Brief Genetics Report

Promoter Polymorphisms of the TNF-α (G-308A) and IL-6 (C-174G) Genes Predict the Conversion From Impaired Glucose Tolerance to Type 2 Diabetes

The Finnish Diabetes Prevention Study

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High levels of cytokines are risk factors for type 2 diabetes. Therefore, we investigated whether the promoter polymorphisms of the tumor necrosis factor-α (TNF-α; G-308A) and interleukin 6 (IL-6; C-174G) genes predict the conversion from impaired glucose tolerance (IGT) to type 2 diabetes in the Finnish Diabetes Prevention Study. Altogether, 490 overweight subjects with IGT whose DNA was available were randomly divided into one of the two treatment assignments: the control group and the intensive, individualized diet and exercise intervention group. The −308A allele of the TNF-α gene was associated with an approximate twofold higher risk for type 2 diabetes compared with the G-308G genotype (odds ratio 1.80, 95% CI 1.05–3.09; $P = 0.034$). Subjects with both the A allele of the TNF-α gene and the C-174C genotype of the IL-6 gene had a 2.2-fold (CI 1.02–4.85, $P = 0.045$) higher risk of developing type 2 diabetes than subjects without the risk genotypes. We conclude that the −308A allele of the promoter polymorphism (G-308A) of the TNF-α gene is a predictor for the conversion from IGT to type 2 diabetes. Furthermore, this polymorphism seems to have a gene-gene interaction with the C-174C genotype of the IL-6 gene. *Diabetes* 52:1872–1876, 2003

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DPS, Diabetes Prevention Study; HOMA, homeostasis model assessment; HOMA-IR, HOMA for insulin resistance; HOMA-IS, HOMA for insulin secretion; IGT, impaired glucose tolerance; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α.

The major known risk factors for developing type 2 diabetes include a previous history of abnormal glucose tolerance, hyperinsulinemia, obesity, hypertension, physical inactivity, and a family history of diabetes (1). Additionally, type 2 diabetes has been linked to low-grade cytokine-associated acute-phase proteins (2). Circulating levels of cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), and acute-phase proteins, such as C-reactive protein, are elevated in obesity, metabolic syndrome, and type 2 diabetes (2,3). In a prospective study, elevated concentrations of cytokines and acute-phase proteins have been associated with the development of type 2 diabetes (4). Compared with the G-308G genotype, the −308A allele of the TNF-α gene has been shown to increase transcription twofold and, therefore, TNF-α concentration (5,6). Furthermore, TNF-α inhibits insulin signaling (7) and impairs insulin secretion (8). The C-174C genotype of the IL-6 gene has been shown to be associated with insulin resistance in normoglycemic subjects (9). TNF-α and IL-6 interact, with TNF-α regulating IL-6 expression and IL-6 downregulating TNF-α (10).

In this study, we investigated whether two polymorphisms in the promoter region of the TNF-α and IL-6 genes predict the conversion from impaired glucose tolerance (IGT) to type 2 diabetes in participants from the Finnish Diabetes Prevention Study (DPS) (11). To this aim, we genotyped 490 participants of the DPS for the G-308A promoter polymorphism of the TNF-α gene and the C-174G promoter polymorphism of the IL-6 gene.

In all study subjects, the frequencies of the genotypes were 74% G-308G, 25% G-308A, and 1% A-308A for the TNF-α gene promoter polymorphism and 32% C-174C, 46% C-174G, and 22% G-174G for the IL-6 gene promoter polymorphism. The frequencies did not differ between the intervention and control groups, and they were in Hardy-Weinberg equilibrium. As only six subjects had the A-308A genotype of the TNF-α gene, they were combined with

