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ORIGINAL PAPER

Association with Leptin Gene c.-2548 G>A Polymorphism, Serum Leptin Levels, and Body Mass Index in Turkish Obese Patients

Deniz Say Sahin · Cemil Tumer · Cemil Demir · M. Murat Celik · Mustafa Celik · Edip Ucar · Ramazan Gunesacar

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Abstract Leptin is a protein hormone which plays a critical role in the regulation of both body-weight through reducing food intake and stimulating energy expenditure. Several polymorphisms in leptin gene (*LEP*), which encodes for leptin, have been described. However, its association with obesity is still controversial. Therefore, in the present study, we aimed to investigate whether *LEP* c.-2548 G>A polymorphism was associated with serum leptin levels, lipid parameters, and body mass index in Turkish obese patients. Forty-seven obese patients and 48 healthy individuals were included in the study. Blood samples were collected for DNA extraction. *LEP* c.-2548 G>A polymorphism were detected using polymerase chain reaction–restriction fragment length polymorphism technique. Serum leptin levels and lipid parameters were measured by ELISA and enzyme colorimetric assay techniques, respectively. GA or AA genotypes and A allele carrier frequencies of the c.-2548 G>A polymorphism in the *LEP* were higher in obese (38.3, 34.0 and 72.3 %) when compared with controls (14.6, 12.5, and 27.1 %; $p = 0.011$, 0.016, and 0.002, respectively). On the other hand, AA or AG genotypes were also related to increased serum leptin levels ($p < 0.001$) and body mass index ($p < 0.0001$). All these consequences showed that *LEP* -2548 AA or AG genotypes are important predictors for increased levels of leptin and BMI in Turkish obese patients and it may be a useful marker for obesity risk in our population.

Keywords Leptin · c.-2548 G>A · Polymorphism · Obesity · Body mass index

D. S. Sahin · C. Demir
Vocational Higher School of Health Services, Mardin Artuklu University, 47100 Mardin, Turkey

C. Tumer
Department of Physiology, Faculty of Medicine, Mustafa Kemal University, 31100 Hatay, Turkey

M. M. Celik · E. Ucar
Department of Internal Medicine, Faculty of Medicine, Mustafa Kemal University, 31100 Hatay, Turkey

M. Celik · R. Gunesacar (✉)
Department of Medical Biology and Genetics, Faculty of Medicine, Kahramanmaras Sutcu Imam University, 46050 Kahramanmaras, Turkey
e-mail: rgunesacar@hotmail.com

Introduction

Obesity is the most prevalent nutritional public health problem. The prevalence of obesity is increasing in most of westernized and developing countries in the world including Turkey [1, 2]. Obesity has a complex pathogenesis that results from interactions between genetic and environmental factors [3, 4]. In the medical literature, at least 37 obesity-related genes have been reported so far. Some of theme such as MC4R, POMC, FTO, and BDNF genes play a major role in the pathogenesis of obesity [5].

Leptin is a hormone that consists of 167-amino acid transcribed by the leptin gene (*LEP*) and secreted primarily by adipocytes [6, 7]. Leptin, an important signal in the regulation of adipose-tissue mass and body-weight, regulates by inhibiting food intake and stimulating energy expenditure. Its expression and secretion are highly correlated with body fat and adipocyte number and size [8–10]. This hormone has not only regulate the food intake and body mass but also several systemic effects such as immunity, reproduction, angiogenesis, wound healing,

bone remodeling, and cardiovascular and respiratory functions [11, 12]. Serum leptin concentration is proportional to the body adiposity and markedly increased in obese individuals [11].

The *LEP* encodes for leptin that spans 14.6 kb of genomic DNA. In humans, it is located on the long arm of chromosome 7 (7q31.3) and consist of three exons separated by two introns [13, 14]. Several studies have suggested that variants in *LEP* may be important to the pathophysiology of human obesity [15, 16]. A common single nucleotide polymorphism within the 5′ promoter region (c.-2548 G>A) of *LEP* has been associated with variations in serum leptin and body mass index (BMI) in obese individuals [17, 18]. It has been shown that the *LEP* c.-2548 G>A polymorphism influences leptin expression, possibly at the transcriptional level, and therefore also adipose secretion levels of the hormone [19]. However, the impact of *LEP* polymorphisms and leptin levels in obesity is still under debate and contradictory results have been reported in different populations. Therefore, in the present study, we aimed to investigate whether *LEP* c.-2548 G>A polymorphism was associated with serum leptin levels, lipid parameters, and BMI in Turkish obese patients.

