

Association between TNF- α promoter G-308A and G-238A polymorphisms and obesity

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Abstract Tumor necrosis factor- α (TNF- α), an adipokine, is produced in adipocytes, and the elevation of its levels has been linked to obesity and insulin resistance in some population. In this study the relationship between TNF- α promoter gene polymorphism and obesity in an Iranian population has been studied. Subjects were randomly selected from Tehran Cohort Lipid and Glucose Study. Adult participants placed in three groups according to their body mass index (BMI): BMI < 25, 25 \leq BMI < 30, BMI \geq 30 and under-18 subjects placed in two groups, under 85th percentile BMI and above 85th percentile. Finally, 244 persons were selected for G-308A and G-238A polymorphisms analysis. The FBS, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride, cholesterol levels and blood pressure and HOMA of all subjects were measured. The polymorphism -308 and -238 were revealed by restriction fragment length polymorphism (RFLP; NCOI and MSPI) after the promoter site was amplified by PCR. The allele frequency of TNF- α polymorphism was in the Hardy-Weinberg equilibrium. There was no relation between BMI and the frequency of this allele. The fact that there is no association between G-308A and G-238A TNF- α promoter polymorphisms and obesity probably shows

that it is not an important risk factor for obesity and consequently for cardiovascular disease.

Keywords Obesity · TNF- α · BMI · Polymorphism

Introduction

There has been an increase of prevalence of obesity which is associated with several adverse health problems, including type 2 diabetes, hyperlipidemia and hypertension. The role obesity plays in the development of obesity-related disorders is complex and probably due to an interaction of genetic, nutritional and metabolic factors [1, 2]. A number of candidate genes have been implicated in the pathogenesis of obesity in humans. Tumor necrosis factor- α (TNF- α) is primarily secreted in adipocytes, and elevated levels of this cytokine have been linked to obesity and insulin resistance. TNF- α messenger RNA levels in skeletal muscle and adipose tissue of humans are positively correlated with body mass index (BMI), fat mass, and insulinemia and inversely correlated with lipoprotein lipase activity [3–5]. The gene which is coded human TNF- α is located on the short arm of chromosome 6 [6]. Recently, attention has been focused on two nucleotide polymorphisms identified in the promoter region of the TNF- α gene: G \rightarrow A substitutions at -308 and -238 respectively. In vitro experiments have demonstrated that the polymorphism -308 which is identified by *NcoI* restriction fragment length polymorphism (RFLP), increases transcriptional activation of the TNF- α gene [7]. The potential functional impact of the polymorphism at position -238 of the TNF- α gene has not yet been investigated in in vitro expression studies. However, the variant is located within a putative Y box, which is a regulatory motif typical of the promoter region of major histocompatibility complex class II

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genes [8]. The purpose of the present study is to determine whether the two mentioned TNF- α promoter polymorphisms are associated with obesity in Iranian population.

Materials and methods

Subjects were randomly selected from Cohort Tehran Lipid and Glucose Study and adults were classified in three groups according to their BMI: BMI < 25, $25 \leq$ BMI < 30, BMI \geq 30. As for –308 polymorphism, 202 adults (81 men, 121 women) and 42 under-18 subjects (21 boys, 21 girls) were placed in two groups according to their BMI: under and above 85th percentile as far as age and sex are concerned. And as for –238 polymorphism, 239 (78 men, 120 women) and 41 under-18 subjects (17 boys, 24 girls) were classified in two groups according to their BMI: under and above 85th percentile as far as age and sex are concerned. BMI, waist to hip ratio, fat mass, and plasma concentration of glucose, serum levels of triglyceride, total cholesterol, and high-density lipoprotein (HDL) cholesterol were analyzed.

In order to genotype the subjects, the standard salting-out Proteinase K method was used to extract the germinal DNA. Both TNF- α promoter polymorphisms were detected using the RFLP method after amplification of the sequences by Polymerase Chain Reaction (PCR).

