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원 저

Study on Relationship between Tumor Necrosis Factor-α Gene Polymorphism and Obese Patients

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Objective : A number of candidate genes have been in implicated in the pathogenesis of obesity in humans. Tumor necrosis factor-alpha (TNF-α) is expressed primarily in adipocytes, and elevated levels of this cytokine have been linked to obesity and insulin resistance. Recently, the A allele of a polymorphism at position 308 in the promoter region of TNF-α (G-308A) has been shown to increase transcription of the gene in adipocytes. Therefore, we designed this study to test whether obese and non-obese subjects differ in TNF-α genotype distribution, and how the genotypes affect anthropometric parameters, including degrees of body mass index (BMI).

Methods : The study included 153 obese but otherwise healthy women (BMI≥25 kg/m², range 25-54.7, age range 15-40 years) and 82 non-obese healthy women (BMI<25 kg/m², age range 15-40 years). Total fat mass and percent body fat were determined by dual-energy X-ray absorptiometry. Genomic DNA was extracted and used for NcoI restriction fragment length polymorphism (RFLP) based genotyping of TNF-α.

Results : No differences were observed for allelic and genotype frequencies between the obese (BMI≥25) and non-obese women. Also, no association of TNF-α polymorphism was observed with body mass index (BMI) for genotype in obese women. In addition, age, percent body fat, BMI, and cholesterol levels did not differ by TNF-α genotype. However, waist-to-hip ratio (WHR) was significantly lower in subjects with TNF-α GA or AA genotype (0.94 0.07 vs. 0.92 0.03, P<0.005).

Conclusion : These results suggest that TNF-α promoter polymorphism at position -308 is not a significant factor for BMI, but affects the WHR in obese healthy women from Koreans.

Key Words: obesity, TNF-α gene, waist-to-hip ratio (WHR)

Introduction

Obesity is increasing in prevalence and is associated with several adverse health problems, including type 2 diabetes, hyperlipidemia and hypertension[1]. The influence of obesity on the development of obesity-related disorders is complex and probably due to an interaction of genetic, nutritional and metabolic factors[2,3].

The cytokine tumor necrosis factor (TNF)-α acting as a modulator of gene expression in adipocytes is implicated in the development of insulin resistance and obesity[4]. Fat tissue is a significant source of endogeneous TNF-α production, and the expression of this cytokine is elevated in human obesity in both adipose[5,6] and muscle tissues[7]. Increased expression of TNF-α strongly correlates with the level of hyperinsulinemia[8] and the glucose disposal rate during euglycemic clamp technique[7].

The gene for human TNF-α is located on the short arm of chromosome 6[9], and a G → A substitution at position -308 upstream from the transcription initiation

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site in the promoter region of the gene has been identified[10]. In vitro experiments have demonstrated that this DNA NcoI restriction fragment length polymorphism (RFLP) increases transcriptional activation of the TNF-α gene[11]. Although controversial, the majority of the data support a direct role for this biallelic polymorphism in the elevated TNF-α levels observed in homozygotes for the -308 A allele[12]. Subsequent studies on the relationship between this polymorphism and insulin resistance have shown inconsistent results[13-16]. Fernandez-Real et al[13] showed that this polymorphism at ñ 308 influenced insulin sensitivity through increased body fat in a group of nondiabetic normotensive Spanish subjects. A recent study on type 2 diabetes mellitus patients showed no difference in the frequencies of allele at -308 compared with healthy control subjects[14]. Very recently, Walston et al[16] reported that TNF-α polymorphism at -308 site did not relate to any traits of obesity and insulin resistance in a group of nondiabetic subjects.

The author designed this study to test whether obese and non-obese subjects differ from TNF-α genotype distribution, and the genotypes affect the anthropometric parameters, including the degrees of body mass index (BMI) in obese healthy women.