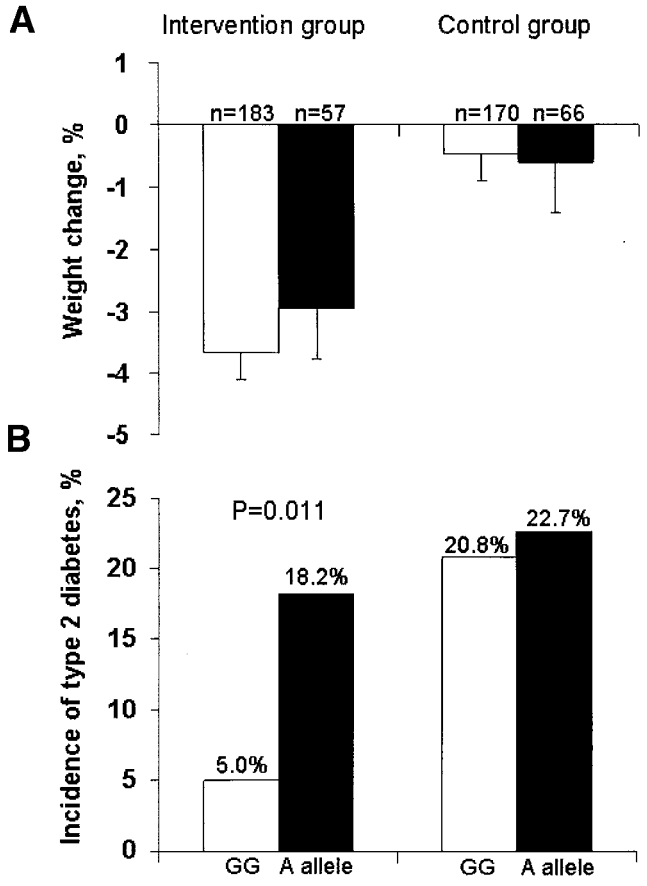

FIG. 1. Three-year weight change (%) (mean ± SE) (*A*) and 3-year incidence (%) (*B*) of type 2 diabetes in the intervention and control groups according to the G-308A polymorphism of the TNF-α gene.

subjects who had the G-308A genotype. There were no differences in clinical characteristics, fasting and 2-h levels of glucose and insulin in the oral glucose tolerance test, or homeostasis model assessment (HOMA) for insulin resistance (HOMA-IR) and insulin secretion (HOMA-IS) at baseline according to the G-308A promoter polymorphism of the TNF-α gene or the C-174G promoter polymorphism of the IL-6 gene (data not shown).

During the 3-year follow-up 69 genotyped subjects (19 subjects in the intervention group and 50 subjects in the control group) developed type 2 diabetes. We found that the −308A allele (G-308A and A-308A genotypes) of the TNF-α gene was associated with a high incidence of type 2 diabetes in all subjects in the DPS ($P = 0.032$) because 12.6% of subjects (44 of 348) with the G-308G genotype and 20.7% of subjects (25 of 121) with the −308A allele converted from IGT to type 2 diabetes. The IL-6 polymorphism was not associated with the progression to type 2 diabetes (14.1% of C-174C genotype subjects, 15.3% of C-174G genotype subjects, and 14.3% of G-174G genotype subjects developed diabetes; $P = 0.796$). When the intervention and control groups were analyzed separately, the presence of the −308A allele of the TNF-α gene was a predictor of type 2 diabetes only in the intervention group ($P = 0.011$) (Fig. 1*B*). No significant difference in weight loss between subjects with the G-308G genotype and −308A allele carriers of the TNF-α gene was found in either the intervention or control group (Fig. 1*A*).

TABLE 1
TNF-α promoter polymorphism (G-308A) as a predictor for the development of type 2 diabetes by the study group (logistic regression analysis)

	Regression coefficient	*P*	OR	95% CI
Model 1 (univariate)				
TNF-α −308A allele				
All participants	0.587	0.034	1.80	1.05–3.09
Control group	0.111	0.751	1.12	0.56–2.22
Intervention group	1.439	0.003	4.22	1.62–11.0
Model 2 (multivariate)				
Intervention group				
TNF-α −308A allele	1.465	0.006	4.39	1.53–12.3
Weight at baseline	0.047	0.005	1.05	1.01–1.08
Weight change	0.181	0.001	1.21	1.08–1.35

TNF-α genotypes were encoded as 0 = the G-308G genotype and 1 = the −308A allele. Weight change was calculated as (weight [kg] 3 years − weight [kg] baseline)/weight (kg) baseline × 100%. If diabetes was diagnosed before the 3-year examination, the weight at the visit when diabetes was diagnosed was used in statistical analyses.

