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PRELIMINARY REPORT

A Tumor Necrosis Factor-β Polymorphism Associated With Hypertriglyceridemia in Non–Insulin-Dependent Diabetes Mellitus

J. Vendrell, C. Gutierrez, R. Pastor, and C. Richart

Non–insulin-dependent (type II) diabetes mellitus is associated with significant abnormalities of lipoprotein metabolism. Control of glycemia rarely completely corrects the alterations in lipid metabolism, suggesting a participation of environmental and genetic factors. The observation that tumor necrosis factor (TNF) can modulate triglyceride metabolism offers a new genetic candidate to be analyzed. Samples of DNA from 91 control subjects and 61 diet-treated type II diabetic patients were analyzed to determine the lipid profile and a possible association with TNF genetic polymorphisms. For TNF restriction fragment length polymorphisms, we used the *Nco* I restriction enzyme and a TNF-α probe obtaining two allelic bands at 10.5 and 5.5 kb. We found a significant association ($P < .01$) of the 10.5-kb homozygous genotype in type II diabetic subjects with high triglyceride levels. Furthermore, these patients showed significant differences in triglycerides as compared with matched control subjects with the same genotype ($P < .001$). This study provides support for considering the TNF locus as a susceptibility genetic region in the hypertriglyceridemia of type II diabetes.

ALTERATIONS OF the lipid profile in non–insulin-dependent (type II) diabetic patients are the result of different influences and represent a complex picture. It is generally accepted that interactions between genetic and environmental factors can affect lipid metabolism. Several genetic variations are probably responsible for the susceptibility to lipid alterations. In support of this view, some polymorphisms in the apolipoprotein (apo) B locus and in the apo AI-CIII-AIV cluster appear to occur in patients who have hypertriglyceridemia. However, this association is not seen in type II diabetic patients.[1] For this reason, other genetic loci have been proposed as possible candidates in type II diabetes,[2] but no major susceptibility gene has been identified up to this moment.

Recent studies support the concept that cytokines might play an important role in lipid metabolism, which could offer the possibility of analyzing new genetic candidate loci in dyslipidemic patients.[3] It has been observed that several cytokines that mediate in the host immune response, such as tumor necrosis factor-α (TNF-α), have important metabolic effects as modulators of triglyceride metabolism.[4] Furthermore, interindividual differences in TNF-α monocyte responses can be accounted for by genetic polymorphisms at the TNF-β locus defined by the *Nco* I restriction enzyme.[5]

In view of the probable polygenic nature of lipid disorders and the cytokine implication in triglyceride metabolism, we have investigated the TNF-β polymorphism in the lipid profile of type II diabetic patients.

SUBJECTS AND METHODS

Subjects

The study population consisted of two unrelated type II diabetic cohorts with 27 patients (12 men and 15 women) in the first one and 34 (16 men and 18 women) in the second, and 91 (43 men and 48 women) control subjects randomly selected. All subjects provided informed consent, and the study was approved by the ethics committee of the University Hospital Joan XXIII of Tarragona. All diabetic patients had less than 5 years' evolution since the clinical diagnosis, none were taking any pharmacologic treatment that could alter lipid parameters, and all were only under diet treatment. They were also free of nephrotic syndrome as ascertained by

From the Endocrinology Unit, Biochemistry Service, and Internal Medicine Service, Hospital Universitari de Tarragona Joan XXIII, School of Medicine of Reus, Universitat Rovira i Virgili, Tarragona, Spain.

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Address reprint requests to J. Vendrell, MD, Endocrinology Unit, Hospital Joan XXIII, Av. Dr. Mallafré Guasch, 4, 43007 Tarragona, Spain.

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Table 1. Clinical and Laboratory Data of the Two Cohorts of Type II Diabetic Patients

