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Short communication

The tumour necrosis factor alpha –238 G → A and –308 G → A promoter polymorphisms are not associated with insulin sensitivity and insulin secretion in young healthy relatives of Type II Diabetic patients

M. Koch, K. Rett, A. Volk, E. Maerker, K. Haist, M. Weisser, A. Rettig, W. Renn, H. U. Häring

Medical Clinic of the University of Tübingen, Section IV, Internal Medicine and Endocrinology, Tübingen, Germany

Abstract

Aims/hypothesis. Tumour necrosis factor-α (TNF-α) is believed to influence skeletal muscle insulin resistance. Two G → A transitions in the promoter region of *TNF-α* at position –238 and –308 have been identified that could play a part in transcriptional regulation of the gene. Insulin resistance is an independent familial trait that predicts the development of Type II (non-insulin-dependent) diabetes mellitus. We investigated the influence on insulin sensitivity and insulin secretion of both polymorphisms in a cohort of young healthy relatives of patients with Type II diabetes.
Methods. We examined 109 first-degree relatives of Caucasian patients with a history of Type II diabetes, who underwent extensive metabolical and anthropometrical phenotyping, and determined the *TNF-α –238* and *–308 G → A* promoter polymorphisms.
Results. For the –238 polymorphism, 83 probands (76.1 %) were homozygous for the G-allele, 25 probands (22.9 %) were heterozygous and 1 proband (0.9 %) was homozygous for the A-allele. For the –308 polymorphism, 83 probands (76.1 %) were homozygous for the G-allele, 24 probands (22.0 %) were heterozygous and 2 probands (1.18 %) were homozygous for the A-allele. Probands with and without the polymorphism did not differ in insulin sensitivity ($p = 0.78$), insulin-concentrations and C-peptide concentrations in oral glucose tolerance tests ($p > 0.05$).
Conclusions/interpretation. We could not detect an association between insulin sensitivity or insulin secretion and *TNF-α* promoter polymorphisms in our cohort. The polymorphisms occur at the same frequencies in probands with either low or high insulin sensitivity. [Diabetologia (2000) 43: 181–184]

Keywords Insulin resistance, Type II diabetes, tumour necrosis factor-α, promotor polymorphisms, first-degree relatives.

Insulin resistance is an independant familial trait that predicts the development of Type II (non-insulin-dependent) diabetes mellitus [1]. The genetic basis of Type II diabetes is polygenic and polyallelic and is clinically apparent only when present in combination with environmental factors. Obesity is one of the major risk factors contributing to the development of insulin resistance and overt Type II diabetes. Signals derived from adipose tissue like tumour necrosis factor-α (TNF-α), are believed to cause skeletal muscle insulin resistance and have a role as mediators of insulin resistance related to obesity [2].

Studies in isolated cells suggested that TNF-α has an anti-insulin effect by interrupting the signals stimulated by insulin thus inhibiting insulin action and increasing insulin resistance [3].

Local and systemical induction of TNF-α expression was observed in adipose tissue from different rodent models of obesity and diabetes, indicating a role for TNF-α in obesity, particularly in the insulin resistance and diabetes that often accompany obesity [2].

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Corresponding author: Dr. M. Koch, Labor Wagner & Partner, Werner-von-Siemens-Str. 10, 37077 Göttingen, Germany
Abbreviations: MCR, Metabolic clearance rate.

Tumour necrosis factor-α is regulated both transcriptionally and post-transcriptionally. Sequence polymorphisms have been identified that could play a part in the transcriptional regulation of the gene. Two G → A mutations in the promoter region of *TNF-α* at position –238 (alleles: *TNF-α-238-G* and *TNF-α-238-A*), and *–308* (called *TNF-2* allele) located in a regulative motif of promoters of the MHC class II genes, have been described [4, 5].

One prerequisite of genetic analysis in complex diseases is to characterize the phenotype of subjects as comprehensively as possible. A history of Type II diabetes in first-degree relatives is associated with an increased risk for the offspring [1]. We therefore examined the prevalence of the –238 and –308 promoter polymorphisms in young healthy non-diabetic first-degree relatives of patients with Type II diabetes. To detect an association between the –238 and –308 sequence variations and insulin sensitivity, the probands underwent extensive metabolic phenotyping by glucose tolerance tests, calculation of the rate of appearance of newly secreted insulin in the portal vein and insulin sensitivity by euglycaemic hyperinsulinaemic glucose clamp and other experiments.