In univariate logistic regression analyses (Table 1), the presence of the −308A allele of the TNF-α gene was associated with an almost twofold higher risk for type 2 diabetes compared with the G-308G genotype (odds ratio [OR] 1.80, 95% CI 1.05–3.09; $P = 0.034$). When we included the presence of the −308A allele of the TNF-α gene and the study group into the regression model, there was a significant interaction ($P = 0.027$) between the TNF-α promoter polymorphism and the study group (interaction term: TNF-α −308A allele × study group). Therefore, we performed statistical analyses separately in both groups. In the control group, the −308A allele did not predict diabetes, but in the intervention group, subjects with the −308A allele had an approximate fourfold increase for the risk of diabetes (4.22, 1.62–11.0; $P = 0.003$). Adjustment for baseline weight and weight change did not change the results. Even after adjustment for the achievement of the goals of the intervention (weight loss >5%, reduction in fat intake <30% of energy, reduction in saturated fat intake <10% of energy, increase in fiber intake >15 g/1,000 kcal, and physical exercise >4 h/week), the −308A allele predicted the conversion to diabetes (4.54, 1.63–12.65; $P = 0.004$).

The IL-6 risk genotype, C-174C, was not associated with increased risk for type 2 diabetes (OR 0.93, CI 0.53–1.62), and there was no interaction between the IL-6 risk genotype and the study group.

Subjects simultaneously having the −308A allele of the TNF-α gene and the C-174C genotype of the IL-6 gene had the highest incidence of type 2 diabetes (26.8%) compared with subjects without these risk genotypes (14.2%, $P = 0.041$). In univariate logistic regression analysis (Table 2), subjects having both the −308A allele of the TNF-α gene and the C-174C genotype of the IL-6 gene had a 2.22-fold (CI 1.02–4.85, $P = 0.045$) higher risk of developing type 2 diabetes than subjects having neither of these risk genotypes (model 1). This risk was not, however, significantly higher than that for the −308 A allele of the TNF-α gene alone. In the intervention group the subjects with both risk genotypes had an approximate sixfold higher incidence of

TABLE 2
TNF-α promoter (−308 A allele) and IL-6 promoter (C-174C genotype) risk genotypes as predictors for the development of type 2 diabetes in 41 subjects with both risk alleles and 240 subjects without any risk allele by the study group (logistic regression analysis)

	Regression coefficient	*P*	OR	95% CI
Model 1 (univariate)				
TNF-α and IL-6 risk genotypes				
All participants	0.798	0.045	2.22	1.02–4.85
Control group	0.225	0.679	1.25	0.43–3.63
Intervention group	1.822	0.001	6.19	2.06–18.6
Model 2 (multivariate)				
Intervention group				
TNF-α and IL-6 risk genotypes	1.644	0.009	5.18	1.51–17.8
Weight at baseline	0.045	0.008	1.05	1.01–1.08
Weight change	0.180	0.001	1.20	1.07–1.34

Genotypes were encoded as 0 = the presence of the G-308G genotype of the TNF-α gene and the −174G allele of the IL-6 gene and 1 = the presence of the −308A allele of the TNF-α gene and the C-174C genotype of the IL-6 gene. Weight change was calculated as (weight [kg] 3 years − weight [kg] baseline)/weight (kg) baseline × 100%. If diabetes was diagnosed before the 3-year examination, the weight at the visit when diabetes was diagnosed was used in statistical analyses.

diabetes (OR 6.19, 95% CI 2.06–18.6). This association did not essentially change, even after the inclusion of baseline weight and weight change into the model.

Insulin sensitivity (HOMA-IR) increased significantly from baseline to 3 years in subjects who converted to type 2 diabetes compared with those who did not independently of the TNF-α genotypes (Fig. 2*A*). In contrast, no significant changes in insulin secretion (HOMA-IS) were found between the converters and nonconverters (Fig. 2*B*). Subjects having both risk alleles (the −308A allele of the TNF-α gene and the C-174C genotype of the IL-6 gene) had a significant decrease ($P = 0.009$) in insulin secretion (Fig. 2*D*) compared with subjects who did not develop type 2 diabetes.

