Brief Genetics Report

Tumor Necrosis Factor-α-238 and -308 Polymorphisms Do Not Associate With Traits Related to Obesity and Insulin Resistance

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Tumor necrosis factor-α (TNF-α) is expressed primarily in adipocytes, and elevated levels of this cytokine have been linked to obesity and insulin resistance. The purpose of this investigation was to test whether the TNF-α-308 polymorphism (previously linked to insulin resistance and increased leptin levels) and the TNF-α-238 polymorphism (linked to decreased insulin resistance) were associated with insulin resistance or obesity-related traits in 424 subjects self-referred to the Johns Hopkins Weight Management Center (JHWMC). There were no differences in allele frequencies of either polymorphism by obesity category in the JHWMC and a lean control group. Despite previous smaller studies that have linked insulin resistance and the 308 allele, we found no such relationship in the JHWMC population. Instead, homozygotes for this allele had a significantly lower BMI than their counterparts without the polymorphism. In addition, we found no relationship between the 238 polymorphism and BMI, fasting glucose, or log of fasting insulin. *Diabetes* 48:2096–2098, 1999

Obesity is increasing in prevalence among Americans and is associated with several adverse health problems, including type 2 diabetes, hyperlipidemia, and hypertension (1–3). The influence of obesity on the development of type 2 diabetes is complex and is likely due to an interaction of genetic, nutritional, and metabolic factors (4). Much attention has been focused on the identification of molecular pathways that contribute to the development of obesity and type 2 diabetes (5–8). The cytokine tumor necrosis factor-α (TNF-α) is expressed primarily in adipocytes, where it is an important modulator of gene expression (9–11). In obese individuals, TNF-α expression is elevated and correlates strongly with hyperinsulinemia (10). Recently, two polymorphisms were identified in the 5′ regulatory region of the TNF-α gene. The first, found at position 308 (guanine [G] adenine [A]) of the TNF-α gene, may increase expression of this cytokine in fat tissue and influence fat mass and insulin resistance (12). Fernández-Real et al. (8) recently reported that in 38 subjects (19 men and 19 women), this polymorphism was associated with increased insulin resistance, elevated percent body fat, and increased serum leptin levels. The second polymorphism was recently reported by Day et al. (13) to associate with decreased insulin resistance among family members who carried the 238 (guanine [G] adenine [A]) polymorphism. The purpose of this study was to determine the relationship of these variant TNF-α alleles to traits related to obesity and type 2 diabetes in an obese cohort.

The Johns Hopkins Weight Management Center (JHWMC) participants (n = 424) were >18 years of age, nondiabetic by American Diabetes Association criterion, and mostly female (65%). All signed institutional review board consent forms for genotyping. Characteristics of this population have been described elsewhere (7). A lean (BMI <25 kg/m^2) control group (n = 210) of age- and sex-matched nondiabetic individuals was identified from the east Baltimore area to study allele frequency differences.

Genotype frequencies in the JHWMC population were 0.18 overall for the 308 variant (Caucasian 0.17, n = 362; African-American 0.18, n = 62). The 238 TNF-α allele frequencies were 0.06 for both Caucasians and African-Americans. The frequencies of each allele were in Hardy-Weinberg equilibrium. To examine whether the variant alleles were more common in patients with morbid obesity, we stratified the JHWMC participants by BMI classifications of obesity according to Wensier (14) and compared with the lean group (BMI <25 kg/m^2) of volunteers. A χ^2 analysis revealed no significant differences in gene frequencies across the four grades of obesity (Table 1) for either the TNF-α-308 or the TNF-α-238 polymorphism. Analysis of covariance (ANCOVA) with adjustment for age, BMI, ethnicity, and sex revealed no significant differences in the JHWMC participants among genotype for waist-to-hip ratio, fasting glucose levels, or log of fasting insulin levels (Table 2). We used a two-factor ANCOVA to test for interactions between these two polymorphisms and found no significant interactions between

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ANCOVA, analysis of covariance; JHWMC, John Hopkins Weight Management Center; PCR, polymerase chain reaction; TNF-α, tumor necrosis factor-α.