	First Cohort			Second Cohort		
	5.5/5.5 (n = 5)	10.5/10.5 (n = 14)	10.5/5.5 (n = 8)	5.5/5.5 (n = 3)	10.5/10.5 (n = 19)	10.5/5.5 (n = 12)
Sex (M/F)	1/4	6/8	5/3	2/1	8/11	6/6
Age (yr)	61.0 ± 2.2	56.4 ± 8.2	50.8 ± 10.8	59.0 ± 4.6	56.0 ± 7.6	52.5 ± 7.2
BMI (kg/m²)	31.4 ± 9.9	28.9 ± 5.8	29.0 ± 3.0	26.0 ± 1.5	28.2 ± 4.8	26.9 ± 4.0
DBP (mm Hg)	96.2 ± 21.4	82.5 ± 12.5	92.2 ± 17.3	81.7 ± 10.4	80.9 ± 13.1	82.5 ± 13.1
SBP (mm Hg)	152.5 ± 22.2	141.5 ± 25.9	154.0 ± 29.9	141.7 ± 16.1	137.1 ± 15.0	123.7 ± 47.1
Glucose (mmol/L)	11.1 ± 4.5	9.8 ± 1.8	8.4 ± 1.7	10.0 ± 2.6	10.5 ± 2.5	12.0 ± 4.4
Cholesterol (mmol/L)	5.7 ± 0.9	6.7 ± 1.4	5.4 ± 0.6	4.9 ± 0.7	5.7 ± 1.2	5.2 ± 1.1
Triglycerides (mmol/L)	1.2 ± 0.2	3.2 ± 1.2*	1.7 ± 0.4	1.7 ± 1.2	2.3 ± 1.0†	1.3 ± 0.4
HDL-c (mmol/L)	1.1 ± 0.3	1.1 ± 0.2	0.9 ± 0.3	1.3 ± 0.2	1.5 ± 0.3	1.4 ± 0.3
Apo AI (g/L)	1.4 ± 0.2	1.5 ± 0.3	1.3 ± 0.2	1.3 ± 0.3	1.5 ± 0.3	1.4 ± 0.3
Apo B (g/L)	1.3 ± 0.3	1.6 ± 0.4	1.4 ± 0.2	1.2 ± 0.1	1.5 ± 0.4	1.3 ± 0.4
LDL-C (mmol/L)	4.0 ± 1.1	4.2 ± 0.9	3.5 ± 0.6	2.5 ± 0.7	3.6 ± 1.1	3.2 ± 1.2
Lp(a) (mg/dL)	7.5 ± 6.1	8.2 ± 9.8	4.6 ± 4.3	7.0 ± 4.8	12.3 ± 12.9	11.9 ± 8.4
IRI (μU/mL)	16.1 ± 5.4	22.8 ± 19.2	17.8 ± 9.7	10.5 ± 8.3	18.1 ± 13.6	14.4 ± 10.9
C-peptide (ng/mL)	2.5 ± 0.7	3.4 ± 1.4	2.8 ± 1.1	2.1 ± 0.8	3.2 ± 2.6	2.1 ± 0.9
HbA_{1c} (%)	8.9 ± 2.4	10.3 ± 2.2	8.3 ± 1.8	9.0 ± 2.8	9.2 ± 1.9	8.7 ± 3.2

Abbreviations: M, males; F, females; DBP, diastolic blood pressure; SBP, systolic blood pressure.

*$P < .005$ (genotype 10.5/10.5 *v* remainder genotypes in type II diabetic group).

†$P < .01$ (genotype 10.5/10.5 *v* remainder genotypes in type II diabetic group).

routine measurement of protein levels in urine (<300 mg/d). Control subjects were over 30 years of age and had fasting glucose levels less than 6 mmol/L. Subjects with an immediate relative with diabetes were excluded.

Methods

After overnight fasting, blood samples for determination of blood glucose, hemoglobin A_{1c} (HbA_{1c}), plasma lipids, lipoprotein (a) [Lp(a)], insulinemia (immunoreactive insulin [IRI]), and C-peptide were obtained. Plasma levels of high-density lipoprotein cholesterol, cholesterol (HDL-C), triglycerides, and apos AI and B were measured by established techniques.[6] Low-density lipoprotein cholesterol (LDL-C) level was calculated using Friedewald's formula. Lp(a) level was measured by enzyme immunoassay (Behring Institute, Marburg, Germany) with a monoclonal antibody against Lp(a) and a polyclonal anti-Lp (a). Intraassay and interassay coefficients of variation for the method were 5% and 10%, respectively. IRI and C-peptide were analyzed by radioimmunoassay. HbA_{1c} level was measured by a chromatographic method (Glico Hb Quick Column Procedure, Helena Laboratories, Beaumont, TX). The normal range for HbA_{1c} values in our laboratory is 6% to 8%.