A 107-bp fragment encompassing the –308 variant site was amplified from genomic DNA by PCR using standard reagents, technique, and primers (5-AGG CAATAG GTTTTGAGGGGCCAT-3) and (5-TCCTCCCTGCTCCG ATTCCG-3) for the –308 variant and (5-ATCTGGA GGAAGCGGTAGTG-3) and (5-AGAAGACCCCCCTCG GAACC-3) for the –238 variant allele. To detect –308 variant alleles the PCR product was digested with *Nco*I at 37°C overnight, leaving a 107 bp fragment when the variant was present and products of 87 and 20 bp fragments for the normal allele visualized on 3% agarose gel. To detect the –238 variant alleles, the PCR product was digested with *Msp*I at 37°C for an hour, leaving a 152 bp fragment when the variant was present and products of 133 and 19 bp fragments for the normal allele visualized on 3% agarose gel.

Statistical analysis

Normality distribution of the data checked by the KS test. The mean levels of all normally-distributed variants were tested by ANOVA and the mean levels of all abnormally-distributed variants were tested by nonparametric Kruskal–Wallis H test and the data were presented as means \pm SD of the differences. All analyses were conducted using the SPSS 16 software.

Results

Genotype frequencies in all groups were in accordance with the Hardy–Weinberg equilibrium. The distribution of TNF- α genotype for –308 site in under-18 subjects was as follows: GG (81%), GA (16.6%), and AA (2.4%). In adults the following results were produced: GG (86.6%), GA (12.4%), and AA (1%). As for the –238 site in under-18 subjects the results were: GG (80.5%), GA (19.5%), and AA (0%), and in adults the following results were recorded: GG (79.3%), GA (19.2%), and AA (1.5%). Clinical and biochemical data of participants, when classified in accordance to G/A polymorphisms at positions –308 and –238 of the TNF- α gene promoter, are presented in Table 1 (–308) and Table 2 (–238). To examine whether the variant alleles were more common in patients with obesity, adults were classified in three groups according to their BMI: BMI < 25, $25 \leq$ BMI < 30, BMI \geq 30 and under-18 subjects were classified in two groups according to their BMI: under 85th percentile BMI and above 85th percentile as far as age and sex are concerned—Table 3 (–308) and Table 4 (–238).

Discussion

The purpose of the present study is to determine whether the G \rightarrow A polymorphisms of the TNF- α gene promoter are associated with obesity and anthropometric parameters in Cohort Tehran Lipid and Glucose study. There are no associations between G-308A and G-238A TNF- α promoter polymorphisms and increases in BMI. TNF- α is one of the cytokines secreted from adipose tissue in obesity; it can be a candidate gene to inspect obesity and related diseases. Obesity is a complex metabolic disorder with a strong genetic component and there are many candidate genes for obesity and its related phenotypes. Most of these genes are candidate because their mutations cause rare genetic syndromes affecting adipocytes differentiation [9]. Recent interest has focused on the role of the TNF- α gene in insulin resistance and obesity. We therefore examined the association between the polymorphism of TNF- α and obesity without metabolic disease. It came as another study reported an association between this genetic marker and excessive fat accumulation in women [10].

Moreover, a potential role for the TNF- α locus in obesity is also supported by a linkage study, which reported a relationship between the TNF- α locus and various global measures of adiposity in Pima Indians and European Caucasians [11, 12]. The functional role of the TNF- α G-308A polymorphism is currently unclear. However, it is located within a consensus sequence for the transcription factor AP-2 in the promoter region of the gene [13].