Materials and Methods

Subjects

This study was performed on two groups. The first group was composed of forty-seven obese patients (mean age: 44.28 ± 9.40 years; mean BMI: 34.80 ± 3.96 kg/m^2, BMI range: 30.05–45.91 kg/m^2), recruited from the Department of Endocrinology, Mustafa Kemal University Hospital and Hatay Government Hospital (Hatay, Turkey). The second group was composed of forty-eight non-related healthy controls (mean age: 41.96 ± 10.05 years; mean BMI: 24.5 kg/m^2, BMI range: 17.56–29.43) living in the community, randomly recruited from the local population register of Hatay city. Weight and height were measured on the subjects barefooted and lightly clothed. BMI was calculated, and obesity was defined as BMI ≥30 kg/m^2 [20].

Biochemical Analyses

A 12 h fasting venous blood samples were collected from each participant for serum leptin and lipids determinations. Triglycerides, total cholesterol and high-density lipoprotein (HDL) were measured by Randox kites (Randox Laboratories, San Francisco, CA, US) enzymatic-colorimetric assays using an Olympus AU 600 autoanalyzer (Olympus Optical Co. Ltd. Tokyo, Japan). Low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) cholesterol were calculated by Friedewald formulae [21]. Serum leptin levels were measured by MICROELISA kit according to manufacturer instructions (Human Leptin ELISA DLL-40-24100f, Texas, USA).

DNA Extraction and *LEP* c.-2548 G>A Genotyping

Genomic DNA was extracted from EDTA-anticoagulated whole blood by a salting out method as described by Miller et al. [22]. *LEP* c.-2548 G>A polymorphism was detected by a PCR–RFLP technique using the endonuclease *Hha*I enzyme. The forward (5′-TTT CTG TAA TTT TCC CGT GAG-3′) and reverse (5′-AAA GCA AAG ACA GGC ATA AAA A-3′) primers were designed based on the *LEP* promoter sequence. Each of the PCR reactions were performed in a total volume of 25 µl, containing 50 ng of DNA, 5 pmol of each primer, 1.5 µl of 10× reaction buffer, 2 mM magnesium chloride, 0.2 mM dNTPs, and 1 Unit of *Taq* DNA polymerase. The PCR consisted of an initial denaturation for 5 min (min) at 94 °C, followed by 35 cycles of denaturation at 94 °C for 45 s (sec), annealing at 52 °C for 45 s, extension at 72 °C for 45 s, and a final extension at 72 °C for 7 min. The PCR products were digested for 6 h at 37 °C with 5 Unit of *Hha*I restriction endonuclease (Fermentas, Vilnius, Lithuania) and restricted fragments were separated by electrophoresis using a 2.5 % agarose gel (Fig. 1).

Statistical Analysis

Genotype and allele frequencies for the studied polymorphisms were compared by Fischer's exact test. The agreement of genotypes frequencies with Hardy–Weinberg

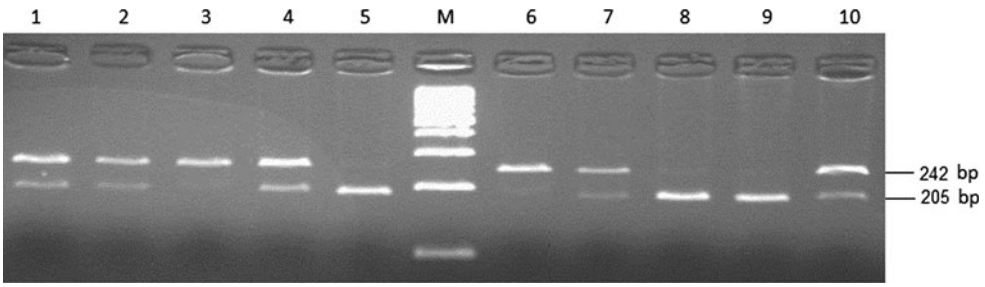

Fig. 1 Genotyping of *LEP* c.-2548 G>A polymorphism by *Hha*I RFLP. *Lane M* 100 bp ladder marker; *lanes 1, 2, 4, 7, and 10* genotype GA (242, 205 and 37 bp); *lanes 3 and 6*, genotype GG (242 bp); *lanes 5, 8 and 9*, genotype AA (205 and 37 bp). 37 bp fragments are not visible on the gel

