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Leptin and leptin receptor polymorphisms are related to body mass index in a Turkish population

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Background/aim: Leptin is a hormone that is known to be related to weight gain and obesity. The soluble leptin receptor has been found in plasma as an important determinant of leptin sensitivity. In this study, our goal was to investigate the association between leptin levels and leptin receptor polymorphisms in a Turkish population.

Materials and methods: The sample pool of this study consisted of 202 subjects. G2548A variant in the promoter region of the leptin gene and Q223R polymorphism of the leptin receptor gene were evaluated by using PCR-RFLP. Leptin levels were determined by ELISA.

Results: Leptin levels were significantly higher in subjects with the A allele than in subjects without the A allele. Leptin receptor levels were lower in subjects with the AA genotype than in those with the AG genotype. There was a higher prevalence of the leptin-2548 AA genotype among subjects with a BMI ≥ 25 kg/m2 than in those with a BMI < 25 kg/m2.

Conclusion: The leptin-2548A allele might be a predisposing factor for obesity.

Key words: Leptin, leptin receptor, obesity

1. Introduction

Obesity is a major public health problem that contributes to the pathogenesis of several severe chronic diseases including cardiovascular disease, diabetes, and certain kinds of cancer. Leptin is an essential hormone related to weight gain and obesity, controlling energy balance and metabolism in the human body. It is a peptide hormone, secreted from white adipose tissue as a signal to the brain, and has a primary influence on body weight by coordinating the metabolic, endocrine, and behavioral responses to starvation (1). Insensitivity of the leptin receptor is known as leptin resistance. Free leptin levels were found to be correlated with leptin resistance, BMI, and fat mass (2). Leptin receptor is a member of the class-1 cytokine receptor family and it is an important determinant of leptin sensitivity originally found in hypothalamic neurons. However, alongside the membrane-bound forms of this receptor, there is also a soluble form in plasma (1). The function of this soluble form is not known yet, but it is thought to contribute to the delay in clearance of leptin (3). An inverse relationship was found between leptin and soluble leptin receptor levels (4). The balance between bound and free leptin depends on the plasma concentration of this soluble leptin receptor (1,5). The ratio between total and soluble leptin concentration was used as the free leptin index, indicating leptin resistance (5).

Genetic studies about leptin and leptin receptor mutations revealed that those patients who had homozygous mutations of these gene regions had severe gonadal and thyroid abnormalities, delayed puberty, and dramatic increase in weight (6). In this aspect, the effects of polymorphisms on leptin and leptin levels were also evaluated and the Gln223Arg (Q223R) polymorphism of the leptin receptor was found to lead to an amino acid change in the extracellular domain that is common for all isoforms of the receptor affecting the charge of the region and leptin binding capacity (7).

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The leptin gene region G-2548A variant exists in the promoter region, affecting the functional capacity of leptin. The 2548A allele was found to be positively correlated with elevated serum leptin concentrations (8,9). The objective of our study was to evaluate the association between serum leptin and soluble leptin receptor levels and their polymorphisms in conjunction with changes in body mass indices.

2. Materials and methods

2.1. Study groups

The study group consisted of 202 subjects chosen from the population of patients who applied to İstanbul Bilim University Hospital's Internal Medicine Clinics for regular health examinations. Subjects without any major chronic disease were included in the study. Written informed consent was obtained from each subject. Clinical variables of the study groups are given in Table 1.

2.2. Specimen supply

Immediately upon arrival, whole blood samples were centrifuged and aliquoted; they were afterwards stored at –30 °C.

2.3. DNA extraction

The genomic DNA samples of the subjects were isolated from venous whole blood samples (from leukocytes) by a

Table 1. Clinical variables of subjects according to BMI.

