Tumor Necrosis Factor Alpha Gene G-308A Polymorphism in the Metabolic Syndrome

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Tumor necrosis factor alpha (TNF-α) is a multifunctional cytokine constitutively produced by adipose tissue that may mediate insulin resistance. Studies in Caucasian subjects have suggested that the G-308A transition in the 5′ region of the TNF-α gene may be associated with insulin resistance and obesity. These factors have been proposed to underlie the clustering of type 2 diabetes, hypertension, and dyslipidemia found in the metabolic syndrome, the prevalence of which is reaching epidemic proportions in Hong Kong Chinese. We investigated the association of this gene polymorphism with the components of the metabolic syndrome including the lipid profile, as well as with the indices of obesity and insulin resistance as measured by the insulin-glucose product, in 440 Chinese subjects (healthy [27.5%] and overlapping groups with type 2 diabetes [54.1%], hypertension [38.8%], dyslipidemia [39.3%], or obesity [39.5%]). The frequency of the mutant A allele was 7.4% in 121 healthy controls and 9.0% in the total population. The mutation was not associated with any component of the metabolic syndrome or with the prevalence of albuminuria and retinopathy in these subjects. Furthermore, there was no difference in anthropometric measures, insulin resistance, or lipid levels between subjects with the GG genotype and those with the mutant allele. In summary, the TNF-α gene G-308A polymorphism is unlikely to play an important role in the development of these disorders in this population.

TUMOR NECROSIS factor alpha (TNF-α) is a multifunctional cytokine constitutively produced by adipose tissue. It has been suggested to mediate insulin resistance by modulating adipocyte gene expression.[1,2] TNF-α is involved in adipocyte metabolism at multiple sites including transcriptional regulation, glucose and fatty acid metabolism, and hormone receptor signaling.[3] TNF-α downregulates the GLUT4 glucose transporter in adipocytes in a manner that correlates with TNF-α–induced insulin resistance.[4] In obese subjects with type 2 diabetes, a similar effect was not found in skeletal muscle, the major site of insulin-mediated glucose uptake.[5] However, TNF-α is expressed in human muscle and at a higher level in subjects with insulin resistance and diabetes.[6,7] TNF-α also has been reported to reduce tyrosine kinase activity of the insulin receptor and insulin-stimulated tyrosine phosphorylation of the insulin receptor substrate-1 without affecting the number of receptors or their insulin-binding capacity.[2]

Hypertension, dyslipidemia, type 2 diabetes mellitus, and obesity are often found clustered together in the same individual.[8-10] Obesity, particularly visceral obesity, and insulin resistance have been considered as important links underlying the close associations among this constellation of metabolic disorders.[10-16] The components of the metabolic syndrome in Chinese subjects are all reported to be insulin-resistant states.[17-19] Furthermore, there is a strong genetic component to these disorders.[10,20-24] Mutations within the TNF-α gene may therefore influence the development of insulin resistance in these disorders. A linkage has been demonstrated between a locus near the TNF-α gene and obesity in Pima Indians.[25] However, a mutation within the promoter region of the gene was not associated with the degree of body fatness.[25] A G-308A transition also in the 5′ region of the TNF-α gene has been associated with a higher transcription rate[26] and with elevated fasting plasma insulin and leptin and percent body fat in a small study of 38 Caucasian subjects.[27] However, no relationship was identified between this polymorphism and type 2 diabetes in a larger study of Caucasian subjects.[28] In a further study in Caucasians, although the polymorphism was not associated with diabetes, there was limited evidence to support the involvement of the gene in modulating insulin resistance.[29]

The evidence concerning the relationship of this polymorphism with insulin resistance is therefore controversial. In this study, we assessed the association of this polymorphism with aspects of the metabolic syndrome in a population of Chinese subjects.

SUBJECTS AND METHODS

The Clinical Research Ethics Committee of The Chinese University of Hong Kong approved the study protocol. All 440 unrelated subjects provided written informed consent. They were of Han Chinese origin, without any known ancestors of other ethnic origin, and were living in the Hong Kong Special Administrative Region of China at the time of the study. The catchment area of The Prince of Wales Hospital has been developed only since the 1960s, and serves a population of over 1 million. The majority of its inhabitants are a typical socioeconomic representation of first- or second-generation migrants from Southern China now living in a westernized environment.