Table 1 Clinical and biochemical data of subjects when classified in accordance to the genotype of the G/A polymorphisms at positions –308 of the TNF- α gene promoter

Trait	Under 18				Above 18			
	GG (34)	GA (7)	AA (1)	<i>P</i>	GG (175)	GA (25)	AA (2)	<i>P</i>
Age (years)	12.51 \pm 2.8	14.21 \pm 2.25	10.5	0.240	43.34 \pm 15.62	40.96 \pm 16.04	51.25 \pm 1.76	0.591
Sex (M/F)	17/17	3/4	1/0	0.572	70/105	10/15	1/1	0.960
BMI (kg/m ²)	19.73 \pm 3.84	19.91 \pm 2.07	26.71	0.179	26.65 \pm 4.55	27.33 \pm 4.82	32.18 \pm 2.7	0.196
FBS (mg/dl)	86.45 \pm 5.62	87.35 \pm 6.51	73.5	0.088	89.00 \pm 22.18	90.50 \pm 8.66	87.5 \pm 7.07	0.865
HOMA	10.1 \pm 5.80	9.45 \pm 2.87	12.15	0.889	12.13 \pm 281.39	12.36 \pm 8.83	10.15 \pm 0.02	0.683
CHO (mg/dl)	158.57 \pm 2.44	169.79 \pm 22.38	178.00	0.421	196.57 \pm 42.73	197.00 \pm 47.91	208.75 \pm 44.90	0.925
HDL-C (mg/dl)	44.30 \pm 8.60	43 \pm 7.34	29	0.208	42.26 \pm 9.63	42.76 \pm 9.20	31.75 \pm 13.78	0.295
LDL-C (mg/dl)	95.96 \pm 20.75	109.69 \pm 22.87	113.40	0.241	124.53 \pm 3.46	118.52 \pm 43.83	128.75 \pm 40.09	0.723
TG (mg/dl)	94.44 \pm 39.00	85.85 \pm 21.80	178.50	0.074	128.50 \pm 81.07	142.50 \pm 154.54	241.25 \pm 44.19	0.053
WHR	0.83 \pm 0.05	0.82 \pm 0.07	0.98	0.053	0.87 \pm 0.08	0.86 \pm 0.09	0.95 \pm 0.06	0.344
SBP (mmHg)	101.31 \pm 8.46	104.21 \pm 5.12	97	0.579	114.50 \pm 17.26	116.50 \pm 11.02	131.25 \pm 22.27	0.426
DBP (mmHg)	67.22 \pm 5.85	71.21 \pm 4.87	57.5	0.059	74.36 \pm 8.09	75.34 \pm 8.98	83 \pm 16.26	0.303

Table 2 Clinical and biochemical data subjects when classified in accordance to the genotype of the G/A polymorphisms at positions –238 of the TNF- α gene promoter

Trait	Under 18				Above 18			
	GG (33)	GA (8)	AA (0)	<i>P</i>	GG (175)	GA (38)	AA (3)	<i>P</i>
Age (years)	13.24 \pm 2.96	12.62 \pm 2.21	–	0.585	42.18 \pm 15.01	39.93 \pm 16.50	42.50 \pm 21.07	0.585
Sex (M/F)	13/20	4/4	–	0.589	97/60	17/21	½	0.745
BMI (kg/m ²)	20.12 \pm 3.58	19.08 \pm 2.88	–	0.454	26.40 \pm 4.37	26.84 \pm 4.71	23.37 \pm 3.12	0.415
FBS (mg/dl)	87.63 \pm 6.24	84.50 \pm 6.83	–	0.218	89 \pm 41.62	87.25 \pm 23.13	89 \pm 13.47	0.766
HOMA	10.79 \pm 6.42	10.28 \pm 3.03	–	0.828	12.13 \pm 8.85	10.15 \pm 9.18	10.19 \pm 1.04	0.158
CHO (mg/dl)	162.18 \pm 23.99	146.38 \pm 26.83	–	0.110	199.08 \pm 40.46	183.78 \pm 37.69	214.17 \pm 92.59	0.090
HDL-C (mg/dl)	43.83 \pm 7.62	43.12 \pm 7.80	–	0.816	42.85 \pm 9.03	42.18 \pm 10.25	41.66 \pm 4.19	0.904
LDL-C (mg/dl)	100.08 \pm 19.52	87.30 \pm 27.62	–	0.134	125.92 \pm 34.11	112.62 \pm 32.22	148.07 \pm 83.89	0.052
TG (mg/dl)	94.30 \pm 37.85	80 \pm 30.23	–	0.328	123.00 \pm 97.55	145.25 \pm 57.70	103.50 \pm 56.73	0.519
WHR	0.82 \pm 0.04	0.8 \pm 0.11	–	0.451	0.87 \pm 0.08	0.87 \pm 0.1	0.83 \pm 0.11	0.780
SBP (mmHg)	100.42 \pm 8.51	104.56 \pm 7.72	–	0.217	115.14 \pm 15.20	115.86 \pm 15.04	103 \pm 21.65	0.757
DBP (mmHg)	66.33 \pm 5.17	70.12 \pm 6.28	–	0.082	73.88 \pm 7.80	75.46 \pm 7.71	70.83 \pm 6.65	0.407