Restriction Fragment Length Polymorphism Analysis

DNA was obtained from peripheral-blood leukocytes, and the polymorphisms were studied using restriction fragment length polymorphism analysis.[7] Briefly, 10 μg DNA was digested with the *Nco* I restriction enzyme. The digested DNA was separated by agarose gel electrophoresis and transferred onto nylon filters using the method reported by Southern.[8] These filters were later hybrid-

Table 2. Clinical and Laboratory Data of Control and Type II Diabetic Groups Related to TNF Genotype

	Control			Type II Diabetics		
	5.5/5.5 (n = 9)	10.5/10.5 (n = 53)	10.5/5.5 (n = 29)	5.5/5.5 (n = 8)	10.5/10.5 (n = 33)	10.5/5.5 (n = 20)
Sex (M/F)	2/7	30/23	11/18	3/5	14/19	11/9
Age (yr)	47.1 ± 11.9	45.4 ± 12.5	45.5 ± 12.5	60.4 ± 3.1	53.6 ± 12.3	50.8 ± 9.0
BMI (kg/m²)	26.4 ± 3.4	25.3 ± 3.9	24.6 ± 4.4	28.9 ± 7.1	27.7 ± 6.9	28.1 ± 3.7
DBP (mm Hg)	81.7 ± 13.7	73.7 ± 13.4	74.1 ± 14.1	90.0 ± 18.0	81.6 ± 10.8	87.2 ± 15.3
SBP (mm Hg)	135.8 ± 16.2	124.4 ± 23.2	125.5 ± 22.3	147.8 ± 19.1	139.0 ± 18.1	144.1 ± 26.5
Glucose (mmol/L)	5.0 ± 0.9	4.8 ± 0.6	4.7 ± 0.6	10.0 ± 3.7	10.6 ± 3.1	10.7 ± 4.0
Cholesterol (mmol/L)	5.5 ± 1.2	5.3 ± 1.0	5.1 ± 1.0	5.4 ± 0.9	5.9 ± 1.3	5.4 ± 1.1
Triglycerides (mmol/L)	1.4 ± 1.3	1.1 ± 0.6	1.2 ± 0.8	1.7 ± 1.0	2.4 ± 1.4*	1.5 ± 0.7
HDL-C (mmol/L)	1.3 ± 0.4	1.5 ± 0.4	1.4 ± 0.4	1.3 ± 0.3	1.5 ± 0.6	1.3 ± 0.4
Apo AI (g/L)	1.5 ± 0.4	1.9 ± 2.5	1.5 ± 0.3	1.4 ± 0.2	1.5 ± 0.3	1.4 ± 0.3
Apo B (g/L)	1.2 ± 0.4	1.2 ± 0.3	1.2 ± 0.2	1.3 ± 0.2	1.4 ± 0.4	1.3 ± 0.4
LDL-C (mmol/L)	3.6 ± 0.8	3.4 ± 0.9	3.1 ± 0.8	3.4 ± 1.2	3.7 ± 1.1	3.6 ± 1.1
Lp(a) (mg/dL)	19.5 ± 16.5	11.3 ± 11.2	12.4 ± 12.6	9.7 ± 8.3	11.4 ± 12.0	8.9 ± 7.8
IRI (μU/mL)	11.0 ± 6.6	10.6 ± 9.6	9.5 ± 5.7	15.5 ± 7.1	19.4 ± 16.2	15.6 ± 10.1
C-peptide (ng/mL)	2.6 ± 1.5	1.9 ± 1.1	1.7 ± 1.0	3.2 ± 2.7	3.1 ± 2.1	2.8 ± 1.8
HbA_{1c} (%)	—	—	—	8.9 ± 2.4	9.4 ± 2.4	8.7 ± 2.7

NOTE. Abbreviations are as in Table 1.

*$P < .01$ (genotype 10.5/10.5 *v* remainder genotypes in type II diabetic group).

ized with a ^{32}P-labeled TNF-α–DNA probe. This probe detects two allelic bands at 10.5 and 5.5 kb corresponding to a mutation within the first intron of the TNF-β gene when using the *Nco* I restriction enzyme. Finally, the filters were exposed to x-ray films for 5 or 7 days.

Statistical Analysis

Data are expressed as the mean ± SD. Results were analyzed using χ^2 with Yates correction when appropriate, and a two-tailed *t* test and one-way ANOVA for comparison of the means. For Lp(a), we used the Kruskall-Wallis and Mann-Whitney nonparametric tests. We also used the Bonferroni correction when necessary, and in this case a *P* value less than .01 was considered significant.

RESULTS

There were no differences in the frequency of genotypic and allelic distributions between the control group and the type II diabetes group (data not shown).

Plasma lipid and metabolic parameters in the control group showed no differences according to genotype.