The subjects presenting with components of the metabolic syndrome were recruited from the medical outpatient clinics at The Prince of Wales Hospital. Seated blood pressure, anthropometric parameters, and plasma biochemical parameters after an overnight fast were measured. The anthropometric parameters required to calculate the body mass index (BMI) and waist to hip ratio (WHR) were measured. Measurements of skinfold thickness were taken at the triceps, biceps, iliac crest, and subscapular sites using digital calipers (Skyndex electronic body fat

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calculator system; Skyndex, Caldwell Justiss, Fayetteville, AR) and the percent body fat was derived.[30]

Subjects with impaired fasting glucose (IFG) and diabetes were diagnosed based on fasting plasma glucose (FPG) levels. FPG less than 6.0 mmol/L was considered normal, 6.0 to 7.0 mmol/L was considered IFG, and 7.0 mmol/L or higher was indicative of diabetes.[31] If the subject had no diabetes-related complaints, then two elevated FPG readings were required for diagnosis. However, if the subject was symptomatic, a single elevated reading was sufficient. In subjects with equivocal plasma glucose levels who underwent a 75-g oral glucose tolerance test (OGTT), a 2-hour postglucose level of 7.8 to 11.1 mmol/L was considered indicative of impaired glucose tolerance (IGT).[31] In this study, subjects with IFG, IGT, and type 2 diabetes were grouped together and considered to have glucose intolerance (GIT), of which 95.9% had type 2 diabetes. Of the type 2 diabetic patients, 224 were examined by an ophthalmologist for retinopathy through dilated pupils. Retinopathy was considered present if there were one or more areas of hemorrhages, microaneurysms, cotton wool spots, and/or laser coagulation scars due to diabetic retinopathy, and it was present in 11.2% of the subjects.

Subjects were defined as hypertensive if their seated systolic blood pressure (SBP) was 140 mm Hg or higher and/or diastolic blood pressure (DBP) was 90 mm Hg or higher on at least two occasions after 5 minutes' rest while off antihypertensive treatment (after a 4-week washout period).[32] A mean of three readings taken 1 minute apart with an automated sphygmomanometer (Dinamap 8100; Critikon, Tampa, FL) was used. No subjects had a history of significant renal, hepatic, or cardiac disease. Normotensive (SBP $<$ 140 mm Hg and DBP $<$90 mm Hg) nondiabetic (FPG $<$6.0 mmol/L) controls were recruited from the hospital staff and their friends.

Obesity was defined as a BMI of 25.0 kg/m^2 or higher or 27.0 kg/m^2 or higher and/or a WHR of 0.85 or higher or 0.90 or higher in females and males, respectively.[31] Dyslipidemia was classified as either a fasting plasma total cholesterol of 6.2 mmol/L or higher or between 5.2 and 6.2 mmol/L with a total cholesterol to high-density lipoprotein (HDL) cholesterol ratio of 5.0 or higher and/or fasting plasma triglycerides, 2.3 mmol/L or higher.[33,34]

The subjects were classified as controls (nondiabetic, nonhypertensive, nondyslipidemic, and non–BMI obese, n = 121, 27.5%) or as overlapping groups of subjects with GIT (54.1%), hypertension (38.8%), dyslipidemia (39.3%), or obesity (39.5%) according to the above-mentioned criteria. Each group also contained subjects with varying degrees of the other conditions. Since these metabolic derangements frequently occur together in the same patient, the combined number of subjects in these groups exceeded 440.

Biochemical Analyses

Fasting plasma samples were obtained for measurement of electrolytes, urate, lipids (triglycerides, total cholesterol, low-density lipoprotein [LDL] cholesterol, and HDL cholesterol), and glucose. Plasma electrolytes were determined by ion-selective electrodes on a parallel-multichannel analyzer (DuPont, Newark, DE). The creatinine level was measured using the Jaffé method on a Beckman Astra-8 Chemistry analyzer (Beckman, Brea, CA). Measurement of urate based on the uricase method was performed using the Dade Dimension clinical chemistry system (Dade International, Deerfield, IL). The plasma glucose level was measured using a standard glucose oxidase method. The insulin level was measured in a 96-well microtiter-plate enzyme-linked immunosorbent assay system (Dako Diagnostics, Ely, UK). Plasma total cholesterol and triglyceride levels were measured enzymatically (Centrichem Chemistry System, Baker Instruments, Allentown, PA). The long-term imprecision of the assay was 3% at 3.3 mmol/L and 2.2% at 6.8 mmol/L for total cholesterol, and 6.9% at 1.02 mmol/L and 4.6% at 2.18 mmol/L for triglycerides. HDL cholesterol was determined following fractional precipitation with dextran sulfate–$MgCl_2$, and LDL cholesterol was calculated using Friedewald's formula.[35] The urinary albumin concentration was measured by immunoturbidimetry.[36] The detection limit was 2.5 mg/L, and the interassay and intraassay coefficient of variation was less than 5%. Midstream urine was collected for culture and microscopy to rule out infection. A single measurement of the spot albumin to creatinine ratio was used as a screening test for albuminuria ($>$3.5 mg/mmol), which was found in 26.4% of the subjects.

