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Clinical Study

Investigation of Associations between Obesity and *LEP* G2548A and *LEPR* 668A/G Polymorphisms in a Turkish Population

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Objective. Obesity is a complex heterogeneous disease that is caused by genes, environmental factors, and the interaction between the two. The leptin (*LEP*) and leptin receptor (*LEPR*) genes have been evaluated for polymorphisms that could potentially be related to the pathophysiology of obesity and its complications. The aim of this study was to investigate the role of *LEP* G2548A and *LEPR* 668A/G polymorphisms in the pathogenesis of obesity. *Subjects.* The study included 127 patients with obesity and 105 healthy controls. Polymerase chain reaction and restriction fragment length analysis for *LEP* G2548A and *LEPR* 668A/G polymorphisms were applied. *Results.* There was no statistically significant difference in the genotype frequencies of the *LEP* gene polymorphism between patients and control groups ($P > 0.05$). We found a difference in the *LEPR* genotypes between patients and controls, but this was not statistically significant ($P = 0.05$). Additionally, we found an increased risk of obesity in the *LEP/LEPR* GG/GG combined genotype ($P < 0.05$). *Conclusion.* Our findings indicate that the *LEP* G2548A polymorphism is not a relevant obesity marker and that the *LEPR* 668A/G polymorphism may be related to obesity in a Turkish population. Further researches with larger patient population are necessary to ascertain the implications of *LEP* and *LEPR* polymorphisms in obesity.

1. Introduction

Obesity is a complex heterogeneous disease that is caused by genes, environmental factors, and the interaction between the two [1]. Obesity is also a multifactorial condition, and many endocrine and inflammatory pathways are involved in its development and in obesity-related diseases [2]. Excess weight in obesity may come from muscles, bone, fat, and/or body water, but obesity specifically refers to having an abnormally high proportion of total body fat [3]. The World Health Organization defines "overweight" as a body mass index (BMI) of 25 or more and "obesity" as a BMI of 30 or more [4]. The prevalence of obesity has been stated as being near epidemic size [1–3, 5–7], and obesity has been associated with type II diabetes, hypertension, coronary artery disease, stroke, and many forms of cancer [8, 9]. Therefore, it is important that the underlying pathophysiology of obesity-related diseases is understood. Obesity results from the combined effects of genes, lifestyle, and the interactions of these factors [10], and both familial and nonfamilial factors play an important role in its development [1]. A genetic predisposition to obesity has been reported as a major risk factor for individuals [7].

With the increasing prevalence of obesity, studies on candidate genes for obesity have increased. Most obesity-predisposing genes encode the molecular components of physiological systems related to energy balance [11]. Leptin is a protein product of the *ob* gene and is expressed and secreted by adipose tissue in amounts proportional to the body weight content; studies on its receptor have greatly advanced the comprehension of the mechanism for regulating body weight and energy homeostasis. The lipostat system,

Table 1: Demographic variables and baseline characteristics of the patients and controls.

Characteristic	Mean
Age of patients (*n*: 127)	44.86 ± 1.51
Age of controls (*n*: 105)	34.25 ± 15.43
Body mass index (BMI) of patients	35.45 ± 4.56
BMI of controls	21.57 ± 1.89
Age of BMI > 35 kg/m^2 patients	45.01 ± 1.38
Age of BMI < 35 kg/m^2 patients	44.7 ± 1.65
BMI of BMI > 35 kg/m^2 patients	38.68 ± 3.11
BMI of BMI < 35 kg/m^2 patients	32.06 ± 1.56

mediated by leptin and its hypothalamic receptor, reduces food intake and increases thermogenesis [10, 12]. The leptin (*LEP*) and leptin receptor (*LEPR*) genes have been evaluated for polymorphisms that could potentially be related to the pathophysiology of obesity and its complications [11]. Although the polymorphisms in these genes have been evaluated [13–15], the association of these polymorphisms with obesity is still controversial. Therefore, we investigated whether the *LEP* gene G2548A polymorphism and *LEPR* gene 668A/G (Q223R) polymorphism might be involved in the pathogenesis of obesity.

2. Materials and Methods

2.1. Study Design. This study included 127 obesity patients (93 women, 34 men) and 105 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, and all patients and controls signed a written consent form. All patients received a complete clinical evaluation, and all individuals in the control group were healthy and were selected by excluding the diagnosis of obesity. All participants, obesity patients and healthy controls, were of Turkish origin. The ethics committee of the Medical Faculty gave approval for this study.

