.6). The intervals overlap to allow more risk estimates to be allocated. Some effect estimates were not used because the CRP range they were based on did not fit in any of the defined intervals. Packages "meta" (18) and "rmeta" (19), running for "R" (20), was used to analyze the data. A random-effects model was used to weight the HRs. A weighted HR was calculated for each interval. First, age- and sex-adjusted HRs and, second, age-, sex-, and BMI-adjusted HRs were used. We investigated whether race is a source of heterogeneity in the association of serum CRP and diabetes by performing a meta-regression using SAS Proc Mixed (21).

We used the Haplo.Stats package running for R (20) to estimate the CRP haplotypes and to investigate the association of inferred haplotypes with serum CRP and risk of diabetes (22). This method assigns the probability for each haplotype pair in each individual and then models an individual's phenotype as a function of each inferred haplotype pair, weighted by their estimated posterior probability so as to account for haplotype ambiguity. The Haplo.glm function was used to calculate adjusted ORs for each haplotype. Haplo.glm is based on a generalized linear model and can be modeled for additive, dominant, or recessive effect of haplotypes (23,24). We restricted the analysis to haplotypes with an inferred frequency >0.02. The first haplotype, which was the most frequent and was associated with the lowest serum CRP, was used as the reference group. To find the inheritance model (dominant or recessive) that fits best to the data, we used the likelihood ratio test carried out on the variation of the log likelihood between two models.

TABLE 1
Baseline characteristics of all participants and case subjects with incident type 2 diabetes

Characteristics	All participants*	Incident type 2 diabetic patients	*P*†
n	5,901	544	—
Age (years)	69.1 ± 9.1	68.7 ± 8.1	0.01
Men	2,388 (40.5)	274 (43.5)	0.13
BMI (kg/m^2)	26.2 ± 3.7	28.2 ± 3.8	<0.001
Waist circumference (cm)	90.2 ± 11.1	95.0 ± 11.0	<0.001
Systolic blood pressure (mmHg)	138.5 ± 22.1	143.2 ± 20.6	<0.001
Diastolic blood pressure (mmHg)	73.8 ± 11.6	75.6 ± 11.2	<0.001
Hypertension	1,872 (32.3)	253 (46.4)	<0.001
History of coronary artery disease	669 (12.1)	64 (13.7)	0.28
Total cholesterol (mmol/l)	6.6 ± 1.2	6.6 ± 1.2	0.80
HDL cholesterol (mmol/l)	1.4 ± 0.36	1.3 ± 0.33	<0.001
Daily alcohol intake in drinkers (g/day)	7.1 (1.5–19.3)	6.2 (1.4–17.7)	0.47
Current smoker	1,320 (22.9)	131 (24.4)	0.22
Former smoker	2,425 (42.1)	235 (43.8)	
Postmenopausal hormone therapy‡	1,187 (20.1)	102 (18.9)	0.47
CRP (mg/l)	1.8 (0.9–3.5)	2.3 (1.3–4.2)	<0.001

Data are means ± SD, *n* (%), and median (interquartile range). Student's *t* test was used for normally distributed continuous variables, Mann-Whitney for non-normally distributed covariates, and χ^2 test for categorical variables. *Contains both healthy participants and incident type 2 diabetic patients; †case subjects compared with nondiabetic participants; ‡history of use in women.

RESULTS

Serum CRP and diabetes in the Rotterdam Study. We compared 5,901 participants who had CRP measurements with those 1,034 participants whose serum CRP measurements were missing. Compared with the population used for analysis, participants with missing values were significantly older and more frequently female, but they had similar mean values for BMI, weight, waist circumference, cholesterol level, systolic and diastolic blood pressure, and daily alcohol consumption (data not shown). Table 1 shows the baseline characteristics of 5,901 participants and individuals with incident diabetes. CRP ranged from 0.2 to 247 mg/l with a right-skewed distribution. Median CRP was 1.86 mg/l in men and 1.78 mg/l in women ($P <$ 0.001). Age, BMI, weight, waist circumference, systolic and diastolic blood pressure, and HDL cholesterol were significantly correlated with serum CRP. Except for HDL cholesterol, the correlations were positive. The highest correlation coefficient was 0.27 for waist circumference.