The G-308A mutation in the promoter region of TNF- α acts in vitro as a much stronger transcriptional activator than the wild-type TNF- α and it is suggested that a higher transcriptional activity would result in higher TNF- α concentrations followed by decreased insulin sensitivity [7]. So this variant could play a role in the development of obesity and related phenotypes [14]. Interestingly, obesity is known to be associated with increased levels of TNF- α mRNA and protein in fat tissue both in humans and in genetically obese or diabetic rodents [15]. Moreover, a study has shown that the TNF- α mRNA level in adipose tissue are significantly correlated with the percentage of body fat in human obese subjects [5]. A recent study, while

confirming linkage between the TNF- α locus and obesity in families of French-Canadian origin, has also found a significant association between this locus and high blood pressure [16]. A significant positive correlation between serum TNF- α concentration and systolic blood pressure has also been reported among individuals of an isolated native Canadian population over a wide range of adiposity [17]. An Italian population study suggests that the G-308A mutation of the TNF- α gene is unlikely to play an important role in the development of obesity and its related metabolic abnormalities such as insulin resistance and dyslipidemia [18]. As for the G-238A variant, previous results are also inconsistent. A study of non-diabetic

Table 3 Clinical and biochemical data of subjects when classified in accordance to BMI for polymorphisms at positions –308 of the TNF- α gene promoter

Trait	Under 18			Above 18			
	Grade 1 BMI < 85% (n = 26)	Grade 2 BMI > 85% (n = 16)	P	Grade 1 BMI < 25 (n = 75)	Grade 2 25 ≤ BMI < 30 (n = 81)	Grade 3 BMI ≥ 30 (n = 46)	P
GG	21(80.8%)	13(81.2%)	–	67(89.3%)	71(87.7%)	37(80.4%)	–
GA	5(19.2%)	2(12.51%)	–	8(10.7%)	10(12.3%)	7(15.2%)	–
AA	–	1(6.2%)	–	–	–	2(4.3%)	–
Age (years)	12.88 ± 2.76	12.53 ± 2.81	0.692	36.37 ± 16.65	45.56 ± 14.36	49.85 ± 11.46	0.000
BMI (kg/m ²)	17.64 ± 2.03	2.60 ± 23.63	0.000	22.27 ± 1.93	27.49 ± 1.42	32.90 ± 3.37	0.000
FBS (mg/dl)	86.34 ± 6.21	86.34 ± 6.21	0.948	87 ± 21.07	91 ± 16.66	91.50 ± 26.02	0.001
HOMA	9.94 ± 4.80	10.22 ± 6.27	0.870	12.34 ± 10.18	12.47 ± 413.40	10.31 ± 5.78	0.071
CHO (mg/dl)	155.9 ± 22.13	169.03 ± 25.49	0.086	181.50 ± 42.11	201.32 ± 43.50	213.51 ± 36.66	0.000
HDL-C (mg/dl)	43.19 ± 7.74	44.59 ± 9.93	0.612	42.53 ± 9.18	36.98 ± 8.37	45.62 ± 11.34	0.005
LDL-C (mg/dl)	96.12 ± 19.71	102.79 ± 23.82	0.332	113.77 ± 36.47	125.82 ± 34.53	136.71 ± 32.79	0.002
TG (mg/dl)	86.78 ± 27.92	108.37 ± 49.33	0.077	108 ± 67.10	154.50 ± 117.21	135.25 ± 70.22	0.000
WHR	0.81 ± 0.05	0.86 ± 0.06	0.008	0.83 ± 0.07	0.89 ± 0.08	0.9 ± 0.07	0.000
SBP (mmHg)	100.78 ± 8.14	103.15 ± 7.65	0.355	111.50 ± 11.78	116 ± 18	119 ± 17.75	0.000
DBP (mmHg)	67.51 ± 6.36	67.87 ± 5.52	0.854	71.57 ± 7.23	75.51 ± 8.11	77.79 ± 8.69	0.000