Subjects and methods

Probands. We examined 109 first-degree relatives (46 men, 63 women) of Caucasian patients with a history of Type II diabetes who were younger than 50 years. The participants were recruited from a study launched for the early diagnosis in families with a history of Type II diabetes at the department of endocrinology at the University of Tübingen. All probands had given written consent to their participation. The study was approved by the local ethics committee and was carried out in accordance with the Helsinki guidelines.

Methods. Insulin sensitivity was determined by euglycaemic hyperinsulinaemic glucose clamp and expressed as clamp-derived glucose metabolic clearance rate (MCR) being virtual blood volume cleared from glucose per min and kg body weight ($ml \cdot kg^{-1} \cdot min^{-1}$). Steady-state glucose concentrations during the clamp showed a coefficient of variation (CV) of 5.6% and glucose infusion rates had a CV of 10.5%. Body composition was measured by bioelectrical impedance as percentage body fat. Glucose tolerance was determined by an oral glucose test using 40 g glucose/m^2. Insulin concentrations were determined with an enzyme immunoassay (IM_X. Insulin, Abott, North Chicago, Ill., USA) and C-peptide was measured by radioimmunoassay (RIA-coat C-Peptid, Byk-Sangtec Diagnostica, Dietzenbach, Germany). Body mass index was calculated as weight divided by the square of height (kg/m^2).

The tumour necrosis factor-α –238 and –308 G → A promoter polymorphisms were determined by PCR and subsequent restriction enzyme analysis with *Msp* I [4].

For statistical analysis, differences between the means of age, MCR, BMI and body fat as well as the OGTT values (insulin, C-peptide, insulin secretion rate) were tested by non-parametric Mann-Whitney U tests. All statistical tests were done with JMP software (JMP Version 3.2.1, SAS Institute, Cary, N.C., USA). To exclude the possibility of bias, all tests were also done on subgroups of the sample chosen at random. A p value of less than 0.05 was considered statistically significant.

Results

For the –238 polymorphism, 83 probands (76.1%) were homozygous for the G-allele (genotype: *TNF-α-238-G/TNF-α-238-G*), 25 probands (22.9%) were heterozygous (genotype: *TNF-α-238-G/TNF-α-238-A*), and 1 proband (0.9%) was homozygous for the A-allele (genotype: *TNF-α-238-A/TNF-α-238-A*). The allele frequencies of the *TNF-α-238-G-* and the *TNF-α-238-A*-alleles were $p = 0.876$ and $p = 0.124$, respectively. For the –308 polymorphism, 83 probands (76.1%) were homozygous for the G-allele (genotype: *TNF1/TNF1*), 24 probands (22.0%) were heterozygous (genotype: *TNF1/TNF2*), and 2 probands (1.18%) were homozygous for the A-allele (genotype: TNF2/TNF2). The allele frequencies of the *TNF1* and the *TNF2* alleles were $r = 0.872$ and $s = 0.128$, respectively. The observed genotype frequencies of the –238 and the –308 polymorphisms were at the Hardy-Weinberg frequencies (χ^2 test: $p = 0.998$ for the –238 polymorphism; $p = 0.999$ for the –308 polymorphism; $p = 1$ for the combined data set).

The age of the probands ranged from 16–49 years with a mean of 32.9 years (women 34.4 years; men 30.9 years). Mean body mass index (BMI) was 25.29 ± 0.46 standard error of the mean (SEM) (women 25.65 ± 0.62 SEM, men 24.18 ± 0.67 SEM). Mean percentage of body fat as determined by bioelectrical impedance was 25.21 ± 0.92 SEM (women 30.27 ± 1.08 SEM, men 18.28 ± 0.86 SEM).

Insulin sensitivity clamp-derived glucose MCR varied between 1.75 and 25.23 $ml \cdot kg^{-1} \cdot min^{-1}$. Probands carrying the rare alleles of the –238 or the –308 polymorphisms (*TNF-α-238-A*, *TNF2*), either heterozygous or homozygous, did not differ significantly in MCR ($p = 0.85$ and $p = 0.74$), BMI ($p = 0.69$ and $p = 0.78$) or total body fat ($p = 0.68$ and $p = 0.79$) from those that are homozygous for the more frequent alleles (*TNF-α-238-G*, *TNF1*) compared with the other probands. The rare alleles of both polymorphisms were no more frequent in the insulin resistant group. Of the probands 14 with an MCR above the median of 7.6 $ml \cdot kg^{-1} \cdot min^{-1}$ and 12 with an MCR below the median possessed the rare allele of the –238 polymorphism. There were 13 probands with an MCR above the median of 7.6 $ml \cdot kg^{-1} \cdot min^{-1}$ and 14 with an MCR below the median who possessed the rare allele of the –308 polymorphism (χ^2 test: $p = 0.982$ for the –238 polymorphism; $p = 0.998$ for the –308 polymorphism).

