Brief Genetics Report

Variants of the Interleukin-10 Promoter Gene Are Associated With Obesity and Insulin Resistance but Not Type 2 Diabetes in Caucasian Italian Subjects

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Interleukin (IL)-10 is a major anti-inflammatory cytokine that has been associated with obesity and type 2 diabetes. The three polymorphisms −1082G/A, −819C/T, and −592C/A in the *IL10* promoter were reported to influence *IL10* transcription. We investigated whether these polymorphisms were associated with type 2 diabetes and related traits in a cohort of Italian Caucasians comprising 551 type 2 diabetic and 1,131 control subjects. The −819C/T and −592C/A polymorphisms were in perfect linkage disequilibrium (r^2 = 1.0). The −1082G/A polymorphism was not associated with type 2 diabetes or related traits. Although the −592C/A polymorphism was not associated with type 2 diabetes, nondiabetic homozygous carriers of the A allele showed increased BMI and insulin resistance and lower plasma IL-10 levels compared with the other genotypes. In the nondiabetic group, the ATA haplotype was associated with an increased risk for obesity (odds ratio 1.28 [95% CI 1.02–1.60]; *P* = 0.02). The ATA/ATA composite genotype was associated with an increased risk for obesity (1.96 [1.16–3.31]; *P* = 0.01) and insulin resistance (1.99 [1.12–3.53]; *P* = 0.01). This study suggests that polymorphisms and haplotypes of the *IL10* promoter may be associated with obesity and insulin resistance in a large sample of Italian Caucasians. *Diabetes* 55: 1529–1533, 2006

Increasing evidence suggests a link between a low-grade inflammatory state and the development of obesity and the coexisting conditions of insulin resistance, type 2 diabetes, and the metabolic syndrome (1–4). Proinflammatory cytokines can cause insulin resistance and anti-inflammatory cytokines can counteract these negative effects, suggesting that an unpaired balance between proinflammatory versus anti-inflammatory cytokines may have a role in the pathogenesis of type 2 diabetes and related traits. Interleukin (IL)-10 is a major anti-inflammatory cytokine that plays a crucial role in the regulation of the immune system. It has strong deactivating properties on the inflammatory host response mediated by macrophages and lymphocytes and potently inhibits the production of proinflammatory cytokines (5–8). Recently, it has been reported that cotreatment with IL-10 prevented IL-6–induced defects in both hepatic and skeletal muscle insulin action in rats (9). Low IL-10 circulating levels have been reported to be associated with obesity and metabolic syndrome (10). Moreover, it has been shown that low IL-10 production is associated with hyperglycemia and type 2 diabetes (11). Some polymorphisms in the promoter of the *IL10* gene have been associated with its transcription levels. The best documented are the *IL10* gene promoter polymorphisms −1082G/A, −819C/T, and −592C/A, which form three major haplotypes (GCC, ACC, and ATA) among Caucasian subjects (12,13). The ATA haplotype has been associated with lower transcriptional activity than the GCC haplotype, and the ATA/ATA genotype was associated with lower IL-10 production under lipopolysaccharide stimulation than other genotypes (14–17). In this study, we have examined whether the −1082G/A, −819C/T, and −592C/A polymorphisms in the *IL10* promoter gene separately or in combination are associated with type 2 diabetes and related quantitative traits.

The −819C/T and −592C/A variants were in perfect linkage disequilibrium (r^2 = 1.0). Hence, only data for the −1082G/A and −592 C/A polymorphisms are presented in the single-variant association studies. Hardy-Weinberg expectations were fulfilled in both nondiabetic and type 2 diabetic subjects for both the −1082G/A and the −592 C/A polymorphisms. Clinical and biochemical characteristics of the 1,131 nondiabetic subjects and the 551 type 2 diabetic patients are shown in Table 1.

The −1082G/A polymorphism. In the type 2 diabetes case-control study, there was no significant difference between genotype frequencies of the −1082G/A variant between type 2 diabetic case and nondiabetic control subjects (Table 1) even after adjustment for age, sex, and waist-to-hip ratio in a logistic regression analysis (odds ratio [OR] 1.17 [95% CI 0.95–1.33]; *P* = 0.17). When the analysis was repeated in a subgroup consisting of 239 obese type 2 diabetic and 429 obese nondiabetic subjects, no association was found between the −1082G/A variant and type 2 diabetes (*P* = 0.11 by χ^2 test). In the nondia-

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HOMA-IR, homeostasis model assessment of insulin resistance; IL, interleukin.

