

Significant Association of the Interleukin-6 Gene Polymorphisms C-174G and A-598G with Type 2 Diabetes

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Elevated blood concentrations of IL-6 have been shown to predict type 2 diabetes. Because the impact of IL-6 gene polymorphisms on diabetes status, parameters of the metabolic syndrome, and low-grade systemic inflammation has not been analyzed in a population-based study, we investigated the association of the IL-6 single nucleotide polymorphisms C-174G and A-598G on these parameters in 704 elderly participants of the Kooperative Gesundheitsforschung im Raum Augsburg/Cooperative Research in the Region of Augsburg (KORA) Survey 2000. Both -174G and -598G alleles were significantly associated with type 2 diabetes (-174G: odds ratio = 1.51, 95% confidence interval = 1.11–2.07, $P = 0.0096$; -598G: odds ratio = 1.56, 95% confidence interval = 1.13–2.15, $P = 0.0069$) but not with impaired glucose tolerance. In subgroup

analyses, the association reached statistical significance in men and in leaner subjects (body mass index ≤ 28.7 kg/m², *i.e.* study median) but not in women or more obese persons. Circulating IL-6 levels were not associated with the IL-6 polymorphisms, but significantly elevated levels of the chemokine monocyte chemoattractant protein-1/CC chemokine ligand 2 in carriers of the protective genotypes indicated an indirect effect of these single nucleotide polymorphisms on the innate immune system. Our findings confirm that immune gene polymorphisms can be considered as independent risk factors in the etiology of type 2 diabetes and suggest that their contribution may be indirect, by influencing the levels of other immune mediators like monocyte chemoattractant protein-1. (*J Clin Endocrinol Metab* 89: 5053–5058, 2004)

IN RECENT YEARS, evidence has accumulated that type 2 diabetes is associated with a subclinical systemic inflammation that might be attributable to a dysregulation of the innate immune system (1). This immune response is characterized by elevated blood levels of markers of the acute-phase response and of its principal mediator IL-6 (1). There is compelling evidence that augmented levels of IL-6 are associated not only with type 2 diabetes but also with impaired glucose tolerance (IGT) and predict the development of the disease (2, 3), indicating a potential role of this cytokine in the etiology of type 2 diabetes. The high rate of plasma clearance of IL-6 suggests that the IL-6 concentration is regulated mainly on the levels of transcription and translation (4). Therefore, the discovery of single nucleotide polymorphisms (SNPs) in the promoter region of the IL-6 gene (5, 6) raised the possibility that certain alleles of the IL-6 gene might be considered as risk factors for the development of

type 2 diabetes. A recent study reported the association of the C-174G polymorphism with type 2 diabetes in Native Americans and Spanish Caucasians (7), but neither this nor the A-598G polymorphism have been investigated in detail with respect to diabetes status, parameters of the metabolic syndrome and subclinical systemic inflammation in a comprehensive, population-based study. Therefore, we analyzed a subset of participants of the Kooperative Gesundheitsforschung im Raum Augsburg/Cooperative Research in the Region of Augsburg (KORA) Survey 2000 for the association of the C-174G and A-598G polymorphisms with these parameters.

Subjects and Methods

The study population has been described extensively in a previous report (8). Briefly, the KORA Survey 2000 studied a population-based sample of 4,261 subjects, 25–74 yr old, during the years 1999–2001 (3). Each study participant signed a consent form to participate in genetic studies. All study methods were approved by the Ethics committee of the Bayerische Landesärztekammer, Munich. The sampling design followed the guidelines of three previous surveys, in the same region, that were part of the multinational World Health Organization-MONICA (Monitoring of Trends and Determinants in Cardiovascular Disease) study. In the age range of 55–74 yr, 1,653 persons participated in a standardized interview followed by biochemical and clinical analyses. An oral glucose tolerance test and biochemical and immunological analyses were performed as described previously (9). Diabetes was diagnosed according to 1999 World Health Organization criteria (9). A total of 230 individuals with type 2 diabetes and 235 individuals with IGT were available for analyses. After frequency matching for age and sex, 239 normoglycemic controls were randomly selected. Of the diabetic

Abbreviations: BMI, Body mass index; CCL, CC chemokine ligand; CI, 95% confidence interval; CRP, C-reactive protein; DPS, Diabetes Prevention Study; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; IGT, impaired glucose tolerance; KORA, Kooperative Gesundheitsforschung im Raum Augsburg/Cooperative Research in the Region of Augsburg; LD, linkage disequilibrium; LDL, low-density lipoprotein; MCP-1, monocyte chemoattractant protein-1; MIP-1 α , macrophage inflammatory protein-1 α ; OR, odds ratio; SAA, serum amyloid A; SNP, single nucleotide polymorphism.