Table 1 shows demographic and metabolic characteristics comparing probands with and without the

Table 1. Demographic and metabolic characteristics of first-degree relatives of Type II diabetic patients for *TNF*-alpha-238 G → A and *TNF*-alpha-308 G → A promoter polymorphisms

	*TNF*α-238 G → A polymorphism Genotype			*TNF*α-308 G → A polymorphism Genotype		
	TNF-α-238-G/ *TNF*-α-238-G	*TNF*-α-238-A[a]	*p* value	*TNF*1/*TNF*1	*TNF*2[a]	*p* value
n (women/men)	83 (47/36)	26 (15/11)		83 (51/32)	26 (12/14)	
Age (years)	33.0 ± 0.91	32.6 ± 1.30	0.76	33.7 ± 0.88	30.3 ± 1.34	0.14
BMI (kg/m^2)	25.2 ± 0.52	25.5 ± 0.95	0.69	25.3 ± 0.55	25.2 ± 0.81	0.78
MCR (ml · kg^{-1} · min^{-1})	7.8 ± 0.38	8.3 ± 0.98	0.85	8.0 ± 0.43	7.7 ± 0.75	0.74
Body fat (%)	25.1 ± 1.10	25.5 ± 1.60	0.68	25.4 ± 1.09	24.5 ± 1.67	0.79

Data are means ± standard error of the mean. Means of the variables age, BMI, MCR and body fat were compared by non-parametrical U tests (Mann-Whitney U Test). [a] either homozygous or heterozygous

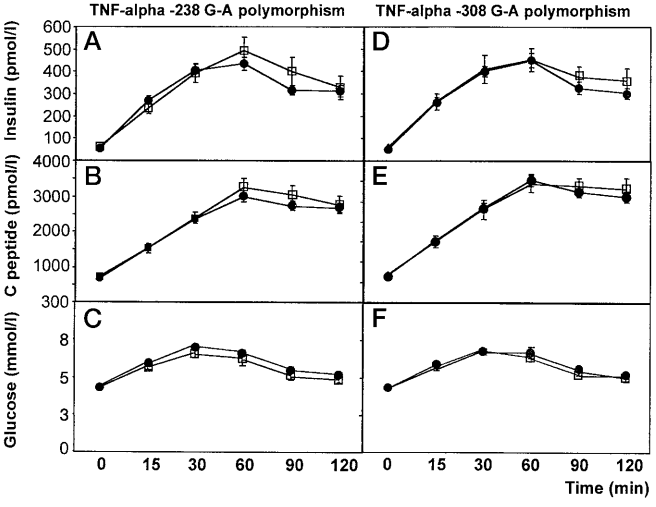

Fig. 1. Profiles of insulin, C-peptide and glucose concentrations in oral glucose tolerance tests of healthy first-degree relatives of patients with Type II diabetes. *TNF*-α –238 G→A promoter polymorphism, *p* values for the insulin curve: ($p_0 = 0.49$; $p_{15} = 0.63$; $p_{30} = 0.96$; $p_{60} = 0.53$; $p_{90} = 0.35$; $p_{120} = 0.96$), and *TNF*-α –308 G→A promoter polymorphism ($p_0 = 0.14$; $p_{15} = 0.91$; $p_{30} = 0.54$; $p_{60} = 0.89$; $p_{90} = 0.22$; $p_{120} = 0.34$). *P* values for the C-peptide curve: *TNF*-α –238 G→A promoter polymorphism ($p_0 = 0.44$; $p_{15} = 0.75$; $p_{30} = 0.65$; $p_{60} = 0.25$; $p_{90} = 0.31$; $p_{120} = 0.61$) and *TNF*-α –308 G→A promoter polymorphism ($p_0 = 0.75$; $p_{15} = 0.74$; $p_{30} = 0.51$; $p_{60} = 0.78$; $p_{90} = 0.37$; $p_{120} = 0.60$). *P* values for the glucose curve: *TNF*-α –238 G→A promoter polymorphism ($p_0 = 0.39$; $p_{15} = 0.88$; $p_{30} = 0.58$; $p_{60} = 0.43$; $p_{90} = 0.66$; $p_{120} = 0.66$), and *TNF*-α –308 G→A promoter polymorphism ($p_0 = 0.99$; $p_{15} = 0.64$; $p_{30} = 0.57$; $p_{60} = 0.66$; $p_{90} = 0.13$; $p_{120} = 0.81$). ●, homozygous for the *TNF*-α-238-G allele, $n = 80$ (**A**, **C**, **D**, **F**), $n = 72$ (**B**), $n = 73$ (**E**), □, either homozygous or heterozygous for the *TNF*-α-238-A allele; ●, homozygous for the *TNF*1 allele; □, either homozygous or heterozygous for the *TNF*2 allele; $n = 26$ (**A**, **D**), $n = 24$ (**B**, **E**), $n = 30$ (**C**, **F**)