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TABLE 1
Clinical and biochemical characteristics of the study subjects

	Nondiabetic control subjects	Patients with type 2 diabetes	*P*
n (male/female)	481/650	278/273	
Age (years)	47 ± 14	61 ± 11	0.46
Age at diagnosis (years)	—	52 ± 11	
Duration of diabetes (years)	—	11 ± 9	
Treatment for diabetes (diet/oral agents/insulin)	—	37/346/168	
BMI (kg/m^2)	29.5 ± 6.5	30.1 ± 5.8	0.03
Systolic blood pressure (mmHg)	122 ± 15	137 ± 18	0.0001
Diastolic blood pressure (mmHg)	78 ± 10	81 ± 10	0.03
Fasting glucose (mg/dl)	92 ± 10	164 ± 71	0.0001
2-h postchallenge glucose (mg/dl) (*n* = 734)	116 ± 32	—	
HbA_{1c} (%)	5.3 ± 0.6	7.5 ± 2.4	0.0001
Total cholesterol (mg/dl)	203 ± 41	205 ± 44	0.47
HDL cholesterol (mg/dl)	52 ± 14	45 ± 15	0.0001
Triglycerides (mg/dl)	125 ± 68	163 ± 100	0.0001
−1082G/A IL-10 variant			
A/A	485 (42.9)	219 (39.7)	0.46
A/G	516 (45.6)	264 (47.9)	
G/G	130 (11.5)	68 (12.3)	
−592C/A IL-10 variant			
C/C	615 (54.4)	301 (54.6)	0.39
C/A	449 (39.7)	226 (41.0)	
A/A	67 (5.9)	24 (4.4)	

Data are means ± SD or *n* (%), unless otherwise indicated. *P* values for comparisons of differences of continuous variables between two genotypes using unpaired Student's *t* test. Differences in genotype frequencies were compared by χ^2 test.

betic group, no phenotypic differences were observed among subjects carrying the three genotypes (Table 2). In the diabetic group, no differences in BMI ($P = 0.11$) were observed among subjects carrying the three genotypes.

The −592 C/A polymorphism. In the type 2 diabetes case-control study, there was no significant difference between frequencies of the −592C/A variant between type 2 diabetic case and nondiabetic control subjects (Table 1) even after adjustment for age, sex, and waist-to-hip ratio in a logistic regression analysis (OR 0.98 [95% CI 0.81–1.18]; $P = 0.84$). When the analysis was repeated in the obese subjects subgroup, no association was found between the −592G/A variant and type 2 diabetes ($P = 0.11$ by χ^2 test). However, in a genotype-quantitative trait study, nondiabetic subjects homozygous for the A allele (A/A) showed increased BMI, waist-to-hip ratio, systolic blood pressure,

TABLE 2
Clinical and biochemical characteristics of the nondiabetic group according to the IL-10 genotype

