

The Investigation of Obesity Susceptibility with IL-4 Gene Intron 3 VNTR and IL-6 Gene -597G/A Polymorphisms in a Turkish Population

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ABSTRACT Obesity results in a proinflammatory state starting within the metabolic cells as adipocyte, monocyte. The bioactive system, including cytokines as IL-4 and IL-6, contributes to the pathogenesis of conditions associated with obesity and favors inflammation. This study aimed to investigate the role of IL-4 gene intron 3 VNTR and IL-6 gene -597G/A polymorphisms in the pathogenesis of obesity. The study included 127 patients with obesity (BMI>30kg/m²) and 110 healthy controls. Genomic DNA was isolated and genotyped using polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) analysis for IL-4 gene and IL-6 gene polymorphisms. There was no statistical significant difference in the allele and genotype frequencies of both genes polymorphism in obesity patients and control groups (p>0.05). Additionally, there was no statistical significant difference in the combine genotype analysis of IL-4 gene intron 3 VNTR and IL-6 gene -597G/A polymorphism (p>0.05). These findings showed that there are not any associations of IL-4 gene intron 3 VNTR and IL-6 gene -597G/A polymorphisms with susceptibility of a person for development of obesity. This is the first report that investigates the relationships between susceptibility of obesity and IL-4 gene intron 3 VNTR, IL-6 gene -597G/A polymorphisms.

INTRODUCTION

Obesity presents as a disorder of the mechanisms of energy balance that caused the genes, environmental factors and interaction between genes and environment (Nirmala et al. 2008; Bircan 2009). And, obesity results in a proinflammatory state starting within the metabolic cells as adipocyte, monocyte (El-Wakkad et al. 2013). It is emphasized that obesity was a complex, multifactorial condition, and many endogenous genetic, endocrine and inflammatory pathways and environmental factors are involved in the development of obesity and obesity related diseases (de Mutsert et al. 2013). The World Health Organization defines overweight as a body mass index (BMI) of 25 or more and obe-

sity as a BMI of 30 or more (Noria et al. 2013). In the literature, the prevalence of obesity states almost reach worldwide epidemic (Eklund et al. 2006; Nirmala et al. 2008; Brunkwall et al. 2013). Obesity is associated type II diabetes, hypertension, coronary artery disease, stroke, musculoskeletal disease, obstructive sleep apnea and many forms of cancer (Bell et al. 2005; Ha et al. 2008).

The excess weight may come from muscles, bone, fat, body water. Obesity specially refers to having an abnormally high proportion of total body fat (Afridi and Khan 2007). Human fat consists of two types of adipose tissue as white and brown. White adipose tissue comprises the majority of the adipose tissue in both obese and normal weight adults. The cells of the adipose tissue are called adipocyte. Adipocytes are endocrine cells that produce a wide range of hormones, growth factors, and cytokines. Cytokines generated by adipose tissue include interleukin (IL)-4, IL-6, IL-8, IL-1 β , tumor necrosis factor- α (TNF- α) and vascular endothelial growth fac-

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tor (VEGF) (Gilbert and Slingerland 2013). In the obese individuals, inflammatory cytokines are observed increased especially in the presence of systemic inflammation related to risk of cardiovascular disease and diabetes (Ha et al. 2008). It has been stated that weight gain is result of lipid accumulation and disruption balance of systemic cell signaling by adipocyte stress-factors (adipokines and cytokines) (Weisberg et al. 2003; Jay et al. 2012). The increase of pre-adipocytes in adipose tissue of obese individuals leads to a significant increase in local and circulating levels of pro-inflammatory cytokines (Gilbert and Slingerland 2013). This bioactive system contributes to the pathogenesis of conditions associated with obesity and favors inflammation (Weisberg et al. 2003). Therefore, it is important that investigate associations between obesity and cytokines that are role in inflammatory process. Based on these findings, the researchers investigated whether the *IL-4* gene intron 3 VNTR polymorphism and *IL-6* gene promoter -597G/A polymorphism might be involved in the pathogenesis of obesity.

METHODOLOGY

Study Population

This study included 127 obesity patients (93 women, 34 men) and 110 controls (62 women, 43 men) provided from the department of Internal Medicine, Gazi Osmanpaşa University in Tokat, Turkey. Informed consent was in accordance with the study protocol, approved by the ethics committee of the Medical Faculty. All patients and controls signed a written consent form after being informed about the details of the study. A complete clinical evaluation was done for all patients. All the individuals in the control group were healthy and were selected by excluding the diagnosis of obesity. All participants, obesity patients and healthy controls were Turkish origin.