This intervention study of Finnish subjects with IGT showed for the first time that the promoter variants of the TNF-α gene and the IL-6 gene predicted the conversion from IGT to type 2 diabetes. When both genes were analyzed separately, the −308A allele of the TNF-α gene predicted the progression to type 2 diabetes, whereas the C-174C genotype of the IL-6 gene alone did not. However, the subjects carrying both the TNF-α and IL-6 risk genotypes had a more than two times higher risk of developing type 2 diabetes than subjects without the risk genotypes, suggesting a gene-gene interaction. We also found that risk genotypes increased the incidence only in the intervention group, suggesting a gene-lifestyle interaction. Whether the TNF-α and IL-6 genes modify the risk for diabetes in usual care compared with "trial setting" remains to be determined.

There is substantial evidence that TNF-α contributes to insulin resistance and thus to type 2 diabetes. Long-term exposure of cultured cells to TNF-α induces insulin resistance (12). TNF-α inhibits insulin receptor signaling by decreasing autophosphorylation of insulin receptor and promoting serine phosphorylation of insulin receptor substrate proteins (7,13). The evidence that TNF-α impairs insulin secretion is much more limited. However, in pancreatic β-cell lines TNF-α decreased glucose-stimulated insulin secretion (8).

The −308A allele of the TNF-α gene has been found to increase TNF-α transcription (5,6) and secretion (14). Thus, high incidence of type 2 diabetes, particularly in obese subjects, may be due to increased production of TNF-α in subjects with the −308A allele. The C-174C genotype has been shown to be associated with insulin resistance (9); therefore, the IL-6 polymorphism could influence the conversion from IGT to type 2 diabetes. However, in our study the C-174C genotype did not

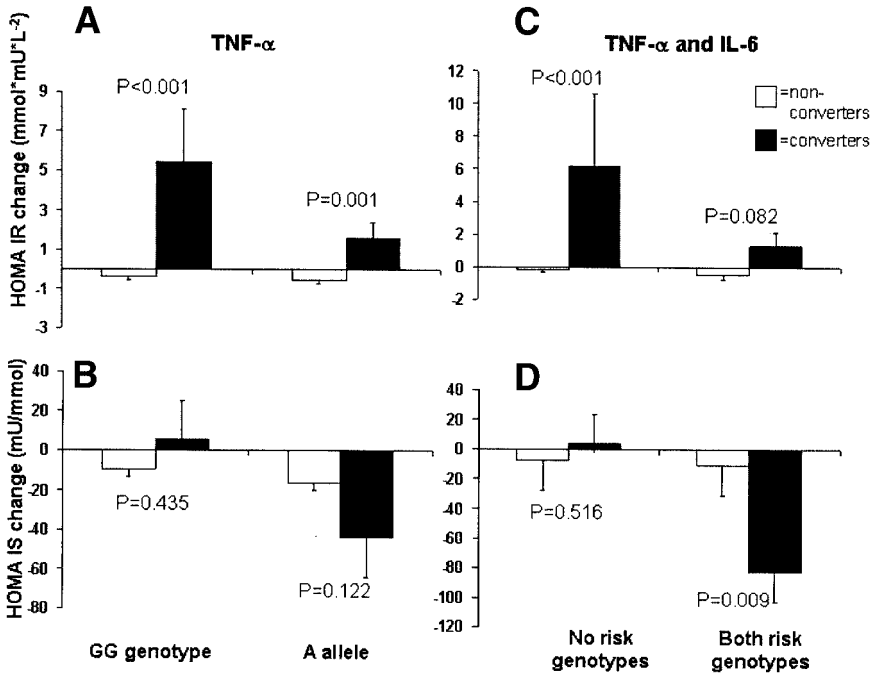

FIG. 2. Three-year change (mean ± SE) in HOMA-IR (*A*) and HOMA-IS (*B*) according to the G-308A polymorphism of the TNF-α gene in subjects who converted and who did not convert to type 2 diabetes. Three-year change (mean ± SE) in HOMA-IR index (*C*) and HOMA-IS index (*D*) according to the presence or absence of risk genotypes of the TNF-α gene (−308A allele) and the IL-6 gene (C-174C genotype) in subjects who converted and who did not convert to type 2 diabetes.

increase the risk of type 2 diabetes, but we found a gene-gene interaction between the TNF-α and IL-6 promoter polymorphisms ($P = 0.05$). Subjects with the C-174C genotype of the IL-6 gene and the −308A allele of the TNF- gene had about a two times higher incidence of type 2 diabetes than subjects without these genotypes.