	−1082G/A polymorphism				−592C/A polymorphism				
	A/A	G/A	G/G	*P*	C/C	C/A	A/A	*P*	*P* (A/A vs. C/A + C/C)*
n (male/female)	207/278	217/299	57/73	0.93	282/333	171/278	28/39	0.04	0.9
Age (years)	48 ± 14	47 ± 14	47 ± 13	0.24	48 ± 14	47 ± 14	49 ± 13	0.52	0.41
BMI (kg/m^2)	29.9 ± 6.7	29.2 ± 6.3	29.3 ± 6.7	0.15	29.3 ± 6.4	29.4 ± 6.6	31.6 ± 6.4†‡	0.01	0.003
Waist-to-hip ratio	0.90 ± 0.09	0.91 ± 0.10	0.90 ± 0.12	0.32	0.90 ± 0.10	0.89 ± 0.08	0.92 ± 0.09§	0.049	0.29
Systolic blood pressure (mmHg)	124 ± 17	121 ± 14	118 ± 13	0.15	121 ± 15	123 ± 16	129 ± 15**	0.04	0.02
Diastolic blood pressure (mmHg)	79 ± 10	78 ± 10	77 ± 9	0.73	77 ± 10	79 ± 9	82 ± 11	0.12	0.13
Fasting glucose (mg/dl)	92 ± 10	92 ± 10	92 ± 11	0.66	92 ± 10	91 ± 10	95 ± 10	0.059	0.04
2-h postchallenge glucose (mg/dl) (*n* = 734)	116 ± 32	115 ± 33	114 ± 29	0.79	115 ± 32	115 ± 33	125 ± 26	0.15	0.053
Total cholesterol (mg/dl)	205 ± 41	203 ± 40	197 ± 39	0.14	202 ± 40	205 ± 42	205 ± 38	0.46	0.76
HDL cholesterol (mg/dl)	52 ± 14	52 ± 13	53 ± 14	0.85	51 ± 13	53 ± 14	49 ± 14	0.02	0.048
Triglycerides (mg/dl)	128 ± 67	124 ± 68	114 ± 69	0.06	122 ± 61	127 ± 74	136 ± 85	0.44	0.26
Fasting insulin (μU/ml)	13 ± 10	13 ± 10	12 ± 8	0.83	13 ± 10	12 ± 8	17 ± 14‖¶	0.001	0.001
HOMA-IR	3.2 ± 2.6	3.1 ± 2.6	2.9 ± 1.8	0.79	3.1 ± 2.6	2.8 ± 2.1	4.3 ± 4.4#††	0.0001	0.0001
HOMA of β-cell function	199 ± 200	194 ± 219	181 ± 150	0.83	201 ± 234	188 ± 168	192 ± 114	0.51	0.26
Plasma IL-10 (pg/ml) (*n* = 236)	1.5 ± 1.5	1.9 ± 2.0	1.9 ± 1.5	0.30	2.1 ± 2.0	1.5 ± 1.2	1.1 ± 0.8	0.04	0.035

Data are means ± SD. *P* values for comparisons of differences of continuous variables between the three genotypes using ANOVA. **P* values for comparisons of differences of continuous variables between two genotypes using unpaired Student's *t* test. Categorical variables were compared by χ^2 test. †$P = 0.01$ vs. C/C; ‡$P = 0.01$ vs. C/A; §$P = 0.04$ vs. C/A; ‖$P = 0.005$ vs. C/C; ¶$P = 0.001$ vs. C/A; #$P < 0.0001$ vs. C/A after Bonferroni correction for multiple comparisons; **$P = 0.04$ vs. C/C; ††$P = 0.002$ vs. C/C.

TABLE 3
Haplotype frequencies in type 2 diabetic patients and nondiabetic control subjects

Haplotype	Nondiabetic control subjects	Patients with type 2 diabetes	*P**	OR (95% CI)†	*P*†
GCC (%)	34.4	36.3		1	
ACC (%)	39.9	38.8		0.88 (0.72–1.06)	0.16
ATA (%)	25.7	24.9	0.53	0.91 (0.74–1.13)	0.42

Haplotype frequencies in nondiabetic control subjects stratified according to BMI

Haplotype	BMI <30 kg/m^2	BMI >30 kg/m^2	*P**	OR (95% CI)†	*P*†
GCC (%)	35.2	33.0		1	
ACC (%)	41.1	38.1		1.00 (0.82–1.22)	0.97
ATA (%)	23.7	28.9	0.02	1.28 (1.02–1.60)	0.02

Association between the number of copies of the ATA haplotypes and BMI

Number of copies of the ATA haplotype	BMI <30 kg/m^2	BMI >30 kg/m^2	*P**	OR (95% CI)†	*P*†
0 ATA (%)	57.0	51.0		1	
1 ATA (%)	38.5	41.0		1.13 (0.88–1.47)	0.32
2 ATA (%)	4.5	7.9	0.025	1.96 (1.16–3.31)	0.01

Association between the number of copies of the ATA haplotypes and HOMA index

Number of copies of the ATA haplotype	Lower quartiles	Upper quartile	*P**	OR (95% CI)†	*P*†
0 ATA (%)	53.1	58.1		1	
1 ATA (%)	42.5	32.7		0.75 (0.54–1.04)	0.08
2 ATA (%)	4.4	9.3	0.001	1.99 (1.12–3.53)	0.01

*P value for overall comparison by χ^2 test. †P values and ORs (95% CI) calculated by a logistic regression analysis with adjustment for age and sex.