During a mean follow-up of 9.8 years in 5,901 participants, diabetes developed in 544 subjects (incidence = 9.4 per 1,000 person-years). The age- and sex-adjusted HR for diabetes per 1 SD increase in ln(CRP) was 1.41 (95% CI 1.29–1.54). The HR attenuated to 1.24 (1.12–1.37) after adjustment for BMI and waist circumference. After further adjustment for systolic blood pressure, diastolic blood

TABLE 2
HRs for diabetes according to level of CRP

Quartile (level in mg/l)	Participants (case subjects)	HR (95% CI) Model 1	Model 2	Model 3
1 (<0.88)	1,463 (77)	1.00 (reference)	1.00 (reference)	1.00 (reference)
2 (0.88–1.8)	1,485 (141)	1.88 (1.42–2.48)	1.59 (1.19–2.11)	1.51 (1.13–2.03)
3 (1.81–3.5)	1,475 (153)	2.16 (1.64–2.84)	1.70 (1.27–2.27)	1.54 (1.15–2.06)
4 (>3.5)	1,478 (173)	2.83 (2.16–3.70)	1.94 (1.45–2.59)	1.73 (1.29–2.33)
P for trend	—	<0.001	<0.001	0.001
1 SD increase in ln(CRP)	5,901 (544)	1.41 (1.29–1.54)	1.24 (1.12–1.37)	1.19 (1.07–1.31)

Model 1 was adjusted for age and sex. Model 2 was adjusted for age, sex, BMI, and waist circumference. Model 3 was adjusted for age, sex, BMI, waist circumference, systolic and diastolic blood pressure, and HDL cholesterol.

pressure, and HDL cholesterol, the HR slightly decreased to 1.19 (1.07–1.31) (Table 2). Considering the lowest CRP quartile as the reference group, age- and sex-adjusted HRs were 1.88, 2.16, and 2.83 for the second, third, and fourth quartiles of CRP, respectively (*P* for trend <0.001). Further adjustment for the above-mentioned covariates attenuated the HRs in models 2 and 3 (Table 2).

Sensitivity analysis. Exclusion of subjects with a history of hormone therapy had a minimal effect on the association. In the age- and sex-adjusted model, the HR for 1 SD increase in CRP attenuated to 1.37 (95% CI 1.25–1.51). To evaluate the effect of adjustment for fasting blood glucose, we selected the third periodical examination of the Rotterdam Study as the baseline measurement. The mean follow-up time was reduced to 5.34 years and the number of incident diabetes cases to 319 individuals. Serum CRP was significantly associated with diabetes in an age- and sex-adjusted model: HR for 1 SD, ln(CRP) 1.53, 95% CI 1.26–1.86. When we additionally adjusted the model for BMI, waist circumference, and fasting blood glucose, the association was attenuated (HR for 1 SD, 1.39, 1.13–1.71).

Meta-analysis. A total of 10 studies were included in the meta-analysis. The studies had been conducted in the U.S., Europe, and Mexico (Table 3). All studies showed a positive association between serum levels of CRP and incident diabetes. For the CRP intervals 0.5–1.8, 1–3.7, and ≥2.6 mg/l, weighted age- and sex-adjusted risk ratios (95% CI) were 1.63 (1.35–1.98), 2.16 (1.81–2.57), and 4.00 (2.83–5.65), respectively (Fig. 1.). After additional adjustment for BMI, the weighted risk ratios decreased to 1.44 (1.16–1.78), 1.72 (1.42–2.08), and 2.37 (1.57–3.58) (Fig. 2). Figure 3 shows that the association was more pronounced in Caucasians than other ethnic groups, although the slope was not significant in our regression (b = 0.01, 95% CI −0.05 to 0.03) (Fig. 3).

CRP gene haplotypes, serum CRP, and diabetes in the Rotterdam Study. We compared 6,157 participants who had CRP genotypes with the 1,826 participants whose CRP gene information were missing. Participants with missing values were older (5.3 years) and more frequently female. They had a lower weight (1.5 kg) and higher HDL cholesterol level (0.03 mmol/l). However, compared with the population used for analysis, there was no difference in their BMI, waist circumference, systolic and diastolic blood pressure, daily alcohol consumption, and serum CRP level.

Genotype distributions of the three tagging SNPs were found to be in Hardy-Weinberg equilibrium. We estimated six allele-specific haplotypes with the Haplo.Stats program. Two of the haplotypes were present in <0.001% of the chromosomes and were therefore not used in our analyses. We coded the other four haplotypes from 1 to 4 according to decreasing population frequency (Table 4).