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patients, 120 were newly detected and did not yet receive antidiabetic treatment (9).

Biochemical analyses

Concentrations of C-reactive protein (CRP), serum amyloid A (SAA), and fibrinogen in plasma were determined by nephelometric assays as described earlier (3, 10, 11). Serum concentrations of cytokines were determined by rigidly evaluated sandwich ELISA; lipid diagnostics were done by standard procedures (3).

Genotyping

Genomic DNA was extracted from leukocytes with a commercial DNA isolation kit (Gentra Systems, Minneapolis, MN) according to the manufacturer's recommendation. Genotyping analyses were carried out by using the MassARRAY system (Sequenom, San Diego, CA). Briefly, genomic DNAs were amplified by PCR using HotStarTaq DNA Polymerase (Qiagen, Hilden, Germany). Genotyping assays were carried out using 5 ng genomic DNA. PCR primers were used at 167-nM final concentrations for a PCR vol of 6 μ l. The PCR condition was 95 C for 15 min for hot start, followed by denaturing at 95 C for 30 sec, annealing at 56 C for 30 sec, extension at 72 C for 1 min for 44 cycles, and finally incubation at 72 C for 10 min. PCR products first were treated with shrimp alkaline phosphatase (Amersham, Freiburg, Germany) for 20 min at 37 C to remove excess deoxynucleotide triphosphates and afterward for 10 min at 85 C to inactivate shrimp alkaline phosphatase. ThermoSequenase (Amersham) was used for the base extension reactions. Extension primers were used at a final concentration of 5.4 μ M in 10- μ l reactions. The base extension reaction condition was 94 C for 2 min, followed by 94 C for 5 sec, 52 C for 5 sec, and 72 C for 5 sec for 40 cycles. The final base extension products were treated with SpectroCLEAN resin (Sequenom) to remove salts in the reaction buffer. This step was carried out with a 96-channel autopipette, and 16 μ l resin-water suspension was added into each base extension reaction, resulting in a total vol of 26 μ l. After quick centrifugation, 10 nl reaction solution was dispensed onto a 384 format SpectroCHIP (Sequenom) prespotted with a matrix of 3-hydroxypicolinic acid using a SpectroPoint nanodispenser (Sequenom). A modified Bruker Biflex matrix-assisted laser desorption ionization-time-of-flight mass spectrometer (Sequenom) was used for data acquisition from the SpectroCHIP. Genotyping calls were made in real time with MASSARRAY RT software (Sequenom).

Statistical analysis

Association of IL-6 genotypes and type 2 diabetes was analyzed by conditional logistic regression of diabetes patients *vs.* age- and sex-matched controls. Conditional logistic regression was carried out with the SAS procedure PROC PHREG (SAS Institute, Cary, NC), which performs an automatic frequency matching of the data. Genotypic data entered the regression model in the form of two dummy variables representing the effect of genotype -174C/G and genotype -174G/G *vs.* the reference category -174C/C, respectively. The same was done for A-598G, where -598A/A was the reference group. In a second approach, genotype data were introduced as a three-categorical variable with the proportional odds assumption that reflects the additive allele effect. Interactions were modeled with interaction terms as well as with subgroup analyses. The components of the metabolic syndrome that showed no major deviation from the normal distribution [waist circumference, body mass index (BMI), total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and leukocyte count] were described by means \pm SD in Table 1 and by means with confidence intervals adjusted for the covariables age, sex, and diabetes status (see Table 4). The *P* values reflecting differences between groups of diabetes, IGT, and control probands and between groups of different genotypes resulted from ANOVA models. Components of subclinical inflammation [IL-6, CRP, SAA, fibrinogen, monocyte chemoattractant protein-1 (MCP-1)/CC chemokine ligand 2 (CCL2), and macrophage inflammatory protein-1 α /MIP-1 α /CCL3] were described by median and interquartile range, and differences between groups were analyzed by the nonparametric Kruskal-Wallis test. The remaining components of the metabolic syndrome [fasting insulin, homeostasis model assessment (HOMA)-insulin resistance, and fasting triglycerides] were treated likewise because of clear deviation from the normal distribution. A *P* value < 0.05 was considered statistically significant. All calculations were performed with the software package SAS 8.