2.2. DNA Extraction and Genotyping. Genomic DNA was isolated from white blood cells by a kit procedure (Invitrogen Life Technologies, Carlsbad, CA, USA) and stored at −20°C. *LEP* G2548A and *LEPR* 668A/G polymorphisms were analyzed by polymerase chain reaction based restriction fragment length polymorphism (PCR-based RFLP) methods. The PCR protocol consisted of an initial melting step of 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 55°C (for *LEP*), 30 s at 60°C (for *LEPR*), and 30 s at 72°C, and a final elongation step of 5 min at 72°C. Amplification was carried out using primers forward 5′-TTT CCT GTA ATT TTC CCG TGA G-3′ and reverse 5′-AAA GCA AAG ACA GGC ATA AAA A-3′ for the *LEP* gene and forward 5′-TCC TCT TTA AAG CCT ATC CAG TAT TT-3′ and reverse 5′-AGC TAG CAA ATA TTT TTG TAA GCA AT-3′ for the *LEPR* gene. PCR was performed with a 25 μL reaction mixture containing 25–50 ng/μL DNA, 1 μL of 10 pmol/μL of each primer, 1 μL of dNTP mixture (5 mM dNTP, 1 μL 2.5 mM $MgCl_2$, 1 U Taq DNA polymerase), 2.5 μL 10X PCR buffer (Mg free, Invitrogen Life Technologies, Carlsbad, CA, USA), and dH_2O. The PCR products were visualized on a 2% agarose gel stained with ethidium bromide. Amplified products were digested with HhaI at 37°C for *LEP* and MspI at 37°C for *LEPR*, and the resulting fragments were separated by 2% agarose gel electrophoresis. The fragments were stained with ethidium bromide and visualized through a Vilber-Lourmat Gel Quantification and Documentation System (QUANTUM-ST4; Vilber Lourmat BP 66, Torcy, France).

Table 2: Distribution of *LEP* gene G2548A polymorphism and allele frequencies between obesity patients and controls.

Genotype	Patients (*n*: 127) (%)	Controls (*n*: 105) (%)	*P*
AA	37 (29)	32 (30)	
GA	61 (48)	52 (49.5)	0.87
GG	29 (22)	21 (20)	
AA + GA : GG	98 : 29	84 : 21	0.60
I GA + GG : AA	90 : 37	73 : 32	0.82
Allele frequency			
A	135 (53)	116 (55)	0.65
G	119 (46.8)	94 (44.7)	

2.3. Statistical Analysis. Analysis of the data was performed using SPSS 16.0 (SPSS, Chicago, IL, USA) and OpenEpi Info (http://www.openepi.com). Continuous data were given as mean ± standard deviation. The frequencies of the alleles and genotypes (Hardy-Weinberg equilibrium) in patients and controls were compared with χ^2 analysis, and 95% confidence intervals were calculated. A P value less than 0.05 (two-tailed) was regarded as statistically significant. The Bonferroni method was applied so that the corrected P value could be calculated.

3. Results

Table 1 shows the demographic variables and baseline characteristics of patients. The mean age and BMI were 44.86 ± 1.51, 35.45 ± 4.56 and 34.25 ± 15.43, 21.57 ± 1.89 in patients and control groups, respectively. The mean age of obesity patients with BMI > 35 kg/m^2 and BMI < 35 kg/m^2 was 45.01 ± 1.38 and 44.7 ± 1.65, respectively, while the mean BMI of these patients was 38.68 ± 3.11 (BMI > 35 kg/m^2) and 32.06 ± 1.56 (BMI < 35 kg/m^2). Patients and controls were genotyped for both the G2548A polymorphism in the *LEP* gene promoter and the 668A/G polymorphism in the *LEPR* gene. The distribution of the *LEP* G2548A and LEPR 668A/G polymorphisms of the patients and control groups are presented in Tables 2 and 3. We found no statistically significant difference in the genotype frequencies of the *LEP* gene polymorphism in patients and control groups (P > 0.05). The *LEPR* genotypes differed between the obesity

Table 3: Distribution of *LEPR* gene 668 A/G polymorphism and allele frequencies between obesity patients and controls.

Genotype	Patients (*n*: 127) (%)	Controls (*n*: 105) (%)	*P*
AA	50 (39)	50 (47.6)	
GA	56 (44)	46 (43.8)	0.05*
GG	21 (16.5)	9 (8.5)	
AA + GA : GG	106 : 21	96 : 9	0.10
I GA + GG : AA	77 : 50	55 : 50	0.20
Allele frequency			
A	156 (61.4)	146 (69.5)	0.08
G	98 (38.5)	64 (30.4)	

*According to Bonferroni correction is not significant.

Table 4: The distribution of combined genotypes *LEP* and *LEPR* gene polymorphism between patients and control groups.

Combined genotypes	Patients (*n*: 127) (%)	Controls (*n*: 105) (%)	*P*
AA-AA	17 (13.39)	19 (18.10)	
AA-GA	15 (11.81)	10 (9.52)	
AA-GG	5 (3.94)	3 (2.86)	
GA-AA	23 (18.11)	22 (20.95)	
GA-GA	27 (21.26)	24 (22.86)	
GA-GG	11 (8.66)	6 (5.71)	
GG-AA	10 (7.87)	9 (8.57)	
GG-GA	14 (11.02)	12 (11.43)	
GG-GG	5 (3.94)	0	**0.047***
	Patients		
	BMI > 35 kg/m^2 (*n*: 65) (%)	30 kg/m^2 < BMI (*n*: 62) (%) < 35 kg/m^2	
AA-AA	8 (12.31)	9 (14.52)	
AA-GA	6 (9.23)	9 (14.52)	
AA-GG	3 (4.62)	2 (3.23)	
GA-AA	13 (20.00)	10 (16.13)	
GA-GA	11 (16.92)	16 (25.81)	
GA-GG	5 (7.69)	6 (9.68)	
GG-AA	6 (9.23)	4 (6.45)	
GG-GA	9 (13.85)	5 (8.06)	
GG-GG	4 (6.15)	1 (1.61)	0.37

*The results that are statistically significant.

patients and controls, but this was not statistically significant after the Bonferroni correction ($P = 0.05$).