Geometric mean of serum CRP level was 1.50 mg/l for carriers of haplotype 1. Mean serum CRP increased per copy of other haplotypes relative to haplotype 1. This increase was 0.3 mg/l for haplotype 2, 0.15 mg/l for haplotype 3, and 0.46 mg/l for haplotype 4. Serum CRP level was significantly higher in participants with haplotype 4 compared with the carriers of haplotype 1 ($P < 0.001$). Elimination of the CRP levels >10 mg/l did not materially change the results (data not shown). A nearly significant higher risk for diabetes was found for carriers of haplotype 4 compared with carriers of haplotype 1. In an additive model, the OR was 1.30 (95% CI 0.99–1.71). Based on the log likelihood ratio test, none of the inheri-

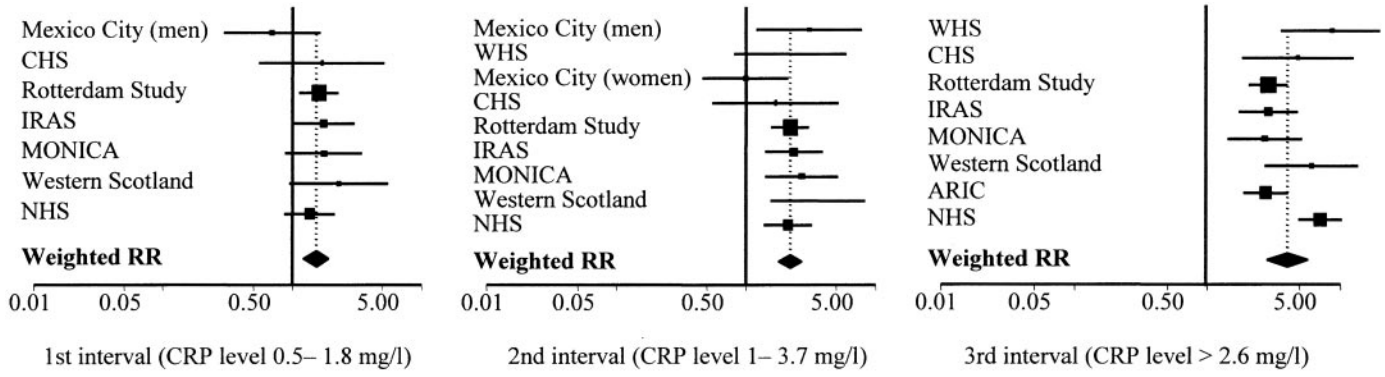

FIG. 1. Age- and sex-adjusted risk ratios for diabetes in different categories of CRP levels compared with the reference category (<0.5 mg/l). Weighted risk ratio (95% CI) was 1.64 (1.35–1.98) for the first interval, 2.16 (1.81–2.57) for the second interval, and 4.00 (2.83–5.65) for the third interval. ARIC, Atherosclerosis Risk in Communities Study; CHS, Cardiovascular Heart Study; IRAS, Insulin Resistance Atherosclerosis Study; NHS, Nurses' Health Study; WHS, Women's Health Study.