Results

The study group contained 704 subjects, 55–74 yr old: 230 type 2 diabetes patients, 235 patients with IGT, and 239 controls frequency-matched for age and sex. Table 1 shows

TABLE 1. Characteristics of KORA Survey 2000 study population: association of diabetes status with components of the metabolic syndrome and subclinical inflammation

Variable	T2D	IGT	Controls	<i>P</i> T2D <i>vs.</i> controls	<i>P</i> IGT <i>vs.</i> controls
Age (yr)	65.1 \pm 5.2 (n = 197)	65.5 \pm 5.3 (n = 175)	65.2 \pm 5.3 (n = 189)	0.80	0.64
Sex (male/female)	121/76 (n = 197)	96/79 (n = 175)	104/85 (n = 189)	0.21	0.96
Waist circumference (cm)	103.4 \pm 11.4 (n = 196)	98.1 \pm 10.4 (n = 175)	93.6 \pm 11.0 (n = 189)	<0.0001	<0.0001
BMI (kg/m ²)	31.0 \pm 4.6 (n = 193)	29.3 \pm 3.8 (n = 175)	27.4 \pm 3.6 (n = 188)	<0.0001	<0.0001
Insulin resistance (HOMA) ^a	4.72 (3.05, 8.07) (n = 102)	2.99 (2.10, 4.51) (n = 174)	2.03 (1.41, 3.06) (n = 189)	<0.0001	<0.0001
Insulin (mU/liter)	18.60 (10.50, 31.80) (n = 193)	11.78 (8.70, 16.35) (n = 174)	8.85 (6.00, 13.05) (n = 189)	<0.0001	<0.0001
LDL cholesterol (mg/dl)	144.9 \pm 41.7 (n = 196)	156.9 \pm 39.7 (n = 175)	155.0 \pm 41.1 (n = 188)	0.022	0.65
HDL cholesterol (mg/dl)	50.8 \pm 14.5 (n = 197)	55.8 \pm 15.8 (n = 175)	58.1 \pm 15.1 (n = 188)	<0.0001	0.10
Total cholesterol (mg/dl)	233.9 \pm 45.6 (n = 197)	244.7 \pm 42.9 (n = 175)	242.9 \pm 42.7 (n = 188)	0.063	0.68
Fasting triglycerides (mg/dl) ^a	137 (104, 186) (n = 102)	125 (93, 179) (n = 174)	109 (84.5, 146.5) (n = 184)	<0.0001	0.0020
Leukocyte count ($\times 10^{-3}/\mu$ l)	6.96 \pm 2.04 (n = 197)	6.27 \pm 1.56 (n = 175)	5.88 \pm 1.27 (n = 189)	<0.0001	0.010
IL-6 (pg/ml)	2.47 (1.25, 4.43) (n = 194)	2.25 (1.09, 3.23) (n = 174)	1.64 (0.65, 2.85) (n = 186)	<0.0001	0.0032
CRP (mg/ml)	2.62 (1.13, 5.89) (n = 192)	2.33 (1.25, 4.40) (n = 174)	1.27 (0.75, 2.99) (n = 188)	<0.0001	<0.0001
SAA (mg/ml)	4.25 (2.7, 7.5) (n = 192)	4.2 (2.5, 7.4) (n = 174)	3.5 (2.2, 5.8) (n = 188)	0.0018	0.0060
Fibrinogen (g/liter)	3.03 (2.61, 3.38) (n = 192)	2.99 (2.58, 3.38) (n = 174)	2.87 (2.53, 3.19) (n = 188)	0.022	0.11
MCP-1/CCL2 (pg/ml)	276.4 (206.4, 358.8) (n = 194)	289.9 (220.0, 370.2) (n = 174)	299.5 (191.7, 420.6) (n = 186)	0.15	0.64

For the variables waist circumference, BMI, LDL cholesterol, HDL cholesterol, total cholesterol, leukocyte count, and age, means \pm SD are shown. The respective *P* values result from ANOVA. The median and quartiles are shown for markers of subclinical inflammation (IL-6, CRP, SAA, fibrinogen, MCP-1/CCL2, and for HOMA-insulin resistance, insulin, and fasting triglycerides). The respective *P* values were obtained with the nonparametric Kruskal-Wallis test. Frequencies of male and female probands are given with a *P* value resulting from conditional logistic regression. The groups were adjusted for age and sex. Only data from subjects which were both genotyped and phenotyped are given. T2D, Type 2 diabetes.

^a Patients with a history of diabetes provided nonfasting blood samples and were excluded.

the association of diabetes status with parameters of the metabolic syndrome and with inflammatory markers.