Materials and Methods

1. Patients

Patients were 153 healthy obese (BMI≥25 kg/m^2, range 25-54.7) women between the ages of 15-40 years. All subjects were nonsmokers and had no evidence of cancer, liver, renal, hematological disease or other metabolic disorder other than obesity. A total of 153 women met all study criteria and were enrolled into the study. All subjects were normotensive and the subjects with dyslipidemia (total cholesterol>250 mg/dl, HDL-cholesterol<35 mg/dl) and/or diabetes were excluded. The control groups (all Korean) consisted of 82 non-obese women (BMI<25 kg/m^2, age range 15-40 years) undergoing routine health screening. Because the age of obese women was relatively low (mean age 30.01± 10.5) the author limited the age of non-obese women below 40 years old. All methods and procedures for the study were approved by the institutional review board of the Wonkwang University Hospital. Each participant provided written informed consent.

2. Phenotype measurements

Anthropometry. Height (in cm) and weight (in kg) were measured to calculate BMI as weight(kg)/height(m) squared. Waist circumference (measured at the narrowest point superior to the hip) was divided by the circumference of the hip (measured at its greatest gluteal protuberance) to obtain the waist-to-hip ratio (WHR).

Dual-energy X-ray absorptiometry. Fat mass was determined by dual-energy X-ray absorptiometry (DEXA).

3. Genotype determination

The genomic DNA was extracted by inorganic procedure[17]. A single base pair polymorphism at position ñ 308 in the promoter region of the TNF-α gene was examined by the NcoI (Takara, Shiga, Japan) restriction fragment length polymorphism (RFLP) method described elsewhere[18]. The following primers were used: 5' -AGGCAATAGGTTTTGAGGGCCAT-3' and 5' -TCCTCCCTGCTCCGATTCCG-3' .

4. Discrimination of Sasang constitution of obese women

Individuals were discriminated into four types through the use of the Questionnaire for the Sasang Constitution Classification (QSCC) II program and clinically important characteristics such as physical frame, facial

features, personalities, emotions, and reactions to herbal medicines. The clinical characteristics were based on the Donguisusebowon (Longevity and Life Preservation in Oriental Medicine) which is a basic book that explains how to identity each constitution.

5. Statistical analysis

The mean levels of all numerical values were tested by the Student t test or ANOVA test. Comparisons of the genotype and allele frequencies of the TNF-α genotypes between groups were carried out using the Pearson chi-square test. All statistical analyses were performed using SPSS v9.00 (SPSS Inc.) statistical analysis software. A p-value less than 0.05 were considered statistically significant.

Results

1. Clinical characteristics of obese women

The clinical characteristics of obese women and controls are presented in Table 1. A total of 27.5% of obese women were classified as BMI 25~26 (n=42), 35.9% were BMI 27~29 (n=55), and 36.6% were BMI 30~40 (n=56). As expected, the values of weight, fat mass, percent body fat (PBF), and WHR differed among the three BMI groups (Table 1).

2. Frequencies of alleles and genotypes

Genotype frequencies in all groups were all in accordance with the Hardy-Weinberg equilibrium. The distribution of the TNF-α genotype was similar in each group investigated, the genotype frequencies for the

Table 1. Characteristics of Obese Women according to BMI(n153)

	Non-obese controls	BMI(kg/m²)			p^a value
		~26	27~29	30~	
N(%)	82	42(27.5)	55(35.9)	56(36.6)	
Age(year)	25.6±7.5	28.0±9.8	31.5±10.0	30.2±11.3	0.258
Weight(kg)	62.2±19.5	64.4±4.9	70.8±6.0	85.7±14.6	<0.001
Height(cm)	161.3±5.2	158.7±6.5	158.2±6.0	163.5±7.0	<0.001
Total cholesterol(mg/dl)	163.7±25.4	182.9±45.3	174.6±34.7	196.2±41.5	0.027
Triglyceride(mg/dl)	77.3±38.8	116.7±112.0	122.2±79.5	163.2±140.6	0.086
Fat mass(kg)	19.5±2.1	23.2±2.6	26.1±3.2	34.4±8.4	<0.001
PBF(%)	33.3±2.6	35.9±2.9	36.1±6.2	39.6±6.0	0.001
WHR	0.85±0.04	0.89±0.03	0.93±0.04	0.98±0.07	<0.001
SBP(mm Hg)	112.9±9.5	110.8±11.6	116.7±17.2	121.7±11.5	0.083
DBP(mm Hg)	72.9±9.5	69.1±7.9	71.3±11.3	77.4±7.5	0.023

Values are means±SD; BMI, body mass index; PBF, percentage body fat; WHR, ratio of waist-to-hip circumstance; SBP, systolic blood pressure; DBP, diastolic blood pressure.
a By one-way ANOVA test (among BMI groups).