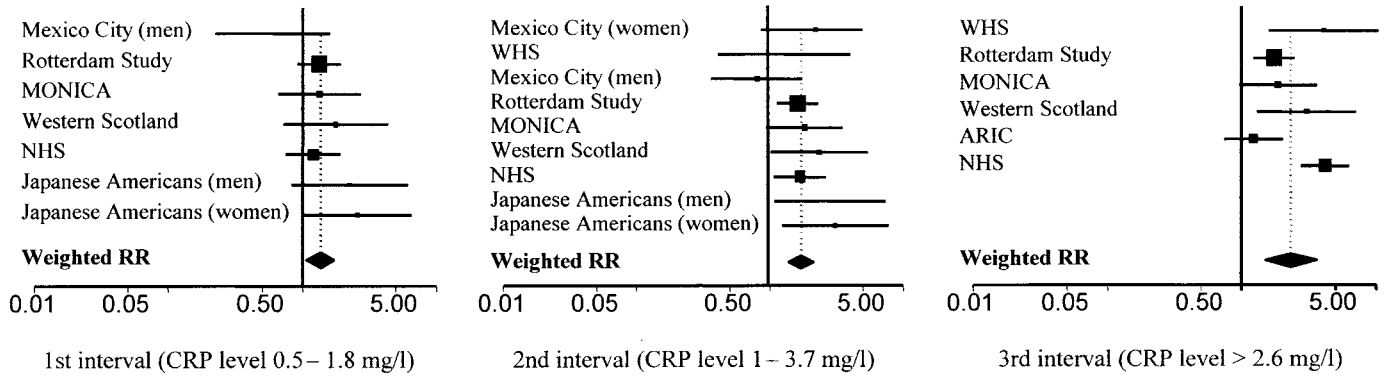

FIG. 2. BMI-, age-, and sex-adjusted risk ratios for diabetes in different categories of CRP levels compared with the reference category (<0.5 mg/l). Weighted risk ratio (95% CI) was 1.44 (1.16–1.78) for the first interval, 1.72 (1.42–2.08) for the second interval, and 2.37 (1.57–3.58) for the third interval. ARIC, Atherosclerosis Risk in Communities Study; CHS, Cardiovascular Heart Study; IRAS, Insulin Resistance Atherosclerosis Study; NHS, Nurses' Health Study; WHS, Women's Health Study.

tance models (dominant or recessive) improved the fit of the model significantly. Other haplotypes did not change the risk of diabetes significantly (Table 5). After adjustment for BMI, waist circumference, systolic and diastolic blood pressure, and HDL cholesterol, the association increased in strength (OR 1.45, 95% CI 1.08–1.96). When the age- and sex-adjusted estimate was further adjusted for serum CRP, the OR decreased (1.20, 0.89–1.60). Using the Mendelian randomization approach, the expected OR for carriers of haplotype 4 compared with carriers of haplotype 1 was 1.09 and was not significantly different from the observed OR.

DISCUSSION

The results of our population-based cohort study and the meta-analysis showed that serum CRP is associated with risk of diabetes independent of obesity. We identified a genetic variant in the human CRP locus that associates with a high serum CRP and an increased risk of diabetes. The latter association reduced after adjustment for serum CRP. These findings support the hypothesis that CRP is etiologically involved in the pathogenesis of diabetes.

In our study including 544 case subjects with incident diabetes, we showed that the association of serum CRP with diabetes remains significant not only after adjustment for obesity indexes but also after adjustment for blood pressure and cholesterol. We adjusted for the latter variables as surrogates of the metabolic syndrome, which could confound the association. Adjustment for obesity indexes in other studies has provided controversial results. The CHS (Cardiovascular Heart Study), the Women's Health Study, the West of Scotland Coronary Prevention Study, and the NHS (Nurses' Health Study) showed significant associations between CRP and incident diabetes, even after adjustment for obesity indexes (1,5,7,9). In contrast, the ARIC (Atherosclerosis Risk in Communities Study), the Monitoring of Trends and Determinants in Cardiovascular Diseases (MONICA)-Augsburg Cohort Study, and the IRAS (Insulin Resistance Atherosclerosis Study) showed nonsignificant associations after adjustment for obesity indexes (3,8,9). To summarize these controversial results, we performed a meta-analysis. Weighted risk ratios showed a significant obesity-adjusted association between serum CRP and diabetes. We believe that the negative result in the three latter reports could be explained by several factors. The Insulin Resistance Atherosclerosis Study and the MONICA-Augsburg Cohort Study had fewer diabetic cases and consequently had less power. In addition, in the Atherosclerosis Risk in Communities Study, nearly one-third of the participants were non-Caucasian. We observed in our meta-analysis that, though not significant, the association was more pronounced in Caucasians than in other ethnic groups (Fig. 3).

We showed that serum CRP is significantly different in carriers of different haplotypes. Several studies have used haplotypes describing the total variation of the CRP gene to examine the issue. Miller et al. (25) resequenced 192 individuals to identify a comprehensive set of common SNP variants. Later, they studied the association of the gene variation with serum CRP level in subsets of three cohorts. The haplotypes that Miller et al. found are comparable to ours. Three of the haplotypes were the same as in our study. Haplotypes 3 and 4 constitute our haplotype 1, and we have not identified their haplotype 6, which was present in 2.1% of their population. Miller et al. found their haplotypes 2 and 5 were associated with higher CRP, and their haplotypes 1, 3, and 4 were associated with lower CRP. This is in agreement with our findings. Carlson et al. (12) also defined all common genetic variation across the human CRP locus by resequencing the region in a multiethnic variation discovery panel, and they selected haplotype tagging SNPs for genotyping in a larger panel (the CARDIA [Coronary Artery Risk Development in Young Adults] Study). Furthermore, they investigated the associations between common haplotypes and serum CRP, rendering significant results. Although they used an approach