DNA Extraction and Genotyping

DNA was extracted from 2 mL venous blood according to kit procedure (Sigma, USA) and stored at -20 °C. To detect 70 bp VNTR polymorphism of *IL-4* gene, polymerase chain reaction (PCR) assay as described by Mout et al. (Mout et al. 1991). PCR was performed with a 25 µL reaction mixture containing 50 ng DNA,

0.8 L M of each primer, 200 IM of each dNTP, 2.5 mM MgCl₂, 0.5 U Taq polymerase, 10× KCl buffer (MBI, Fermentas). Amplification was carried out using primers 52 -AGGCTGAAAGGG GGAAAGC-32, 52 -CTG TTCACCTCAACT GCTCC-32 with initial denaturation at 95 °C for 5 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 45 s, extension at 72 °C for 1 min and final extension at 72 °C for 10 min. The PCR products were visualized on 3% agarose gel stained with ethidium bromide. PCR product was of 183 bp for P1 allele and 253 bp for P2 allele. The analysis of the -597G/A polymorphism of the *IL-6* gene (rs1800797) was performed using the following primers 5'-GGAGACGCCTTGAAGTAAC TGC-3' and 5'-GAGTTTCCTCTGACTCC ATC-3' to generate a PCR fragment of 163 bp (Snoussi et al. 2005). Genotyping was resolved by PCR product digestion with the *FokI* enzyme. The rare *IL-6* A allele has a *FokI* restriction cutting site. The resulting fragments have the size of 116 and 47 bp. In order to validate the accuracy and reproducibility of this method, each PCR reaction included internal controls for each genotype. Second PCR was performed to confirm samples which results are not clear. Also, to confirm the accuracy of the genotyping, repeated analysis was performed on randomly selected samples and discrepancies were not found.

Statistical Analysis

Analysis of the data was performed using the computer software SPSS 16.0 (Chicago, IL, USA) and OpenEpi Info software package program (www.openepi.com). Continuous data were given as mean ± SD (standard deviation). The frequencies of the alleles and genotypes (Hardy-Weinberg equilibrium) in patients and controls were compared with χ^2 analysis. 95% confidence intervals (CIs) were calculated. p value less than 0.05 (two-tailed) were regarded as statistically significant.

RESULTS

Table 1 shows demographic variables and baseline characteristics of patients. The mean age and BMI were 44.78±1.33 and 34.27±1.46 in patients group respectively. While the mean age of BMI>35 kg/m² and BMI<35 kg/m² patients were 45.02±1.69 and 44.51±2.12 in obesity patients respectively, the mean BMI of BMI>35

kg/m² and BMI<35 kg/m² patients were 38.51±0.50 and 31.98±0.19 in obesity patients respectively. Patients and controls were genotyped for both *IL-4* intron 3 VNTR and *IL-6* gene promoter -597G/A polymorphisms. The distribution of *IL-4* intron 3 VNTR and the *IL-6* -597G/A polymorphisms genotypes and alleles of the patients and control groups are presented in Table 2 and 3. It is determined that there was no statistical significant difference in the allele and genotype frequencies of both genes polymorphism in patients and control groups ($p>0.05$) (Tables 2, 3). There was no statistical significant difference in the combine genotype analysis of *IL-4* and *IL-6* genes ($p>0.05$) (Table 4).

Table 1: Demographic variables and baseline characteristics of the obesity patients

Characteristic	Mean
Age of patients (n:127)	44.78±1.33
Body Mass Index	34.27±1.46
Age of BMI>35 kg/m ² patients	45.02±1.69
Age of BMI<35 kg/m ² patients	44.51±2.12
BMI of BMI>35 kg/m ² patients	38.51±0.50
BMI of BMI<35 kg/m ² patients	31.98±0.19

DISCUSSION

Obesity results in a proinflammatory state starting in the metabolic cells and also recruiting

Table 4: The distribution of composite genotypes *IL-4* and *IL-6* gene polymorphisms between patients and control groups

Composite genotypes	Patients (n)	Controls (n)	Total	p value
<i>P1P1-AA</i>	0	0	0	0.61
<i>P1P1-GA</i>	3	1	4	
<i>P1P1-GG</i>	1	4	5	
<i>P1P2-AA</i>	1	0	1	
<i>P1P2-GA</i>	6	5	11	
<i>P1P2-GG</i>	16	16	32	
<i>P2P2-AA</i>	7	3	10	
<i>P2P2-GA</i>	25	19	44	
<i>P2P2-GG</i>	68	62	130	

immune cells with the consequent release of inflammatory cytokines. The link between obesity and inflammation has been determinate that proinflammatory cytokines are over-expressed in obesity. Adipose tissue is a heterogeneous mix of adipocytes, stromal pre-adipocytes, immune cells, and endothelium, and it can responds rapidly and dynamically to alterations in nutrient (Emanuella et al. 2012). *IL-6* has an important role in the regulation of whole-body energy homeostasis and inflammation. *IL-6* receptor is also expressed in several regions of the brain, such as the hypothalamus, in which it controls appetite and energy intake (Stenlöf et al. 2003; Emanuella et al. 2012). Also, it is emphasized in different studies that *IL-4*, like *IL-6*, is associ-

Table 2: Distribution of *IL-4* gene 70bp VNTR polymorphism and allele frequencies between obesity patients and controls