For the C-174G SNP, the frequencies of the CC, CG, and GG genotypes were 0.174, 0.508, and 0.318, respectively, with allele frequencies of 0.428 for C-174 and 0.572 for -174G. For the A-598G SNP, almost identical results were obtained with frequencies of the AA, AG, and GG genotypes of 0.180, 0.507, and 0.313, respectively, and allele frequencies of 0.433 for A-598 and 0.567 for -598G. Analysis of linkage disequilibrium (LD) revealed that the C-174G and A-598G SNPs were in LD in 97.5% of subjects. A bias due to the genotyping rates of 92.3% (C-174G) and 86.2% (A-598G) appears unlikely because both SNPs were in Hardy Weinberg equilibrium and the data are consistent with previously published results for comparable Caucasian populations regarding allele frequencies (12–14) and LD (15).

Conditional logistic regression demonstrated that both C-174G and A-598G SNPs exhibited a statistically significant association with diabetes. Because of the high degree of LD and the higher call rate for the C-174G SNP, only data for the C-174G SNP are shown. The frequencies for the genotypes -174C/C, C/G, and G/G were 0.196, 0.536, and 0.268, respectively, for the controls; 0.181, 0.476, and 0.343, respectively, for subjects with IGT; and 0.144, 0.509, and 0.347, respectively, for patients with type 2 diabetes. The genotype frequencies for the A-598G SNP were almost identical.

With the reference category of -174C/C, conditional logistic regression yielded an odds ratio (OR) of 1.32 with a 95% confidence interval (CI) of 0.78–2.23 ($P = 0.31$) for genotype C/G and an OR of 1.81 (CI, 1.02–3.21; $P = 0.044$) for genotype G/G (Table 2). The additional consideration of BMI as covariable increased the OR in both genotypes C/G and G/G *vs.* C/C (genotype C/G: OR = 1.61, CI = 0.89–2.94, $P = 0.12$; genotype G/G: OR = 2.34, CI = 1.22–4.47, $P = 0.010$) (Table 2).

The figures suggest an additive allele effect; therefore we

continued our analyses with conditional logistic regression in an additive model. The effect of the G allele *vs.* the C allele was then estimated with an OR of 1.35 (CI, 1.02–1.79; $P = 0.037$), which was again more accentuated after correction for BMI (OR, 1.51; CI, 1.11–2.07; $P = 0.0096$) (Table 2).

Subanalyses showed that the association of the C-174G SNP with diabetes status reached statistical significance in men (OR, 1.46; $P = 0.042$) but not in women (OR, 1.20; $P = 0.42$) and also in leaner subjects (BMI \leq median, 28.7 kg/m² of KORA Survey 2000) compared with subjects with BMI > 28.7 kg/m² (OR, 1.67; $P = 0.022$ *vs.* OR, 1.36, $P = 0.14$). When physical activity was considered as a covariable in addition to age, sex, and BMI, the significant association of the C-174G SNP with type 2 diabetes persisted (OR, 1.44; CI, 1.05–1.99; $P = 0.023$). Further subdivision of the study group by sex and BMI confirmed that this association remained significant for men with BMI \leq 28.7 kg/m² (OR, 1.71) but not for men with BMI > 28.7 kg/m² or for all women regardless of BMI.

However, the interaction between BMI and genotype that might be proposed by the subgroup analysis of Table 2 cannot be verified on a statistically significant basis (OR, 0.83; CI, 0.46–1.50; $P = 0.54$; Table 3). A model containing an interaction term for sex and *IL-6* genotype also shows no significant interaction (data not shown).

The same analyses for both SNPs were performed for the IGT group against controls, but no significant differences between the IGT and the control groups could be found. Analyses with diabetes patients and IGT probands in one group *vs.* the controls reduced the genotype's effect, indicating that the sharp distinction between the diabetic and normal groups emphasizes the effect.

The groups with genotypes -174C/C, C/G, and G/G showed a marginally significant impact of genotype on BMI but no difference regarding other key parameters characterizing the metabolic syndrome, such as waist circumference, total cholesterol, LDL cholesterol, HDL cholesterol, leuko-

TABLE 2. Association of the C-174G SNP with type 2 diabetes: conditional logistic regression on type 2 diabetes patients and controls (subgroup analysis)