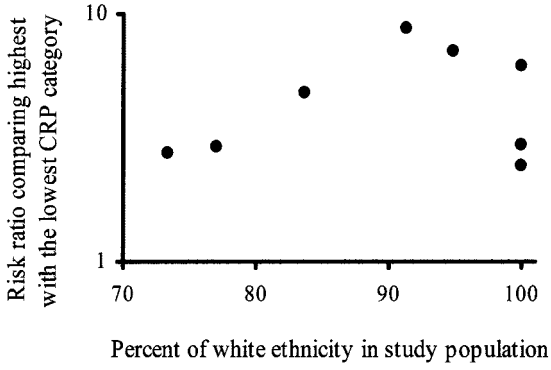

FIG. 3. The percent of the study population comprised of white ethnicity and the risk ratio for diabetes comparing the highest with the lowest category of CRP in the meta-analysis.

TABLE 3
Published studies on serum CRP and risk of diabetes

Study name	Study design	Diabetic/nondiabetic (*n*)*
Cardiovascular Heart Study (CHS)	Follow-up	45/4,436
Women's Health Study (WHS)	Nested case-control	188/362
West of Scotland Coronary Prevention Study (WOSCOPS)	Follow-up	127/5,118
Japanese Americans Study	Follow-up	122/825
Nurses' Health Study (NHS)	Nested case-control	737/785
Mexico City Diabetes Study	Follow-up	86/1,158
Insulin Resistance Atherosclerosis Study (IRAS)	Follow-up	144/903
Atherosclerosis Risk in Communities Study (ARIC)	Case cohort	581/572
Monica Augsburg Cohort Study (MONICA)	Follow-up	101/1,951

*Nondiabetic is the number of control subjects in the case-control studies. In cohort studies, it does not contain the diabetic cases.

similar to our study, Carlson et al. investigated a mixed population, partly European and partly African American, whereas the Rotterdam Study cohort is of almost exclusively European descent. As a result, Carlson et al. found three haplotypes that were not present in the European-descent populations. The other haplotypes identified by Carlson et al. were similar to the haplotypes we defined in the Rotterdam Study. Timpson et al. (26) selected four SNPs based on published reports. They constructed four haplotypes by use of a genetic data analysis program named SimHap. Their haplotypes (CGT, GGT, CAC, and CGC) were close to the haplotypes we used in our study (Table 4). Timpson et al. found a significant association between haplotypes and serum CRP. Their results were comparable to those of our study. Several other studies observed a relation between genetic variation in the human CRP locus and serum CRP. For instance, Szalai et al. (27) constructed their haplotypes based on the bi-allelic −409G/A (rs3093032) and tri-allelic −390C/T/A (rs3091244) CRP gene promoter polymorphisms. Interesting to note is that these haplotypes affect transcription factor binding, alter transcription activity, and influence the variation of the serum CRP. The T and A allele of the tri-allelic −390C/T/A polymorphism is present in participants with haplotypes 2 and 4 in our study (13). Thus, these functional SNPs may partly explain the higher serum CRP in carriers of these two haplotypes in our study. Other studies that examined the association of CRP gene haplotypes and serum CRP are not comparable with our study because they used different SNPs to build up their haplotypes, or they used different populations in terms of health status, race, or age (28–30).

We showed that participants carrying haplotype 4 have a significantly higher risk of diabetes. Thus, genetic susceptibility to high serum CRP increases the risk of diabetes. Our findings show that the association of genetic susceptibility with diabetes was independent of BMI and waist circumference, suggesting that obesity indexes do not explain the genetic susceptibility. Moreover, in a model adjusted for age, sex, and CRP, the association diminished, suggesting that the effect of haplotype 4 is likely to be explained by the variation in serum CRP level.

We found no significant difference between the observed OR and the one calculated based on Mendelian randomization. This provides evidence of an independent role of CRP in developing diabetes. The Mendelian randomization approach is a tool to assess the nature of associations. Because gene alleles that influence the intermediate phenotype are inherited at random, potential confounders for the association will be evenly distributed in those who do, and those who do not, have the alleles. Consequently, any difference between these two groups should be free of confounding by environmental factors. Furthermore, regression dilution and reverse causation are not probable to occur because the genotype is constant over time and is determined before the onset of disease (31). However, this approach has certain limitations, such as the potential for confounding the gene-phenotype association by linkage disequilibrium with other genes. Similarly, population origin can confound the gene-disease association (32). In addition, pleiotropic effects of the SNP in more than one biologic pathway can violate one of the assumptions of Mendelian randomization.