	Patients (%)	Controls (%)	χ^2	p value	OR (95%CI)
<i>Genotype</i>					
<i>P₁P₁</i>	4 (3.1)	5 (4.5)	0.37	0.82	
<i>P₁P₂</i>	23 (18.1)	21 (19)			
<i>P₂P₂</i>	100 (78.7)	84 (76.3)			
<i>P₁P₁+P₁P₂:P₂P₂</i>	27:100	26:84	0.07	0.77	0.87 (0.47-1.61)
<i>P₁P₂+P₂P₂:P₁P₁</i>	123:4	105:5		0.82	1.46 (0.36-6.26)
<i>Allele Frequency</i>					
<i>P₁</i>	31 (12.2)	31 (14)	0.3	0.54	0.84 (0.49-1.45)
<i>P₂</i>	223 (87.7)	189 (85.9)			

Table 3: Distribution of *IL-6* promoter -597G/A polymorphisms and allele frequencies between obesity patients and controls

	Patients (%)	Controls (%)	χ^2	p value	OR (95%CI)
<i>Genotype</i>					
<i>AA</i>	8 (6.2)	3 (2.7)	2.49	0.28	
<i>GA</i>	34 (26.7)	25 (22.7)			
<i>GG</i>	85 (66.9)	82 (74.5)			
<i>AA+GA:GG</i>	42:85	28:82	1.2	0.25	1.44 (0.82-2.56)
<i>GG+GA:AA</i>	119:8	107:3		0.32	0.41(0.08-1.57)
<i>Allele Frequency</i>					
<i>A</i>	50 (19.6)	31 (14)	2.6	0.10	1.49 (0.91-2.45)
<i>G</i>	204 (80.3)	189 (85.9)			

ated with obesity (Wernstedt et al. 2004; Cardellini et al. 2005; Qi et al. 2007; Ha et al. 2008; El-Wakkad et al. 2013; Ladefoged et al. 2013; Lucas et al. 2013).

In this study, it was analyzed associations between *IL-4* intron 3 VNTR and the *IL-6* -597G/A polymorphisms and obesity. And, the researchers demonstrated that these polymorphisms are not associated with obesity susceptibility in a Turkish population ($p>0.05$). Although there is not any previous study that these polymorphisms had analyzed together in terms of obesity risk in the literature, there are some studies that are investigating associations between the other polymorphism and proteins of the *IL-4* and *IL-6* and obesity susceptibility. Ha et al. (2008) has reported that *IL-4* receptor rs180275 polymorphism was associated with an increase in BMI in Korean population while no association was found between rs1805010 and obesity (Ha et al. 2008). El-Wakkad et al. (2013) found an increase between *IL-4* and *IL-5* and obese adolescent girls with central obesity (El-Wakkad et al. 2013). In the Sobti et al. (2010) study, it is showed that no significant association was found *IL-4* intron 3 VNTR polymorphism and central type obesity (Sobti et al. 2010). There are concordance between our results and Sobti et al.'s study results.

Lucas et al. (2013) has stated that plasma levels of *IL-6* are significant elevated in obese pre-diabetic individuals (Lucas et al. 2013). Ladefoged et al. (2013) denoted that levels of *IL-6* are increased in obesity (Ladefoged et al. 2013). It is known that obesity increased to risk development of type 2 diabetes (Cardellini et al. 2005; Ha et al. 2008). In the Cardellini et al. (2005) study, it is stated that *IL-6* gene -174 G/C polymorphism was associated with insulin sensitivity. Also, Cardellini et al. (2005) has denoted that *IL-6* gene -174 G/C polymorphism are influence on the levels of *IL-6* expression in adipose tissue (Cardellini et al. 2005). It is important information that *IL-6* gene polymorphisms leads to alterations on levels of expression in especially fat tissue. This information can mean that obese individuals will be affected more than normal individuals from these gene polymorphisms. Wernstedt et al. (2004) has expressed that *IL-6* -174 G/C polymorphism was associated with overweight (Wernstedt et al. 2004). In a meta analysis of Qi et al. (2007), the seven polymorphisms of the *IL6* gene, including the *IL-6* -597G/A polymorphism, is significantly as-

sociated with adiposity and greater waist circumference (Qi et al. 2007). There was no concordance between our results and Qi et al. (2007) study results. Phillips et al. (2010) investigated to associations between *IL-6* -597G/A polymorphism and the metabolic syndrome that consist from any diseases as obesity, insulin resistance, hypertension. And, no differences were noted for the *IL-6* -597G/A polymorphism (Phillips et al. 2010). Slattery et al. (2008) stated that mean level of BMI was not associated with any of the *IL6* markers as the *IL-6* -597G/A polymorphism (Slattery et al. 2008). It seen that there was a concordance between our results and the results of Phillips et al. (2010) study and Slattery et al. (2008) study.

CONCLUSION

These findings showed that there are not any associations of *IL-4* gene 70bp VNTR and *IL-6* gene -597G/A polymorphism with susceptibility of a person for development of obesity ($p>0.05$). The results of this study are important because of this is the first report that investigates the relationships between susceptibility of obesity and combined effect of *IL-4* gene intron 3 VNTR, *IL-6* gene promoter -597G/A polymorphisms. Additional analyses with larger populations are required to confirm these findings in different study populations.

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