Table 2. Distribution of TNF-α Genotypes in Obese and Non-Obese Women

	Genotypes			Statistics [a]
	GG	GA	AA	
Non-obese (n=82), n(%)	69(84.1)	11(13.4)	2(2.4)	p=0.338, χ^2=2.168, df=2
Obese (n=153), n(%)	129(84.3)	23(15.0)	1(0.7)	

[a] By χ^2 test (two-sided).

obese women (BMI≥25), non-obese women controls (BMI<25) and random controls being 83.2, 15.2, and 1.6%; 83.3, 14.3, and 2.4%; 83.4, 14.1, and 2.5% for the GG, GA, and AA genotypes, respectively. The allele frequencies of obese women were not significantly different from the distribution in two control groups (Table 2).

3. Characteristics of obese women according to TNF-α genotypes

Table 3 describes relationship between the TNF-α genotype and anthropometric parameters in obese women. Of interest, WHR was lower in women with GA+AA genotypes than in those with the GG genotype (0.92±0.03 vs. 0.94±0.07, $p<0.005$). The remaining variables were similar between GG and GA+AA genotypes as well as among the three genotypes.

4. TNF-α polymorphism and BMI

The author investigated the association of the TNF-α polymorphism with BMI. This resulted in a failure to detect any statistically significant association between the different genotypes (Table 4). However, compared with obese women (BMI<30) and extremely obese women (BMI≥30), the considerable differences were observed in the frequencies of GG and GA+AA genotypes, although these differences were not statistically significant. In extremely obese women (BMI≥30), only a trend was observed for carriers of GA+AA genotypes to exhibit a low frequency than did

Table 3. Characteristics of Obese Women according to TNF-α Genotypes (n=153)

	Genotypes	
	GG	GA+AA
N(%)	129(84.3)	24(15.7)
Age(year)	30.1±11.3	29.4±8.2
Weight(kg)	74.8±13.7	72.4±11.5
Height(cm)	160.3±6.5	159.6±9.1
Total cholesterol(mg/dl)	186.2±43.2	178.9±26.7
Triglyceride(mg/dl)	135.6±115.3	125.0±84.22
Fat mass(kg)	28.7±7.9	26.6±4.6
BMI(kg/m2)	29.2±4.1	28.3±2.2
PBF(%)	37.5±5.9	36.8±3.6
WHR	0.94±0.07	0.92±0.03*
SBP(mm Hg)	119.0±14.6	112.2±6.7

Values are means±SD; BMI, body mass index; PBF, percentage body fat; WHR, ratio of waist-to-hip circumstance; SBP, systolic blood pressure; DBP, diastolic blood pressure.
*$p<0.005$ by t-test (GG genotype vs. the other genotypes).

Table 4. Frequencies of TNF-α Genotypes according to BMI in Obese Women (n=153)

BMI (kg/m²)	Genotypes		Statistics[a]
	GG	GA+AA	
25~26, n (%)	34(81.0)	8(19.0)	$p=0.780$, $\chi^2=0.496$, df=2
27~29, n (%)	47(85.5)	8(14.5)	
30~40, n (%)	48(85.7)	8(14.3)	
<30, n (%)	81(83.5)	16(16.5)	$p=0.717$, OR=0.84, CI=0.34-2.12
≥30, n (%)	48(85.7)	8(14.3)	

[a] By $\chi 2$ test (two-sided).

Table 5. Distribution of Sasang Constitutions in Obese and Controls

Sasang constitution	Obese n=150	Controls n=887[a]	Statistics[b]
Taeumin, n(%)	144(96.0)	327(36.9)	p <0.001
Soyangin, n(%)	1(0.7)	314(35.4)	
Soeumin, n(%)	5(3.3)	246(27.7)	
Taeyangin, n(%)	0(0)	0(0)	

The a was quoted from Park et al.19).
[b] By χ^2 test (two-sided).
In obese, 150 of 153 cases were valid, while the data of three cases were missing.

obese women (BMI<30) (85.7 and 14.3% vs. 83.5 and 16.5% for the GG and GA+AA genotypes, respectively.