In the first cohort of type II diabetic patients, metabolic parameters were similar in the three genotypic groups, except for triglyceride levels, which were significantly higher ($P < .005$) in 10.5-kb homozygous subjects (Table 1). To confirm this result, we analyzed a second cohort of type II diabetic patients with the same clinical characteristics and obtained similar results (higher triglyceride levels in 10.5-kb homozygous subjects, $P < .01$; Table 1). Since the two cohorts were homogeneous, we analyzed the results together (Table 2). This difference in triglyceride levels was maintained when comparing the 10.5-kb homozygous group with the remaining genotypes matched for HbA_{1c}, age, and body mass index (BMI). When we analyzed the results according to gender, we observed that the differences were fundamentally attributed to the male group. No differences in clinical characteristics, metabolic control, or alcohol consumption were observed between both sexes that could justify this gender difference.

When analyzing the plasma lipids of type II diabetic patients as compared with the control group matched for sex, age, BMI, and TNF genotype, we observed differences only between both 10.5-kb homozygous groups, with significantly higher levels of triglycerides ($P < .001$) in type II diabetic patients (Fig 1). These differences were similar in both sexes.

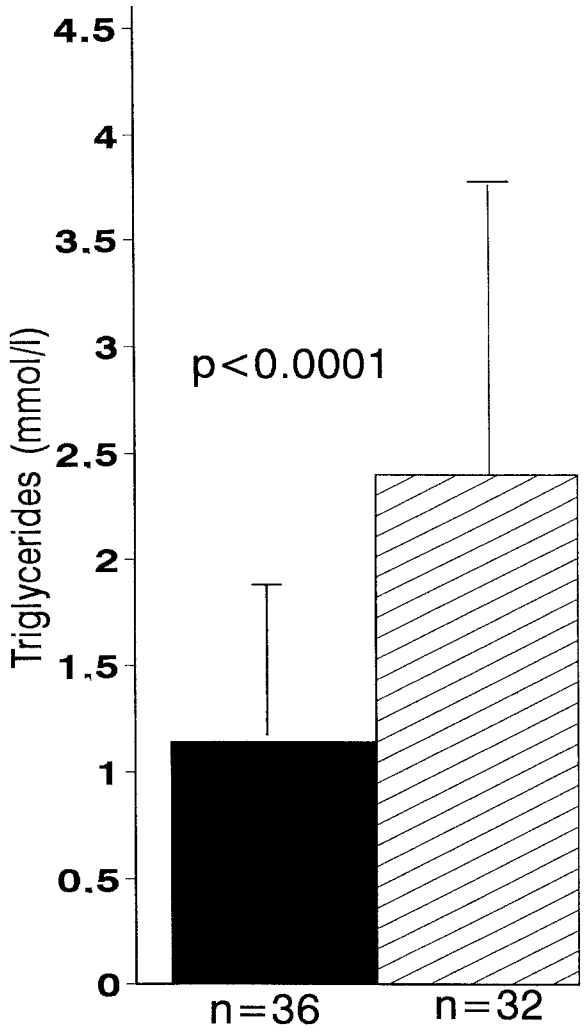

Fig 1. Genotype 10.5/10.5 matched for gender, age, and BMI. (■) Controls (n = 36) *v* (▨) type II diabetic patients (n = 32).

DISCUSSION

We have investigated the association between a genetic polymorphism in the TNF locus and the lipid profile in a group of type II diet-treated diabetic patients. Our overall results were consistent with an association of higher triglyceride levels for patients homozygous at the 10.5-kb fragment. We did not find significant differences in the remaining genotypes in either of the population groups exclusively attributed to the TNF genotype. In the diabetic patients, clinical parameters and glycemic control, which are factors that could influence the lipid profile, were similar among the three genotypes, suggesting an implication of the TNF gene in the triglyceride levels of patients homozygous for the great allele, mainly in the men.

The observed association between triglycerides and this genotype is of special interest if we consider that several studies have demonstrated a relationship between monokine secretion by monocytes and the TNF genotype, with a higher activity in subjects with the 10.5-kb homozygous genotype.[5,9] On the other hand, the ability of TNF to induce hepatic triglyceride synthesis in vivo[4] could lead to a functional relationship between these polymorphisms and lipid metabolism in type II diabetic patients. However, the lack of differences between the three TNF genotypes in the control group led us to believe that the genotype is necessary but not sufficient to explain the lipid changes observed in 10.5-kb homozygous diabetic patients. These preliminary results encourage us to consider the TNF region as a susceptibility locus for analysis of the dyslipidemia of type II diabetes.

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