Model	Covariables	Subgroup	Odds ratio	CI	P
Additive			1.35	1.02–1.79	0.037
Additive		Men	1.46	1.01–2.10	0.042
Additive		Women	1.20	0.77–1.87	0.42
Additive	BMI		1.51	1.11–2.07	0.0096
Additive		BMI \leq 28.7	1.67	1.08–2.60	0.022
Additive		BMI > 28.7	1.36	0.91–2.04	0.14
Additive	BMI, physical activity		1.44	1.05–1.99	0.023
C/G <i>vs.</i> C/C			1.32	0.78–2.23	0.31
G/G <i>vs.</i> C/C			1.81	1.02–3.21	0.044
C/G <i>vs.</i> C/C		Men	1.86	0.94–3.69	0.077
G/G <i>vs.</i> C/C		Men	2.26	1.07–4.77	0.032
C/G <i>vs.</i> C/C		Women	0.78	0.34–1.79	0.55
G/G <i>vs.</i> C/C		Women	1.25	0.51–3.11	0.62
C/G <i>vs.</i> C/C	BMI		1.61	0.89–2.94	0.12
G/G <i>vs.</i> C/C	BMI		2.34	1.22–4.47	0.010
C/G <i>vs.</i> C/C		BMI \leq 28.7	3.03	1.15–8.01	0.025
G/G <i>vs.</i> C/C		BMI \leq 28.7	3.71	1.33–10.35	0.013
C/G <i>vs.</i> C/C		BMI > 28.7	1.09	0.52–2.26	0.83
G/G <i>vs.</i> C/C		BMI > 28.7	1.79	0.78–4.11	0.17
C/G <i>vs.</i> C/C	BMI, physical activity		1.55	0.85–2.84	0.15
G/G <i>vs.</i> C/C	BMI, physical activity		2.14	1.11–4.13	0.023

Considered are models with an additive allele effect (*upper half*) as well as models with individual estimates for -174C/G and -174G/G genotypes *vs.* the -174C/C genotype.

cyte count, insulin, insulin resistance (as estimated by the HOMA), and fasting triglycerides (the latter two parameters determined only in individuals without a history of diabetes) (Table 4). Conditional logistic regression showed that there was no association of the C-174G SNP with hypertension (defined as blood pressure \geq 140/90 or antihypertensive treatment) or smoking habits (data not shown).

The analysis of inflammatory mediators demonstrated that the protective $-174C/C$ genotype showed a trend for an association only with increased levels of MCP-1 ($P = 0.065$), whereas no differences could be found for IL-6, CRP, SAA, fibrinogen, and MIP-1 α /CCL3 (Table 4). Because diabetes and diabetes-related factors, such as poor glycemic control and antidiabetic therapy, might affect these variables, the analyses were repeated after exclusion of all patients with type 2 diabetes. Median levels of MCP-1 were almost identical for the genotypes $-174C/C$, C/G , and G/G with 299.0 pg/ml ($n = 81$), 291.5 pg/ml ($n = 218$), and 274.6 pg/ml ($n = 131$), respectively ($P = 0.14$).

We investigated the serum levels of MCP-1/CCL2 separately in both men and women and also in leaner and more obese subjects. As shown in Table 5, the association of the

C-174G SNP with type 2 diabetes closely reflected the association of this SNP with MCP-1 levels. For the protective $-174C/C$ genotype, both men and subjects with BMI \leq 28.7 kg/m² had increased MCP-1 levels. This increase was most pronounced when both variables were combined, *i.e.* in men with BMI \leq 28.7 kg/m² (median, 382.6 pg/ml compared with 292.5 pg/ml in all $-174C/C$ carriers). These findings correlated with the diabetes prevalence among carriers of the $-174C/C$ genotype, which was 27.4% for the whole study group, but was reduced to 14.3% in men with BMI \leq 28.7 kg/m². The respective values for $-174G/G$ carriers were 36.2% (study group) and 35.0% (men with BMI \leq 28.7 kg/m²). After exclusion of individuals with type 2 diabetes, median MCP-1 levels in men with BMI \leq 28.7 kg/m² were not altered significantly and reached 399.2 pg/ml for the genotype $-174C/C$ ($n = 30$), 290.2 pg/ml for C/G ($n = 74$), and 296.8 pg/ml for G/G ($n = 39$) ($P = 0.051$).

The same analyses, as summarized in Tables 4 and 5, were also performed for the A-598G SNP. Due to the high degree of LD, results comparable with the C-174G SNP would have to be expected and were indeed found.