Table 1 The genotype distribution and allele frequencies for the *LEP* c.-2548 G>A polymorphism of the obese and control subjects

Genotype Carrier frequency	Obese ($n = 47$)	Controls ($n = 48$)	X^2	OR	p value
GG	13 (27.7 %)	35 (72.9 %)	19.458	0.142 (0.06–0.35)	Reference
GA	18 (38.3 %)	7 (14.6 %)	06.887	3.630 (1.34–9.82)	0.011
AA	16 (34.0 %)	6 (12.5 %)	06.193	3.613 (1.27–10.29)	0.016
G (GG + GA)	31 (65.9 %)	42 (87.5 %)	10.27	0.28 (0.12–0.62)	Reference
A (AA + GA)	34 (72.3 %)	13 (27.1 %)	10.27	3.54 (1.60–7.8)	0.002

equilibrium expectation was tested using the χ^2 test. Continuous variables were presented as mean ± SD and compared by t test. Statistical tests were performed by Statistical Package for Social Sciences (SPSS 17.5 for Windows; SPSS Inc. Chicago, IL, USA). The results were considered statistically significant when the p value was less or equal to 0.05.

Ethics

The study was approved by the institutional review board and written informed consent was obtained from the study participants. The study was done in accordance with the principles of Helsinki Declaration.

Results

The studied genotype and allele frequencies were in Hardy–Weinberg equilibrium in both obese and healthy control groups. The genotype distribution and carrier frequencies in obese and healthy controls for the *LEP* C.-2548 G>A polymorphism are presented in Table 1. The GA and AA genotypes and A allele carrier frequencies of the LEP c.-2548 G>A polymorphism were significantly higher in obese (38.3, 34.0, and 72.3 %) when compared with controls (14.6, 12.5, and 27.1 % respectively, $p = 0.011$, 0.016, and 0.002 respectively). The antropometric and biochemical characteristics of the obese and control subjects are presented in Table 2. Levels of mean serum leptin, total cholesterol, LDL, VLD, triglycerides, and mean BMI were higher in obese than that of the controls. There were also relations between the *LEP* AG or AA genotypes with BMI, leptin, total cholesterol, LDL, and VLDL levels (Table 3). No association was found between the LEP c.-2548 variant and HDL or triglycerides ($p > 0.05$). All subjects participating in the study were evaluated, carriers of the *LEP* AG or AA genotypes exhibited higher serum leptin, total cholesterol, LDL, and VLDL levels compared to those with carriers of the *LEP* GG genotype (61.94 ± 26.67 ng/dl vs 18.92 ± 15.10 ng/dl, $p < 0.0001$ for leptin, 164.63 ± 46.69 ng/dl vs 134.50 ± 36.98 ng/dl, $p < 0.001$ for total cholesterol; 128.78 ± 46.73 ng/dl vs 104.72 ± 43.33 ng/dl, $p < 0.01$ for LDL; 36.92 ± 15.82 ng/dl vs 30.48 ± 18.02 ng/dl, $p < 0.03$ for VLDL). Separate analysis by gender revealed that obese females carrying the *LEP* AA or AG genotypes showed also significantly higher serum leptin levels (82.43 ± 13.57 ng/dl) than subjects *LEP* GG genotypes (44.85 ± 9.75 ng/dl, $p < 0.0001$). The significant relationship between the *LEP* AA or AG genotype and higher leptin levels was also found in obese male (64.47 ± 15.70 ng/dl) when compared with non-obese male (36.58 ± 8.71 ng/dl, $p < 0.0001$). When adjusting for BMI, the association between the *LEP* variant and serum leptin persisted in obese women ($p < 0.02$) but disappeared in obese men ($p > 0.05$).