There are several possibilities how TNF-α and IL-6 polymorphisms could interact with each other. The synthesis of IL-6 is tightly regulated, and a multiple response element of the IL-6 gene promoter (−173 to 145) is also controlled by TNF-α. TNF-α stimulates transcription of the IL-6 gene (15,16) and induces the production of IL-6 and its receptor (17). On the other hand, IL-6 has been suggested to negatively control TNF-α production (18).

Although the HOMA model is not a gold standard for the measurement of insulin sensitivity and insulin secretion, our results indicated that HOMA-IR was significantly higher among converters to diabetes than among nonconverters independently of the G-308A polymorphism of the TNF-α gene (Fig. 2). Interestingly, in subjects with both risk genotypes of the TNF-α and IL-6 genes, the reduction in insulin secretion (HOMA-IS) was clearly more pronounced than in subjects carrying only one risk genotype, suggesting that the effect on insulin secretion was additive.

In summary, we have demonstrated that the G-308A promoter polymorphism of the TNF-α gene is a predictor of type 2 diabetes. Furthermore, we have shown that this promoter polymorphism of the TNF-α gene has a gene-gene interaction with the IL-6 promoter polymorphism (C-174G), further increasing the risk of type 2 diabetes. Finally, the G-308A promoter polymorphism of the TNF-α gene seems to have an interaction with lifestyle changes.

RESEARCH DESIGN AND METHODS

Subjects and research design. The DPS is a multicenter longitudinal study carried out in five participating centers in Finland (11). Subjects with IGT according to the World Health Organization 1985 criteria (fasting glucose <7.8 mmol/l and 2-h plasma glucose 7.8–11.0 mmol/l [19]) who were at high risk for progression to type 2 diabetes were recruited. All 522 participants were overweight and had IGT. Subjects were randomly divided into one of two groups: the control group and the intensive, individualized diet and exercise intervention group (11,20). Oral glucose tolerance tests were performed annually, and a diagnosis of diabetes was confirmed in two subsequent tests. DNA was available from 490 subjects (161 men and 329 women). Their mean BMI was 31.1 ± 4.6 kg/m^2 and age 55.4 ± 7.1 years. All participants gave written informed consent, and the ethics committee of the National Public Health Institute in Helsinki, Finland, approved the study protocol.

Measurements. Medical history and physical examination were done at baseline and during annual follow-up visits, as previously described (11,20). HOMA-IR was calculated using the formula fasting plasma glucose (mmol/l) × fasting serum insulin (mU/l)/22.5, and HOMA-IS was calculated as 20 × fasting serum insulin (mU/l)/(fasting plasma glucose [mmol/l] − 3.5) (21).

DNA analysis. The G-308A polymorphism of the TNF-α gene was screened by the restriction fragment–length polymorphism after digestion with *Nco*I restriction enzyme, as previously described (22). The genotyping of the C-174G polymorphism of the IL-6 gene was performed by PCR with published primers (23) followed by the single-strand conformation polymorphism analysis as previously reported in detail (24).

Statistical analysis. Data were analyzed with the SPSS/Win programs (version 10.0; SPSS, Chicago, IL). Data are given as means ± SD, unless otherwise indicated. Students' t test for independent samples was used to compare the two groups, and ANOVA was used to compare the three genotypes. χ^2 test was used in comparison of categorical variables. Insulin concentrations were log transformed before statistical analyses to achieve a normal distribution. Logistic regression analysis was performed to evaluate if the TNF-α or IL-6 polymorphisms predict the development of type 2 diabetes.

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