Genotyping Protocol

The TNF-α gene *Nco*I polymorphism was determined using a polymerase chain reaction (PCR)-restriction fragment length polymorphism–based protocol as described by Wilson et al.[37] A 107-bp fragment of the 5′ region was amplified with 35 cycles of 1 minute at 95°C, 1 minute at 60°C, and 1 minute at 72°C. The PCR product was then digested with 10 U of the restriction enzyme *Nco*I (Promega, Madison, WI) overnight at 37°C. The presence of a G to A transition at position-308 introduced a restriction site that, after digestion, produced two fragments of 87 and 20 bp that were visualized on a 2.5% agarose gel.

Statistical Analyses

Data from normally distributed parameters are presented as the mean ± SD, whereas skewed data were logarithmically transformed and expressed as the geometric mean with 95% confidence interval. Differences in biochemical and anthropometric markers between cohorts were compared using Student's *t* test. The Statistics Package for Social Sciences (SPSS for Windows, Version 7.5.1, 1996, SPSS, Chicago, IL) was used for the analyses.

RESULTS

The TNF-α gene G-308A polymorphism was identified in 440 Chinese subjects with varying components of the metabolic syndrome. The frequency of the mutant A allele was determined to be 9.0% (Table 1). When the genotype and allele frequency of the polymorphism was compared in the control subjects versus subjects with GIT, hypertension, dyslipidemia, obesity, retinopathy, or albuminuria using the χ^2 test, no difference was identified. The frequencies in each group of patients were all in

Table 1. TNF-α G-308A Genotype and Allele Frequencies in the Groups With GIT, Hypertension, Dyslipidemia, or Obesity and Those Without Any of The Components of the Metabolic Syndrome in 440 Chinese Subjects

Group	No. of Subjects	Genotype Frequency (%)			Allele Frequency (%)	
		GG	GA	AA	G	A
Control	121	85.1	14.9	0.0	92.6	7.4
Hypertensive	168	85.1	13.7	1.2	92.0	8.0
GIT	238	83.2	15.1	1.7	90.8	9.2
Dyslipidemic	173	80.9	19.1	0.0	90.5	9.5
Obese	174	83.9	16.1	0.0	91.2	8.0
Total population	440	83.2	15.7	1.1	91.0	9.0

NOTE. No significant differences were identified for either the genotype or allele frequencies between control subjects and subjects with components of the metabolic syndrome.

Hardy-Weinberg equilibrium. The anthropometric and plasma and urinary biochemical parameters in subjects with and without the mutant A allele were compared using Student's *t* test. Due to the low prevalence of the AA genotype (n = 5), these subjects were combined with the mutant carriers for the analysis. No differences among the genotypes were identified either in the total population or within each of the subgroups (Table 2). This includes the index of renal function, the albumin to creatinine ratio, the mean values of which were similar in both groups of subjects. One-way ANOVA was used to test for linear differences between the clinical parameters; however, no significant differences were identified.

The frequency for genotypes GG (80.7%), GA (17.5%), and AA (1.8%) and alleles G (89.5%) and A (10.5%) of the US Caucasian control subjects described by Hamann et al[28] was not significantly different from the rates in our Chinese control subjects. However, the genotype distribution in a similar UK Caucasian population with genotypes GG (68.4%), GA (30.0%), and AA (1.6%) and alleles G (83.3%) and A (16.6%) showed a higher frequency of the mutation ($P < .005$).[29] The genotype frequencies of the two Caucasian populations did not differ.