Table 4 Clinical and biochemical data of subjects when classified in accordance to BMI for polymorphisms at positions –238 of the TNF- α gene promoter

Trait	Under 18			Above 18			
	Grade 1 BMI < 85% (n = 26)	Grade 2 BMI > 85% (n = 16)	P	Grade 1 BMI < 25 (n = 75)	Grade 2 25 ≤ BMI < 30 (n = 81)	Grade 3 BMI ≥ 30 (n = 46)	P
GG	21(80.8%)	13(80%)	–	65(81.2%)	60(77.9%)	32(78%)	–
GA	5(19.2%)	2(20%)	–	13(16.2%)	16(20.8%)	9(22%)	–
AA	–	–	–	2(2.5%)	1(1.3%)	–	–
Age (years)	13.50 ± 2.75	12.46 ± 2.90	0.264	38 ± 16.61	42.42 ± 13.85	50.24 ± 12.16	0.000
BMI (kg/m ²)	18.05 ± 2.28	23.15 ± 2.63	0.000	22.27 ± 1.89	27.50 ± 1.43	32.57 ± 3.18	0.000
FBS (mg/dl)	86.86 ± 7.21	87.30 ± 4.88	0.837	87 ± 54	89 ± 26.03	92 ± 15.04	0.011
HOMA	10.97 ± 5.69	10.21 ± 6.39	0.696	11.32 ± 8.92	12.14 ± 9.73	10.44 ± 6.73	0.385
CHO (mg/dl)	154.36 ± 22.76	167.30 ± 27.41	0.112	186.30 ± 41.59	200.50 ± 42.15	208.26 ± 34.04	0.011
HDL-C (mg/dl)	42.19 ± 6.31	46.30 ± 8.99	0.094	43.35 ± 8.64	40.66 ± 8.26	45.30 ± 11.39	0.023
LDL-C (mg/dl)	95.35 ± 19.41	101.44 ± 25.09	0.391	117.18 ± 36.66	126.27 ± 34.73	131.60 ± 30.38	0.071
TG (mg/dl)	87.8 ± 29.78	97.93 ± 46.60	0.4	110.50 ± 69.11	144.50 ± 111.46	135 ± 69.81	0.000
WHR	0.80 ± 0.06	0.85 ± 0.05	0.012	0.83 ± 0.08	0.89 ± 0.08	0.89 ± 0.08	0.000
SBP (mmHg)	99.4 ± 8.42	104.4 ± 7.71	0.067	111.75 ± 12.67	115 ± 14.84	118.5 ± 17.34	0.001
DBP (mmHg)	66.94 ± 5.68	67.30 ± 5.45	0.845	72.11 ± 7.22	74.13 ± 7.54	78.09 ± 7.87	0.000

relatives and control subjects in Britain showed that the –238 variant was associated with increased insulin sensitivity as estimated by two independent methods [19]. A recent meta-analysis (2,106 cases and 2,920 controls) of the rs361525 (G-238A) variant has not detected a significant association with type 2 diabetes [20], nor has the study

of Caucasian and African American subjects [21]. Be'ne'dicte Fontaine-Bisson and co-workers suggest that the –238G → A and –308G → A polymorphisms of TNF- α alter circulating free fatty acids and insulin resistance in obese subjects with type 2 diabetes mellitus [22].

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