Discussion

The results from several studies on the association between the C-174G and A-598G SNPs and type 2 diabetes and insulin resistance are still controversial. Analyses of smaller cohorts of Native American and Spanish Caucasian origin found the G allele of the C-174G SNP to be associated with higher risk of type 2 diabetes (7), but this SNP has been reported not to be linked with diabetes in the course of the Finnish Diabetes Prevention Study (DPS) (16). Furthermore, one small study with nondiabetic subjects described an association of the $-174C/C$ genotype with higher insulin sensitivity (17), whereas a different study in normoglycemic individuals came to the opposite result (18). The comparison of these studies is hampered by the fact that study groups

TABLE 3. Conditional logistic regression on diabetes status using type 2 diabetes patients and controls only (interaction model)

Variable	Covariable	Odds ratio	CI	P
Diabetes	IL-6/ -174	1.59	1.03–2.44	0.036
	BMI	4.42	2.00–9.78	0.0002
	Sex	0.66	0.44–0.99	0.046
	Interaction IL-6/ -174 * BMI	0.83	0.46–1.50	0.54
Diabetes	IL-6/ -598	1.63	1.05–2.52	0.030
	BMI	4.39	1.94–9.93	0.0004
	Sex	0.63	0.42–0.96	0.033
	Interaction IL-6/ -598 * BMI	0.81	0.44–1.49	0.50

TABLE 4. Association of the C-174G SNP with components of the metabolic syndrome and subclinical inflammation

Variable	$-174C/C$	$-174C/G$	$-174G/G$	P (C/C vs. C/G vs. G/G)
Age (yr)	64.1 (63.1–65.1) ($n = 113$)	65.2 (64.6–65.7) ($n = 330$)	65.5 (64.8–66.2) ($n = 207$)	0.067
Waist circumference (cm)	100.1 (98.2–102.0) ($n = 111$)	97.4 (96.3–98.5) ($n = 330$)	98.2 (96.8–99.6) ($n = 207$)	0.059
BMI (kg/m ²)	30.3 (29.5–31.0) ($n = 111$)	29.1 (28.7–29.6) ($n = 328$)	29.4 (28.9–30.0) ($n = 204$)	0.039
LDL cholesterol (mg/dl)	152.6 (144.9–160.2) ($n = 113$)	154.7 (150.2–159.2) ($n = 328$)	149.5 (143.8–155.2) ($n = 206$)	0.37
HDL cholesterol (mg/dl)	53.2 (50.5–55.9) ($n = 113$)	56.0 (54.4–57.6) ($n = 328$)	55.8 (53.8–57.8) ($n = 207$)	0.21
Total cholesterol (mg/dl)	241.8 (233.7–249.9) ($n = 113$)	243.0 (238.2–247.7) ($n = 329$)	237.8 (231.8–243.8) ($n = 207$)	0.41
Leukocyte count ($\times 10^{-3}/\mu$ l)	6.50 (6.19–6.81) ($n = 113$)	6.44 (6.26–6.62) ($n = 330$)	6.24 (6.01–6.47) ($n = 207$)	0.31
Sex (male/female)	65/48 ($n = 113$)	184/146 ($n = 330$)	120/87 ($n = 207$)	0.47
Insulin resistance (HOMA) ^a	2.93 (1.81, 4.56) ($n = 97$)	2.54 (1.70, 4.50) ($n = 278$)	2.86 (1.87, 4.11) ($n = 169$)	0.70
Insulin (mU/liter)	11.55 (8.25, 20.25) ($n = 111$)	11.48 (7.50, 19.28) ($n = 328$)	12.23 (8.10, 20.25) ($n = 206$)	0.62
Fasting triglycerides (mg/dl) ^a	125 (88, 168) ($n = 97$)	118 (87, 172) ($n = 274$)	126 (89, 166) ($n = 167$)	0.64
IL-6 (pg/ml)	2.22 (1.29, 4.06) ($n = 112$)	2.13 (0.85, 3.62) ($n = 326$)	2.25 (1.15, 3.52) ($n = 205$)	0.58
CRP (mg/ml)	2.48 (0.99, 4.65) ($n = 112$)	1.84 (0.92, 4.04) ($n = 325$)	2.25 (1.02, 4.39) ($n = 206$)	0.38
SAA (mg/ml)	4.15 (2.8, 6.9) ($n = 112$)	4.0 (2.4, 6.7) ($n = 325$)	3.75 (2.5, 6.7) ($n = 206$)	0.55
Fibrinogen (g/liter)	3.03 (2.52, 3.39) ($n = 112$)	2.94 (2.57, 3.36) ($n = 325$)	2.92 (2.53, 3.21) ($n = 206$)	0.53
MCP-1/CCL2 (pg/ml)	292.5 (216.8, 420.4) ($n = 112$)	290.8 (206.4, 376.5) ($n = 326$)	271.1 (194.2, 346.8) ($n = 205$)	0.065