Allele frequencies in the *LEPR* gene showed no statistically significant association ($P > 0.05$) (Table 3). The *LEPR* gene A allele was 61.4% in patients and 69.5% in the control group, while the G allele frequency was 38.5% in patients and 30.4% in the control group. In the combined analysis of the *LEP* and *LEPR* genes, the *LEP/LEPR* GG/GG combined genotype was found to increase the risk of obesity compared to the controls ($P < 0.05$) (Table 4). In the combined genotype analysis based on the mean BMI of obesity patients, there was no association of the *LEP/LEPR* combined genotype and obesity between patients with a BMI ≥ 35 kg/m^2 and patients with a BMI near 30 kg/m^2 ($P > 0.05$) (Table 4).

4. Discussion

Human obesity is a complex trait determined by the interaction of multiple genes and environmental factors [1]. Obesity may arise as a result of increased energy intake, decreased energy expenditure, or increased partitioning of nutrients into fat, either alone or in combination [16]. The prevalence of obesity and being overweight continues to increase worldwide, not only causing serious personal health problems but also imposing a substantial economic burden on societies [17]. Although the development of obesity has a genetic component, the mechanism is unknown. Genetic influences are difficult to elucidate, and identification of the involved genes is not easily achieved [3].

In the present study, we analyzed the frequencies of *LEP* G2548A and *LEPR* 668A/G polymorphisms in obesity

patients in a Turkish population. There was no statistically significant difference between the groups with respect to the *LEP* genotype distribution ($P > 0.05$) and allele frequencies ($P > 0.05$). Hoffstedt et al. suggested that the *LEP* G2548A variant may influence gene expression of leptin and leptin secretion by adipose tissue [18]. Mammès et al. noted that the *LEP* G2548A polymorphism may influence a BMI increase by means of its effects on leptin secretion [19]; however, they identified a significant and independent association between the *LEP* 2548GG carrier status and higher leptin levels. An association of the *LEP* G2548A polymorphism and increased BMI was reported in overweight Europeans and in Taiwanese subjects with obesity [15] and the combined *LEP* 759C/T and *LEP* G2548A genotype may be a determinant of obesity [20]. The results of our study do not support the results of these studies but do support those of other studies that showed no association between the *LEP* G2548A polymorphism and obesity-related phenotypes [11, 14, 15, 21]. We found that *LEPR* genotypes show a difference, but not statistically significant, between obesity patients and controls. We attribute this lack of significance to the low number of patients included in our study, but finding obese patients that have no other disease is difficult. Some researchers have proposed that the polymorphisms of the leptin receptor gene (especially *LEPR* 668A/G polymorphism) may contribute to common forms of human obesity [11, 14, 22–24]. Our results with respect to the LEPR polymorphism are in agreement with the results of these studies.

Our results showed a statistically significant difference between groups with respect to the distribution of the *LEP/LEPR* GG/GG combined genotype. Obesity results from both gene-gene and gene-environment interactions [1], and in our study we examined the gene-gene interactions of the *LEP/LEPR* genes and their link to obesity. Duarte et al. demonstrated that the haplotype association of the *LEP* G2548A and *LEPR* Q223R variants was related to a 58% increase in obesity risk, and they considered the interactions between *LEP* and *LEPR* gene polymorphisms to intensively influence modulation of energy homeostasis [11]. In agreement with the findings of our study, Boumaiza et al. reported that the *LEP* G2548A and *LEPR* Q223R polymorphisms and haplotype combination were associated with a metabolic syndrome and obesity risk in Tunisian subjects [25]. The G2548A and 3′HVR variants of the *LEP* gene have been noted as being in linkage disequilibrium, and I/G combined genotypes are associated with obesity [26]. In addition, the interactions between the polymorphisms of the *LEP* and *LEPR* genes have been shown to increase the risk of non-Hodgkin's lymphoma and influence insulin plasma concentrations and blood pressure levels [11].

Our findings indicate that the *LEP* G2548A polymorphism is not a relevant obesity marker and that the *LEPR* 668A/G polymorphism may be related to obesity in a Turkish population. Additionally, the *LEP/LEPR* GG/GG combined genotype was found to increase the risk of obesity in patients compared to controls. However, the association of these polymorphisms with obesity is still controversial, and further research with larger patient populations is necessary.

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