	BMI < 25 (n = 83)	BMI ≥ 25 (n = 119)	
		25 ≤ BMI < 30	BMI ≥ 30
Age (years)	44.29 ± 20.72	51.52 ± 16.44	
		51.05 ± 16.72	52.57 ± 15.88
Sex (F/M)	56/27	52/67	
		23/44	29/23
Waist circumference (cm)	80.85 ± 8.91	100.55 ± 10.20	
		94.92 ± 7.6	106.61 ± 10.67
Waist/hip ratio	0.83 ± 0.07	0.89 ± 0.08	
		0.89 ± 0.07	0.88 ± 0.08
BMI (kg/m^2)	21.57 ± 2.27	31.01 ± 6.12	
		27.34 ± 1.48	35.61 ± 6.95
Leptin (ng/mL)	21.98 ± 21.61	36.30 ± 35.56*	
		28.05 ± 31.89	47.11 ± 37.40
Leptin receptor (ng/mL)	30.24 ± 17.99	23.39 ± 10.72**	
		24.74 ± 12.86	22.31 ± 9.42
Total cholesterol (mg/dL)	192.84 ± 41.53	217.83 ± 44.19	
		217.79 ± 42.55	214.42 ± 45.57
Triglyceride (mg/dL)	83.50 ± 40.75	135.87 ± 76.41	
		136.49 ± 90.2	132.51 ± 47.28
LDL-cholesterol (mg/dL)	117.67 ± 37.79	139.50 ± 42.22	
		139.31 ± 36.2	136.71 ± 48.91
HDL-cholesterol (mg/dL)	58.33 ± 13.17	49.79 ± 12.18	
		51.34 ± 12.78	47.93 ± 10.88
HbA1c (%)	5.15 ± 0.646	5.79 ± 0.93	
		5.66 ± 0.8	5.94 ± 1.04
Insulin (μU/mL)	7.2 ± 3.02	14.69 ± 8.41	
		13.72 ± 9.19	15.45 ± 6.88

Number of individuals in parentheses. Values are reported as mean ± standard deviation (SD) or number of patients. $P < 0.05$ was considered significant.

*Leptin levels were significantly higher in BMI ≥ 25 kg/m^2 group than in < 25 kg/m^2 one ($P = 0.003$).

**Leptin receptor levels were significantly lower in BMI ≥ 25 kg/m^2 group than in < 25 kg/m^2 one ($P = 0.002$).

method based on sodium dodecyl sulfate lysis, ammonium acetate extraction, and ethanol precipitation (10).

2.4. Polymorphism analyses

We followed previously described methods for leptin and leptin receptor polymorphisms (9).

The primers used for PCR for the leptin G2548A polymorphism were F(5′-TTTCCTGTAATTTTCCCGTGA-3′) and R(5′-AAAGCAAAGACAGGCATAAAA-3′); and for the leptin receptor Q223R polymorphism, the primer sequences were F(5′-ACCCTTTAAGCTGGGTGTCCCAAATAG-3′) and R (5′-AGCTAGCAAATATTTTTGT AAGCAATT-3′) from IDT (Integrated DNA Technologies, Inc.). Leptin gene regions were amplified under following conditions: a reaction mixture containing approximately 100 ng of template DNA, 10 pmol of each primer, all 4 deoxyribonucleoside 5′ triphosphates (each at 1 mM), 2 mM $MgCl_2$, and 0.5 U of *Taq* polymerase in 10X reaction buffer (MBI Fermentas). For leptin receptor gene amplification, the following reaction mixture was used: approximately 100 ng of template DNA, 10 pmol of each primer, all 4 deoxyribonucleoside 5′ triphosphates (each at 1 mM), 2 mM $MgCl_2$, and 0.3 U of *Taq* polymerase in 10X reaction buffer (MBI Fermentas). For both leptin and leptin receptor gene amplifications, reactions were carried out with an initial melting step of 3 min at 94 °C, followed by 33 cycles of 30 s at 94 °C, 30 s at 60 °C, 30 s at 72 °C, and a final elongation step of 5 min at 72 °C. PCR and restriction products were electrophoresed in 2% (w/v) agarose gels and stained with ethidium bromide. The restriction endonuclease was Hin6I to distinguish the leptin-2548 G/A polymorphism. Hin6I digestion generated 181 bp and 61 bp fragments for the G allele, but an uncut 242 bp fragment for the A allele. CfoI restriction endonuclease enzyme was used for the leptin receptor gene. The expected products were 294 bp and 127 bp fragments for the R allele and a single uncut fragment of 421 bp for the Q allele.

2.5. Serum level detection

Leptin and soluble leptin receptor levels were determined by using commercial ELISA kits (Biovendor for leptin receptor and Biosource for leptin).

2.6. Statistical analysis

Statistical analyses were performed using SPSS version 11.5 (SPSS Inc, Chicago, IL, USA) including the chi-square test, Fischer's exact test, and the Pearson correlation test. Odds ratios and 95% confidence intervals were calculated. Mean values were compared among subjects by unpaired Kruskal-Wallis analysis. Values of $P < 0.05$ were considered statistically significant.

3. Results

The study group was divided into subgroups according to body mass index (BMI); demographic details of those subgroups are given in Table 1.