Table 2 Antropometric and biochemical characteristics of the obese and control subjects

	Controls ($n = 48$)	Obese ($n = 47$)	p value
Age	41.96 ± 10.05	44.28 ± 9.40	
BMI (kg/m^2)	24.54 ± 3.54	34.80 ± 3.96	<0.0001
Leptin (ng/dl)	13.05 ± 12.57	58.70 ± 19.73	<0.0001
Total cholesterol (mg/dl)	123.96 ± 27.35	171.35 ± 44.34	<0.0001
LDL (mg/dl)	90.31 ± 28.17	143.75 ± 46.88	<0.0001
VLDL (mg/dl)	23.48 ± 15.39	42.51 ± 12.95	<0.0001
Triglycerides (mg/dl)	137.51 ± 65.31	187.03 ± 73.23	<0.002
HDL (mg/dl)	43.68 ± 14.96	41.01 ± 16.20	>0.05

Discussion

In the present study, we hypothesize that genetic variation in the leptin gene may increase the serum leptin concentration and contribute to the development of obesity. *LEP* c.-2548 G>A polymorphism were successfully determined in all subjects, 72.3 % of obese patients and 29.1 % controls had *LEP* AA or LEP AG genotypes and carriers of the AA or AG exhibited higher levels of serum leptin compared to those with carriers of the *LEP* GG genotype. Our

Table 3 Analyses of body mass index, serum leptin, and lipid parameters according to the LEP c.2548 genotypes in obese and healthy controls

Parameter	LEP c.-2548 genotypes				*p* value
	Controls ($n = 48$)		Obese ($n = 47$)		
	GG ($n = 34$) (Mean ± SD)	GA + AA ($n = 14$) (Mean ± SD)	GG ($n = 13$) (Mean ± SD)	GA + AA ($n = 34$) (Mean ± SD)	
BMI (kg/m^2)	24.90 ± 3.56	23.90 ± 3.04	35.20 ± 4.45	34.90 ± 4.09	<0.0001
Leptin (ng/dl)	14.02 ± 4.22	25.34 ± 8.55	40.40 ± 9.79	75.56 ± 16.70	<0.001
Total cholesterol (mg/dl)	122.05 ± 26.14	122.07 ± 24.00	168,84 ± 41.41	179.55 ± 46.44	<0.001
LDL (mg/dl)	94.08 ± 34.95	88.00 ± 18.63	130.21 ± 51.92	146.76 ± 43.55	<0.0001
Triglyceride (mg/dl)	147.58 ± 80.46	136.57 ± 41.68	160.70 ± 80.47	141.81 ± 96.21	ns
HDL (mg/dl)	40.38 ± 15.32	45.85 ± 16.23	38.38 ± 12.42	41.91 ± 14.85	ns

results may suggested that *LEP* -2548 AA or AG carriers have a significantly higher risk for obesity against those carrying the -2548 GG genotype, which supports the hypothesis for leptin involvement in ethiopathogenesis of obesity. Moreover, this study revealed significant differences in BMI, and serum lipid concentrations among the *LEP* -2548 AA or GA genotypes in both obese and normal weight samples, indicating that this genetic variant may also relevant marker of obesity in the Turkish population. The previous studies analyzing the association between the *LEP* variant and obesity or BMI have been controversial. For example, the LEP -2548 G allele was found to be associated with increased serum leptin levels through an interaction between fat mass and gender in healthy Greek individuals [23]. Conversely, carriers of *LEP* G allele had lower leptin concentrations adjusted for fat mass in overweight healthy men from French population [17] and -2548 AA genotype was associated with increased in serum leptin in obese individuals from French cohorts [24] like our study. However, the relationship between *LEP* -2548 G allele was associated serum leptin levels through an interaction with adiposity and gender in Brazilian [25], European [18], and Asian [26] populations. These different results may arise from interactions of *LEP* c.-2548 G>A polymorphism with other gene polymorphisms in leptin, leptin receptor, adiponectin or others, sample size and ethnic background of population. Because of the limited sample size in this work, further investigations with larger groups from the same ethnic origin is necessary to confirm these findings. In conclusion, AA or AG genotype carriers which is linked to high transcriptional activity of the *LEP* gene are important predictors for increased levels of leptin and BMI in Turkish population and it may be a useful marker for obesity risk in our population.

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