For the variables waist circumference, BMI, LDL cholesterol, HDL cholesterol, total cholesterol, leukocyte count and age, means adjusted for the covariables age, sex and diabetes status are shown with CI. The respective P values result from ANOVA. The median and quartiles are shown for markers of subclinical inflammation (IL-6, CRP, SAA, fibrinogen, MCP-1/CCL2, MIP-1 α /CCL3) and for HOMA-insulin resistance, insulin and fasting triglycerides. The respective P values were obtained with the nonparametric Kruskal-Wallis test. Further analyses showed no evidence for confounding by diabetes so that the Kruskal-Wallis test was conducted in the whole study group. Sex distribution was compared by conditional logistic regression. Only data from subjects which were both genotyped and phenotyped are given.

^a Patients with a history of diabetes provided nonfasting blood samples and were excluded.

TABLE 5. Serum levels of MCP-1/CCL2 for the different genotypes of the C-174G SNP in subdivisions of the study group

Subdivision	–174C/C	–174C/G	–174G/G	<i>P</i>
Men	314.8 (221.7, 433.2) (n = 65)	289.6 (194.6, 368.9) (n = 181)	276.0 (200.8, 355.4) (n = 119)	0.055
Women	276.4 (174.7, 393.2) (n = 47)	292.1 (222.4, 389.8) (n = 145)	253.2 (176.4, 338.2) (n = 86)	0.71
BMI ≤ 28.7	335.0 (228.9, 433.2) (n = 51)	286.1 (210.1, 373.3) (n = 181)	274.6 (203.4, 352.9) (n = 97)	0.069
BMI > 28.7	271.0 (216.3, 404.4) (n = 61)	311.7 (203.4, 380.9) (n = 145)	269.2 (182.3, 342.4) (n = 108)	0.96
Men, BMI ≤ 28.7	382.6 (252.0, 447.4) (n = 35)	289.6 (198.0, 388.8) (n = 111)	277.6 (208.9, 351.8) (n = 60)	0.029
Men, BMI > 28.7	265.4 (217.2, 365.7) (n = 30)	289.1 (194.2, 349.9) (n = 70)	272.8 (184.9, 355.4) (n = 59)	0.96
Women, BMI ≤ 28.7	273.3 (199.7, 297.3) (n = 16)	269.9 (221.9, 355.4) (n = 70)	247.8 (185.8, 352.9) (n = 37)	0.92
Women, BMI > 28.7	299.0 (174.7, 411.5) (n = 31)	334.0 (243.4, 424.0) (n = 75)	253.4 (175.6, 331.0) (n = 49)	0.25

Shown are median and quartiles in picograms per milliliter. The *P* values result from the nonparametric Kruskal-Wallis test (CC vs. CG/GG).

differed in age, degree of obesity, glucose tolerance, and sex distribution.

Therefore, we used the population-based KORA Survey 2000 to reevaluate the relevance of *IL-6* SNPs for type 2 diabetes. We extended our analysis by testing for influence of sex, BMI, and impact on parameters of the metabolic syndrome and subclinical systemic inflammation, which might indicate a potential mediator that links the *IL-6* gene SNPs with diabetes. First of all, our results demonstrate the association of the C-174G and A-598G SNPs with type 2 diabetes in a German Caucasian population. We did not find an association of the *IL-6* SNPs with insulin resistance (HOMA) and IGT, which might indicate that the association with type 2 diabetes may have been a false positive. However, this appears very unlikely because our main finding is confirmed by the study by Vozarova *et al.* (7), which describes the association of the –174G allele with type 2 diabetes independently in smaller populations of Native Americans and Spanish Caucasians. Our study considerably extends the previous findings by subgroup analyses that indicate that the association of *IL-6* genotype and diabetes is statistically significant in men and lean to moderately overweight subjects, *i.e.* that the G alleles of the C-174G and A-598G SNPs might be associated with an elevated diabetes risk, whereas for women and more obese individuals, the SNPs do not appear to affect diabetes risk. Interaction models, however, cannot confirm the association of genotype and BMI or sex. Concerning the association of *IL-6* genotype and type 2 diabetes, the outcome of our study differs from the DPS (16). However, it should be noted that the DPS participants were selected for IGT, which led to a study group being more obese than the KORA participants, which therefore might have possessed additional risk factors that could have obscured the impact of the SNP. In addition, the comparison between subjects with IGT who progressed or did not progress to diabetes might not be as suitable to detect subtle risk factors as our comparison of diabetes patients, subjects with IGT, and age- and sex-matched healthy controls.