fasting insulin levels, and insulin resistance, estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) compared with homozygous (C/C) and heterozygous (C/A) carriers of the C allele (Table 2). After Bonferroni correction for multiple comparisons, differences in BMI, fasting insulin levels, and HOMA-IR remained significant (Table 2). By contrast, no differences were observed between subjects carrying the −592C/A genotype and those carrying the −592C/C genotype, thus suggesting a recessive effect of the A allele. Differences in BMI ($P = 0.02$), fasting insulin levels ($P = 0.0001$), and HOMA-IR ($P = 0.002$) among the three genotypes remained significant after adjusting for age and sex. After adjusting for BMI in addition to age and sex, the differences in fasting insulin levels ($P = 0.02$) and HOMA-IR ($P = 0.008$) among the three genotypes remained significant, indicating that the effect of the polymorphism on insulin sensitivity was not mediated by increased body weight. Plasma IL-10 concentration was assayed in a subgroup of subjects ($n = 236$), for which samples were available. IL-10 levels were significantly lower in carriers of the −592A/A genotype ($n = 12$) compared with carriers of the −592C/A ($n = 93$) and −592C/C genotype ($n = 131$).

In the diabetic group, carriers of the −592A/A genotype showed a tendency toward increased BMI (30.8 ± 6.4 kg/m^2) compared with subjects carrying the −592C/A genotype (29.8 ± 5.6 kg/m^2) and those carrying the −592C/C genotype (29.9 ± 4.9 kg/m^2), although the difference did not reach statistical significance ($P = 0.2$). It is possible that in diabetic subjects secondary weight loss induced by either diet or pharmacological treatments could mask the effects of this polymorphism on BMI.

Haplotype analysis. We found only three of eight different theoretically possible allele combinations in our study group, i.e., GCC, ACC, and ATA (Table 3). Frequencies of the haplotypes were not significantly different between type 2 diabetic case and nondiabetic control subjects ($P = 0.53$ by χ^2 test). By contrast, the ATA haplotype was significantly associated with an increased risk for obesity ($P = 0.02$ by χ^2 test). No association was observed between the ATA haplotype and insulin resistance, defined as the highest quartile of HOMA-IR index ($P = 0.53$ by χ^2 test). A logistic regression analysis with adjustment for age and sex showed a nominally significant association between the ATA haplotype and obesity (OR 1.28 [95% CI 1.02–1.60]; $P = 0.02$). We next analyzed the effect of the number of copies of the ATA haplotype on phenotype. In the nondiabetic group, because all subjects carrying the −592A/A genotype were also bearing the −1082A/A genotype, the phenotypic characteristics of subjects with the ATA/ATA composite genotype were identical to those of carriers of the −592A/A genotype reported in Table 2.

In the nondiabetic group, a higher number of copies of the ATA haplotype was associated with a nominally significant increased risk for obesity ($P = 0.025$ by χ^2 test) and insulin resistance ($P = 0.001$ by χ^2 test) (Table 3). A logistic regression analysis with adjustment for age and sex showed that subjects with two copies of the ATA haplotype have a nominally significant risk for both obesity (OR 1.96 [95% CI 1.16–3.31]; $P = 0.01$) and insulin resistance (1.99 [1.12–3.53]; $P = 0.01$) compared with subjects with no copy of the ATA haplotype.

Overall, we provide evidence that single nucleotide polymorphisms and haplotypes of the *IL10* promoter are associated with obesity and insulin resistance but not with type 2 diabetes. The genotype-quantitative trait interaction study in nondiabetic subjects suggested that the −592C/A, but not the −1082G/A, *IL10* promoter polymorphism contributes to the interindividual variation in BMI and insulin sensitivity. Furthermore, in the obesity case-con-

trol study, we found that the ATA haplotype is more frequent in obese subjects than among nonobese subjects and that subjects with two copies of the ATA haplotype have a nominally significant increased risk also for insulin resistance.