Studies that found independent association between CRP and diabetes suggested various pathways. Many studies argued that the association reflects the effects of cytokines, such as IL-6 and tumor necrosis factor-α, on insulin resistance (2,5,8,33,34). Some others explained the association through oxidative stress or the innate immune system (6,7). Nevertheless, none of the proposed mechanisms provide a causal role for CRP. A recent study that investigated the association of CRP and metabolic syn-

TABLE 4
CRP haplotypes and their frequencies in the Rotterdam Study and published studies

The Rotterdam Study		Miller et al. (25)		Carlson et al. (12)		Timpson et al. (26)	
Haplotype	Frequency	Haplotype	Frequency	Haplotype	Frequency	Haplotype	Frequency
H1 (CTC)*	0.33	H4	0.07	H1	0.06	GGT	0.07
		H3	0.27	H2	0.28	CGT	0.26
H2 (TCC)*	0.32	H2	0.29	H4	0.28	CAC	0.30
H3 (CCC)*	0.29	H1	0.27	H5	0.29	CGC	0.37
H4 (CCG)*	0.06	H5	0.06	H7	0.06		

*Coding from 1184C/T, 2042C/T, and 2911C/G, respectively.

TABLE 5
ORs for diabetes in different CRP haplotypes

CRP haplotypes	Number of alleles (diabetic alleles)	Age and sex adjusted	Multivariate adjusted*	Age, sex, and CRP adjusted
Haplotype 1 (reference)	3,490 (317)	1	1	1
Haplotype 2	3,340 (327)	1.09 (0.92–1.28)	1.10 (0.92–1.32)	1.07 (0.90–1.27)
Haplotype 3	3,198 (308)	1.06 (0.90–1.25)	0.99 (0.82–1.19)	1.03 (0.86–1.23)
Haplotype 4	624 (72)	1.30 (0.99–1.71)	1.45 (1.08–1.96)	1.20 (0.89–1.60)

*Adjustments were performed for age, sex, BMI, waist circumference, systolic and diastolic blood pressure, and HDL cholesterol.

drome pointed to the direct harmful effects of CRP on vessel walls, which may alter endothelial permeability and eventually lead to insulin resistance (35). However, further studies are necessary to find a reasonable mechanism.

To our knowledge, this is the first study in which the association between diabetes and serum CRP was partly explained by variations in the CRP gene. Wolford et al. (36) showed that variation within the CRP locus might play a role in diabetes susceptibility in Pima Indians. Their findings, in line with our study, were consistent with the hypothesis that CRP may play an etiologic role in the development of diabetes. In a recent study, Timpson et al. (26) investigated the causal role of CRP in the development of metabolic syndrome and reported that their findings provide evidence for CRP not to be causally involved in the pathogenesis of the metabolic syndrome. We believe that their study is not in contrast with our study. Timpson et al. combined haplotypes 4 and 3 as the CGC haplotype, whereas we found an association exclusively with haplotype 4. Haplotype 4 is a rare allele, and grouping it with another common haplotype dilutes the effect. Furthermore, diabetes has a well-defined nature compared with the metabolic syndrome, which may have resulted in a stronger association. However, replication of our findings is necessary to establish the relationship.

The strengths of our study include a large sample size, a long follow-up period, a considerable number of incident diabetes cases, and the availability of detailed genotype information. Haplotypes provide more information on genetic variation compared with single SNPs. In addition, we used information on the gene and the protein together in one study. However, several limitations need to be discussed. In the Rotterdam Study, we screened the cohort for prevalent diabetes at baseline by use of a nonfasting glucose level and oral glucose tolerance test. Our baseline measurements revealed 10.8% of prevalent diabetes, which is similar to the expected prevalence of diabetes in our population (37). The studies included in the meta-analysis all used different categories of CRP, and the geometric mean of the CRP level in the reference category ranged from 0.23 to 1.41 mg/l. To group the risk ratios, we allocated the risk estimates to three different CRP intervals, but variation in mean CRP level within categories remained. However, we believe that this minor variation did not result in a sizeable under- or overestimation of our results.

In conclusion, our meta-analysis showed that serum CRP is a risk factor for diabetes, independent of obesity. Furthermore, genetic variation in CRP was associated with the level of CRP and the risk of diabetes. These results support the hypothesis that CRP plays a role in the pathogenesis of diabetes.

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