We were not able to demonstrate an association of the SNPs with insulin resistance and IGT tolerance, which precede the development of overt type 2 diabetes, both in the whole study group and in the subgroups divided by sex and/or BMI. Thus, the results from our large, population-based study do not confirm the aforementioned associations of –174 genotypes with insulin resistance (17, 18). Importantly, the subanalyses in our study emphasize that immune gene polymorphisms may be considered as risk factors only

in certain population segments and that comprehensive studies with a high number of participants may indeed be required to detect SNP-related increased risks in these groups, whereas smaller studies might include patient selection-inherent biases that can skew the outcome and do not reflect the general risk of a population.

The analysis of type 2 diabetes-related inflammatory markers could not reveal a direct mechanism of the *IL-6* SNPs on circulating *IL-6* levels. Although some data indicate that there might be an association between *IL-6* –174C/G genotype and circulating *IL-6* levels (5, 15), our study is in line with the majority of studies on *IL-6* SNPs, which do not observe a significant impact of –174G/C genotypes on *IL-6* levels in serum (12, 14, 18, 19). Interestingly, it has been shown that the regulatory effect of SNPs within the *IL-6* promoter on *IL-6* expression is cell-type specific (6). It is therefore reasonable to hypothesize that the impact of *IL-6* SNPs on *IL-6* expression by various cell types might depend on parameters such as acute inflammation or obesity (and therefore also on the study group), which exert different effects on *IL-6*-producing cells. The view that as-yet-undefined parameters can enhance or suppress the impact of SNPs on *IL-6* levels is supported by the observation that the –174C allele was found to be associated with significantly elevated *IL-6* expression after coronary artery bypass graft surgery; whereas, regarding the baseline *IL-6* levels, no difference between the genotypes could be detected before surgery (15). It cannot be excluded that the presence of carrier proteins or soluble receptors in the blood, such as soluble glycoprotein 130 (20), might have obscured a subtle, but biologically relevant SNP-mediated genetic predisposition to slightly augmented *IL-6* expression (17).

Our investigations, however, demonstrate that the stronger association of the C-174G SNP in subgroups of the study cohort is closely mirrored by elevation of MCP-1/CCL2 blood concentration in carriers of the protective genotype –174C/C. Because elevated levels of MCP-1 have recently been shown to be associated with a lower risk of diabetes (H. Kolb, C. Herder, S. Müller-Scholze, unpublished observations), MCP-1 appears a potential candidate to link *IL-6* gene polymorphisms with diabetes status by an as-yet-unknown mechanism. This mechanism of MCP-1 up-regulation seems to be prone to disturbances by obesity and sex-related influences and might thus be suppressed in women and obese subjects. In this context, it should be noted that individuals taking antiinflammatory drugs were not excluded from our study because, in the age group studied, a large proportion has been taking statins and antihypertensive and antidiabetic

compounds. Their impact appears limited, because systemic levels of inflammatory mediators are elevated in individuals with type 2 diabetes, metabolic syndrome, or atherosclerosis (1, 21) despite their more frequent use of these drugs.

Taken together, this study demonstrates that the *IL-6* gene SNPs C-174G and A-598G are associated with type 2 diabetes and indicates that the chemokine MCP-1 may act as potential protective mediator, although the mechanism still remains to be elucidated. The SNP-related association reached statistical significance in men and in the absence of obesity, thereby underlining the relevance of comprehensive, population-based studies that allow for subgroup analysis.

Acknowledgments

We thank Michaela Bunge and Petra Weskamp for excellent technical assistance. The genetic part of the work was performed in the Genome Analysis Centre of the GSF-National Research Centre for Environment and Health.

Received February 24, 2004. Accepted June 28, 2004.

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The KORA research platform was initiated and funded by the GSF-National Research Centre for Environment and Health, which is funded by the German Federal Ministry of Education, Science, Research and Technology and by the State of Bavaria. This work was supported by grants from the German National Genome Research Net given to the genotyping platform Munich as well as by the Deutsche Forschungsgemeinschaft, the European Foundation for the Study of Diabetes, the Federal Ministry of Health, the Ministry of Science and Research of North Rhine-Westphalia, and the Department of Internal Medicine II-Cardiology at the University of Ulm.

The KORA Group consists of H. E. Wichmann (speaker), H. Löwel, C. Meisinger, T. Illig, R. Holle, J. John, and their co-workers who are responsible for the design and conduct of the KORA studies.

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