TABLE 1
Characteristics of obese JHWMC subjects by TNF-α-308 and TNF-α-238 genotypes

Trait	TNF-α-308			TNF-α-238		
	TNF1/TNF1 (normal)	TNF1/TNF2 (heterozygote)	TNF2/TNF2 (homozygote)	TNF1/TNF1 (normal)	TNF1/TNF2 (heterozygote)	TNF2/TNF2 (homozygote)
n	288	121	15	376	47	1
Age (years)	41.9 ± 10.6	45.0 ± 12.2	43.8 ± 8.5	42.7 ± 11.2	43.9 ± 9.9	36
Female (%)	67.0	60.3	60.0	65.2	61.7	100
BMI (kg/m^2)	40.8 ± 11.7	38.6 ± 9.0	34.4 ± 7.5*	40.1 ± 10.8	39.2 ± 12.2	27.7
Waist-to-hip ratio						
Women	0.82 ± 0.07	0.82 ± 0.07	0.80 ± 0.03	0.82 ± 0.07	0.82 ± 0.08	0.81
Men	0.98 ± 0.07	1.00 ± 0.07	0.98 ± 0.06	0.98 ± 0.07	1.04 ± 0.07†	NA
Fasting plasma glucose (mmol/l)	5.2 ± 1.1	5.5 ± 2.1	5.1 ± 0.6	5.3 ± 1.5	5.1 ± 1.0	4.7
Log fasting insulin (pmol/l)	2.1 ± 0.5	2.6 ± 0.6	2.7 ± 0.6	2.7 ± 0.6	2.6 ± 0.5	2.5

Data are unadjusted means. ANCOVA was used to adjust age for BMI, ethnicity, and sex; BMI for age, ethnicity, and sex; and waist-to-hip ratio, glucose, and insulin for age, BMI, ethnicity, and sex. *P = 0.03, †P = 0.002 vs. normal subjects.

the variants and dependent measures. We found that 49.4% of the obese population had neither variant, whereas 28.2% had TNF-α-308, 8.6% had TNF-α-238, and 3.5% had both variant alleles. BMI was not different across these four groups (P = 0.780). To evaluate a potential genotype–diabetes interaction, we ran a series of two-factor ANCOVAs adjusting for age, sex, and race on all obesity-related variables in the 49 diabetic subjects not included in the initial analysis. As expected, we found that the diabetic subjects had significantly higher BMI, weight, and waist-to-hip ratios than their nondiabetic counterparts. However, our two-factor ANCOVAs found no significant main effects for genotype or genotype by diabetic status interactions in any of the variables of interest.

In conclusion, our data indicate that neither the TNF-α-308 nor the TNF-α-238 variant allele correlates with increased BMI or influence traits related to obesity and insulin resistance in this obese cohort. This does not support the conclusions of a previous study in smaller cohorts that found an association between the 308 allele and increased insulin resistance (8). In fact, the BMI of the group homozygous for the 308 variant allele is significantly lower. Because the influence of a given gene variant in complex genetic disorders is dependent on environmental influences and genetic background, further studies will be required to rule out an effect of this allele in other ethnic groups or in combination with other variant alleles.

RESEARCH DESIGN AND METHODS

The subjects were genotyped using the polymerase chain reaction (PCR)-restriction fragment length polymorphism method as described by Wilson et al. (12). A 107-bp fragment encompassing the 308 variant site was amplified from genomic DNA by PCR using standard reagents, technique, and primers (5′-AGGCAATAG GTTTTGAGGGGCCAT-3′) and (5′-TCCTCCCTGCTCCGATTCCG-3′) for the 308 variant and (5′-ATCTGGAGGAAGCGGTAGTG-3′) and (5′-AGAAGACCCCC CTCGGAACC-3′) for the 238 variant allele. To detect the 308 variant allele, the PCR product was digested at 37°C overnight, leaving a 107-bp fragment if the variant was present and products of 87- and 20-bp fragments for the normal allele. To detect the 238 variant allele, the PCR product was digested at 37°C overnight, leaving a 152-bp fragment if the variant was present and products of 133- and 19-bp fragments for the normal allele.

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TABLE 2
TNF-α genotype frequencies among lean Caucasian adults and patients of the JHWMC with varying degrees of obesity

Degree of obesity	*n*	TNF-α-308			TNF-α-238		
		TNF1/TNF1 (normal)	TNF1/TNF2 (heterozygote)	TNF2/TNF2 (homozygote)	TNF1/TNF1 (normal)	TNF1/TNF2 (heterozygote)	TNF2/TNF2 (homozygote)
Grade 0 (BMI <25 kg/m^2)	208	153 (73.2)	48 (22.5)	9 (4.3)	189 (90.4)	19 (9.1)	1 (0.5)
Grade 1 (BMI 25–29.9 kg/m^2)	63	42 (66.7)	17 (27.0)	4 (6.3)	57 (90.5)	5 (7.9)	1 (1.6)
Grade 2 (BMI 30–40 kg/m^2)	192	129 (67.2)	58 (30.2)	5 (2.6)	165 (85.9)	27 (14.1)	—
Grade 3 (BMI >40 kg/m^2)	169	124 (73.4%)	41 (24.3)	4 (2.4)	154 (91.1)	15 (8.9)	—

Data are *n* (%). Obesity is classified according to Weinsier (14). Data for grade 0 are taken from a healthy lean free-living population in east Baltimore.

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