rare allele of the –238 and the –308 polymorphisms according to insulin sensitivity (MCR), age, BMI or percentage body fat. The course of insulin, C peptide and glucose (Fig. 1) response curves to an oral glucose load, as analysed by Mann-Whitney U tests, did not show significant differences between the genotypes.

Discussion

Relations of the two G → A transitions in the promoter region of *TNF-α* at position –238 and –308 to insulin resistance and Type II diabetes have been investigated with conflicting results. In one study [4], an association of the –238 but not of the –308 polymorphism to decreased insulin resistance in non-diabetic relatives of diabetic patients was found but no association to overt Type II diabetes could be found [6]. The G → A mutation in the promoter region of *TNF-α* at position –308 [5], resulting in the *TNF2* allele acts, at least in vitro, with reporter genes as a much stronger transcriptional activator than the common *TNF1* allele [7]. These in vitro studies would suggest that an increased transcriptional activity could lead to raised TNF-α blood concentrations followed by a lowered insulin sensitivity. We did not measure TNF-α blood concentrations in this study but in previous experiments [8] it was shown by our group that even if such a role of TNF-α is assumed there is a large gap between the circulating concentrations in the range of 10 pg/ml and those required to block insulin signalling in isolated cells (5 ng/ml). Therefore it seems unlikely that the concentration of TNF-α measured in serum or plasma is high enough to exert a functional effect on insulin signalling at the receptor or post-receptor level [8]. Although the –308 G → A polymorphism was not associated with insulin sensitivity in one study [4] it was in another [9]. Contradictory results have also been published of the circulating TNF-α concentrations in Type II diabetic patients: increased concentrations of TNF-α have been measured [10] whereas no correlations between insulin sensitivity and TNF-α concentrations could be found in young healthy first-degree relatives of patients with Type II diabetes [8].

We could not detect an association between insulin sensitivity or insulin secretion and the –238 G → A or

the –308 G → A promoter polymorphisms in our cohort of young healthy offspring of Type II diabetic patients. The polymorphisms occur at the same frequencies in probands with either low or high MCR of glucose as a measure of insulin resistance. Conflicting results in studies evaluating the association of genetic variables with metabolic traits could be due to different methods used for the determination of insulin resistance. In our study, we used the well-accepted euglycaemic hyperinsulinaemic glucose clamp technique, the "gold standard" for measuring insulin sensitivity that is generally thought to give reliable estimates of insulin sensitivity. The major advantage of this method is that insulin sensitivity is determined at a steady state of both insulin and glucose concentrations. The sensitivity and reproducibility of the euglycaemic clamp can detect differences in insulin resistance upwards of around 10%. Other methods like the homeostasis model assessment (HOMA) which was used in [4], and determination of an insulin sensitivity index, as measured by the frequently sampled intravenous glucose tolerance test with minimal model analysis [9] correlate with the index of insulin resistance derived from the euglycaemic hyperinsulinaemic clamp [4] but, as with all statistical correlations, a number of factors could be responsible for discrepant results gained with different methods. It should also be kept in mind that the results of euglycaemic hyperinsulinaemic clamp experiments themselves could be questionable because in these experiments unphysiological conditions are created but this is the case for all other methods measuring insulin sensitivity.

In summary, our observations do not exclude a role of TNF-α in the regulation of insulin sensitivity but make it unlikely that promoter polymorphisms are responsible for this trait in people prone to develop Type II diabetes.

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