Table 2. Comparison of Fasting Plasma and Urine Biochemical and Anthropometric Parameters Between TNF-α G-308A Genotypes

Parameter	TNF-α G-308A Genotype	
	GG	GA/AA
No. of subjects	366	74
Age (yr)	48.5 ± 15.0	51.7 ± 14.6
Gender (% male)	39.6	35.1
MAP (mm Hg)	93 ± 16	94 ± 16
BMI (kg/m²)	23.6 ± 3.5	23.7 ± 3.5
Waist circumference (cm)	82.6 ± 9.4	82.2 ± 9.6
WHR	0.87 ± 0.07	0.87 ± 0.07
Body fat (%)	26.1 ± 8.1	27.8 ± 6.5
Glucose (mmol/L)	7.3 (7.0-7.6)	7.2 (6.5-7.9)
Insulin (pmol/L)	77.6 (69.2-87.1)	90.2 (64.9-125)
Insulin-glucose product	550 (473-631)	661 (437-1,011)
Total cholesterol (mmol/L)	5.4 ± 1.3	5.4 ± 1.2
HDL cholesterol (mmol/L)	1.4 ± 0.4	1.4 ± 0.4
LDL cholesterol (mmol/L)	3.4 ± 1.0	3.4 ± 1.1
Triglyceride (mmol/L)	1.35 (1.25-1.46)	1.49 (1.23-1.79)
Albumin to creatinine ratio	2.4 (1.9-2.9)	2.1 (1.4-3.1)
% with GIT	54.1	54.1
% with dyslipidemia	38.0	44.6
% with hypertension	38.9	33.8
% with obesity	52.7	49.1

NOTE. Results are the mean ± SD or the geometric mean (95% confidence interval). No significant differences were identified between subjects with the GG genotype and those with the mutant allele (GA, n = 69; AA, n = 5).

Abbreviation: MAP, mean arterial pressure.

DISCUSSION

The constellation of disorders associated with the metabolic syndrome is reaching epidemic proportions in Hong Kong.[10,18,38] Type 2 diabetes (based on 75-g OGTT) is found in 10% of the adult population (25 to 74 years), hypertension (SBP ≥ 140/DBP ≥ 90 mm Hg) in 17%, a BMI of at least 25 kg/m² in more than one third, and dyslipidemia (total cholesterol ≥5.2 mmol/L and/or triglycerides ≥2.0 mmol/L) in over 50%.[38] The close association between these metabolic diseases led to the proposal that obesity and/or insulin resistance may be an important link in the development of multiple disorders within an individual.[10-16] There is evidence to implicate TNF-α in the development of obesity and insulin resistance in both animal models and humans.[3]

Mutations within the TNF-α gene may be associated, in part, with the development of these disorders and the differential distribution of the disorders within individuals. We investigated a G-308A transition in the 5′ region of the TNF-α gene, which is reportedly a functional mutation associated with higher in vitro transcription rates.[26] The mutant A allele frequency was 7.4% in the Chinese control subjects in whom type 2 diabetes, hypertension, dyslipidemia, and obesity (by BMI criteria) were absent, and 9.0% in the total population of 440 subjects. The A allele frequency was similar to the rate reported by Hamann et al[28] of 10.5% in US Caucasian control subjects, but was lower than that reported in a similar population from the United Kingdom of 16.6%.[29]

In this study, no relationship was identified between this polymorphism and the component disorders of the metabolic syndrome or possible underlying factors such as obesity or insulin resistance as assessed by the insulin-glucose product. Similar findings have been reported in Caucasian subjects, in which two studies found no relationship between this polymorphism and type 2 diabetes/IFG.[28,29] There is limited evidence to support the involvement of the polymorphism in obesity, with the mutant allele found at a higher frequency in obese Irish subjects, but this was not the case in French subjects from the same study.[39] Hawrami et al[40] described a similar lack of association of the polymorphism with type 2 diabetes in Southern Indians, but reported a significant association with microangiopathy as indicated by retinopathy. However, we found no relationship with microangiopathies, including retinopathy, and albuminuria in this Chinese population.

In summary, the relatively low frequency of the mutant allele despite the high frequency of metabolic disorders in Hong Kong Chinese and the lack of association with any component of the metabolic syndrome suggest that the TNF-α gene G-308A polymorphism is unlikely to play an important role in the development of these disorders in this population.

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