The mechanisms by which the *IL10* promoter polymorphisms might cause an increase in BMI are unknown. The ATA haplotype has been associated with lower transcriptional activity than the GCC haplotype, and the ATA/ATA composite genotype has been associated with lower IL-10 production by peripheral-blood mononuclear cells than other genotypes (14–17), although these results have not been replicated in another study showing that the −1082A allele or the ATA haplotype were associated with a decreased production of IL-10 (18). We found that carriers of the −592A/A genotype (as well as carriers of the ATA/ATA composite genotype) have lower levels of circulating IL-10 compared with other genotypes supporting in vitro data, suggesting that these allele combinations may affect *IL10* transcription. Because proinflammatory cytokine such as tumor necrosis factor-α and IL-6 have been associated to obesity, insulin resistance, and type 2 diabetes, and IL-10 downregulates the production of these proinflammatory cytokines (5–7,8), it is tempting to speculate that impaired IL-10 production in carriers of the ATA/ATA composite genotype may result in increased production of proinflammatory cytokines, which in turn affect insulin action in peripheral tissues. Interestingly, we failed to show any impact of *IL10* promoter polymorphisms on type 2 diabetes, although they are associated with both obesity and insulin resistance, two well-known risk factors for type 2 diabetes. This apparent paradox might partly be related to the fact that *IL10* promoter polymorphisms did not affect insulin secretion, as estimated by HOMA of β-cell function index, thus allowing compensatory response to increased peripheral demand by pancreatic β-cells.

This study has some limitations. We were not able to measure plasma IL-10 concentrations in all of the involved subjects, in particular in type 2 diabetic subjects. Also, a possible linkage disequilibrium of the three polymorphisms of the *IL10* promoter with other functional coding or noncoding variants in the region cannot be excluded. Furthermore, we did not correct for multiple testing in our haplotype analysis so that these findings are only nominally significant. Finally, the present findings obtained in a cross-sectional study are explorative in nature, and replication in independent prospective population-based studies with different ethnicity is needed to determine whether these *IL10* promoter polymorphisms influence insulin action and whether they are truly implicated in the development of obesity.

In conclusion, we show that the −592A/A genotype and the ATA/ATA composite genotype are associated with low circulating IL-10 levels and increased risk of both obesity and insulin resistance in a large sample of Italian Caucasians.

RESEARCH DESIGN AND METHODS

The study involved two groups of Caucasian subjects: *1*) a group of type 2 diabetic patients and *2*) a group of nondiabetic subjects. Subjects with type 2 diabetes were consecutively recruited according to the following criteria: onset of diabetes after age 35 years, absence of ketonuria at diagnosis, and anti-GAD antibody negative, as previously described (19). Type 2 diabetes was diagnosed according to the American Diabetes Association criteria (20). The nondiabetic subjects were participating in a metabolic disease prevention campaign for cardiovascular risk factors including age, hypertension, dyslipidemia, glucose tolerance, and obesity, as previously described (21). A 75-g oral glucose tolerance test was performed in a subset of the nondiabetic control subjects (734 of 1,131 subjects).

Biochemical measurements. Plasma glucose was measured by the glucose oxidation method (Beckman Glucose Analyzer II; Milan, Italy). Plasma insulin concentration was determined by a specific radioimmunoassay (Adaltis, Bologna, Italy). IL-10 concentrations were measured using a high-sensitivity enzyme immunoassay (Quantikine kit; R&D Systems, Minneapolis, MN). In the nondiabetic group, insulin sensitivity was estimated by the HOMA-IR index (22). Quartiles for the population distribution for the HOMA-IR were Q1, 0.40–1.60; Q2, 1.61–2.50; Q3, 2.51 −3.84; and Q4, 3.85–34.04 units. Nondiabetic subjects with insulin resistance were defined as the highest quartile of HOMA-IR. Insulin secretion in the fasting state was estimated by the HOMA for β-cell function index (22).

Genotyping. Genomic DNA was isolated from human leukocytes using standard methods. *IL10* −1082G/A, −819C/T, and −592C/A promoter variants (rs1800896, rs1800871, and rs1800872, respectively) were genotyped by direct sequencing using an ABI Prism 3100 Genetic Analyser (Applied Biosystems).

Statistical analysis. Variables not normally distributed were logarithmically transformed before statistical analyses. ANOVA was used to compare the effect of genotypes on continuous variables with Bonferroni correction. Student's t test was used to compare phenotypic differences between two groups. Categorical variables were compared by χ^2 test. Haplotypes, as well as their relative frequencies, were inferentially reconstructed by PHASE 2.0 (23). Linkage disequilibrium between polymorphisms was calculated by using Haploview 3.2 (24). Logistic regression analysis with adjustment for age and sex was used to test for significant association between genotype or haplotypes frequencies and type 2 diabetes, insulin resistance, or obesity. For all analyses, a P value ≤ 0.05 was considered to be statistically significant. All analyses were performed using SPSS software program Version 12.0 for Windows.

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