5. Distribution of Sasang constitutions

The distribution of individual constitution in 150 obese women, minus three omitted cases, was as follows: Taeyangin, 0 (0%); Taeumin, 144 (96.0%); Soyangin, 1 (0.7%); and Soeumin, 5 (3.3%). This was significantly different from the distribution in 887 control subjects quoted from Park et al.: Taeyangin, 0 (0%); Taeumin, 327 (36.9%); Soyangin, 314 (35.4%); and Soeumin, 246 (27.7%) ($\chi 2$=15.425, p<0.001)[19] (Table 5). The frequency of Taeumins in obese individuals was higher than that of Taeumins in controls.

Discussion

The purpose of the present study was to determine whether the G/A polymorphism of TNF-α gene was associated with obesity and anthropometric parameters in women. Obesity is a complex metabolic disorder with a strong genetic component. There are many candidate genes for obesity and its related phenotypes. Most of these genes are candidates for obesity because mutations in them cause rare genetic syndromes affecting adipocyte differentiation[20]. Recent interest has focused on the role of the TNF-α gene in association with the insulin-resistant state and obesity[21-23]. Thus, the author focused on the association between the polymorphism of TNF-α and obesity without metabolic disease. However, the author did not find the significant differences between obese women and non-obese women in the frequencies of TNF-α polymorphism.

Of interest, the author found that WHR was lower in women with GA+AA genotypes than in those with the GG genotype. These results are inconsistent with those reported by Rosmond et al.[24], who found no association between the TNF-α, obesity (BMI), and body fat distribution (WHR and abdominal sagittal diameter). Morris et al. also reported that the AA variant in TNF-α gene (-308A/G) did not influence the amount of weight loss in overweight and obese men and women on a 30% energy restricted diet[25]. Contrary to that, some studies suggested the TNF-α polymorphism associated with obesity in several European populations[13,26,27]. However, the design and ethnic population of these studies were quite different from this one and the results are, thus, hardly comparable. Moreover, obesity in women (BMI ≥25) was not a main objective in the studies.

The author have selected TNF-α, a candidate gene for obesity in Pima Indians, mainly because of the reported overexpression of TNF-α mRNA in adipose tissue of rodents that are genetic models for insulin resistance and associated obesity[8]. The levels of TNF-α mRNA and protein in the adipose tissue of obese human subjects were found to be 2.5 times higher than the levels in lean controls and highly corrected with fasting

insulin concentration[6]. In addition, it was reported that substitution of adenine (A) for guanine (G) at position ñ 308 in the promoter region of TNF-α enhances the transcription of this cytokine in culture cells[11]. Indeed, some of the previous studies suggest that homozygotes for the -308 variant are more obese compared with the other genotype groups[13,28,29]. Thus the author expected the result that was higher WHR in GA+AA subjects. However, the author obtained an unexpected result, lower WHR in women with GA+AA genotypes. Nonetheless, this result tends to confirm some of the previous studies which suggest that the direct paracrine effect of adipose-derived TNF-α seems to be inhibition of the leptin production[30,31]. Leptin is a protein proposed to be an "adipocity signal". Although the effect on TNF-α on leptin production is complex, the observation that TNF-α inhibits leptin release from the adipose tissue could have important physiological implications in the regulation of adipose tissue deposition. Fawcett et al. suggest that the attenuation in leptin secretion appears to be mediated through a reduction in ob gene expression[30].

In summary, no differences were observed for allelic and genotype frequencies between obese women and non-obese women. Also, no association of TNF-α polymorphism was observed with BMI for genotype in obese women. However, WHR was significantly decreased in subjects with TNF-α GA or AA genotype. These results suggest that TNF-α promoter polymorphism at position -308 is not a significant factor for BMI, but affects the WHR in obese healthy women from Koreans. The author also demonstrated that Taeumins in obese individuals was higher than that of controls.

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