Mean circulating leptin and leptin receptor levels according to the leptin G2548A genotype and allele are shown in Table 2. Leptin levels were significantly higher in subjects with the AA genotype than in those with the AG or GG genotype ($P < 0.01$). However, we found leptin receptor levels to be lower in subjects with the AA genotype than in those with the AG genotype ($P < 0.01$). Leptin receptor levels did not significantly differ between subjects with the A allele and those without the A allele ($P > 0.05$). Leptin levels were significantly higher in subjects with the A allele than in subjects without the A allele ($P = 0.026$). Furthermore, leptin levels were higher in subjects without the G allele than in those with it ($P < 0.01$). In contrast, decreased leptin receptor levels were found in subjects without the G allele in comparison to those who carried the G allele ($P < 0.01$). Mean circulating leptin and leptin receptor levels according to leptin receptor Q223R genotypes and alleles are given in Table 3. There were no statistically significant differences among any of these groups ($P > 0.05$).

Table 2. Mean circulating leptin and leptin receptor levels according to leptin G2548A genotypes and alleles.

	Leptin G2548A genotypes			Leptin G2548A alleles			
	AA (n = 22)	AG (n = 65)	GG (n = 25)	A+	A–	G+	G–
Leptin levels (ng/mL)*	63.73 ± 48.4	28.96 ± 27.61	24.84 ± 19.12	37.75 ± 37.03	24.84 ± 19.12	27.82 ± 25.50	63.73 ± 48.43
Leptin receptor levels (ng/mL)**	16.42 ± 5.62	26.48 ± 13.84	27.27±10.58	23.94 ± 13.03	27.27 ± 10.58	26.70 ± 12.97	16.42 ± 5.62

Data presented as mean ± standard deviation.

*Leptin levels were higher for the AA genotype than the others ($P < 0.01$). Leptin levels were higher in subjects with the A allele than in those without it ($P = 0.026$) and higher in subjects without the G allele than in those with it ($P < 0.01$).

**Leptin receptor levels were lower for AA compared to the others ($P < 0.01$). Leptin receptor levels were lower in subjects without the G allele than in those with it ($P < 0.01$).

Table 3. Mean circulating leptin and leptin receptor levels according to leptin receptor Q223R genotype and allele.

	Leptin receptor Q223R genotypes			Leptin receptor Q223R alleles			
	QQ (n = 47)	QR (n = 51)	RR (n = 13)	Q+	Q–	R+	R–
Leptin levels	35.31 ± 35.30	33.68 ± 32.14	39.17 ± 41.67	34.46 ± 33.53	39.17 ± 41.67	34.80 ± 34.00	35.31 ± 35.30
Leptin receptor levels	25.42 ± 11.65	23.67 ± 14.15	25.56 ± 8.23	24.51 ± 12.97	25.56 ± 8.23	24.06 ± 13.13	25.42 ± 11.65

Data presented as mean ± standard deviation.

Combined analysis was conducted to assess the cumulative effects of possible risk or protective attributes of the alleles. When combined analysis of leptin G2548A and leptin receptor Q223R genotypes was evaluated, there were no significant differences in the subjects (P = 0.497) (Table 4).

Moreover, we analyzed leptin and leptin receptor levels among the subjects, who were divided into 2 groups according to the BMI results < 25 kg/m^2 and ≥ 25 kg/m^2. The latter group was further divided into 2 subgroups with 25 kg/m^2 ≤ BMI < 30 kg/m^2 and BMI ≥ 30 kg/m^2 (Table 1). We found that leptin levels were significantly higher and leptin receptor levels were significantly lower in subjects with BMI ≥ 25 kg/m^2 than in those with BMI < 25 kg/m^2 (P = 0.003, P = 0.002). When we assessed other clinical parameters in the study group, there were no statistically significant differences among the subjects according to their BMI values (P > 0.05) (Table 1).

4. Discussion

Leptin is an essential hormone secreted from adipose tissue and it is related to weight gain and obesity. The serum levels of adipose tissue-derived hormones are known to affect insulin sensitivity (11). In a previous study, it was found that serum leptin concentrations were decreasing while soluble leptin receptor concentrations were increasing after a very-low-calorie diet in obese women (11). In type 2 diabetes cases, it was found that circulating leptin receptor levels were inversely correlated with the risk of type 2 diabetes (3).

Enhanced release of leptin was found to be accompanied with decreased soluble leptin receptor levels, causing leptin resistance over time (5,12). Our results were in accordance with these previous findings. In our study, we evaluated the differences between normal (<25 kg/m^2), overweight (25 kg/m^2 ≤ BMI < 30 kg/m^2), and obese (≥ 30 kg/m^2) subjects. Comparing those 3 groups with each other, leptin levels were found to be higher in obese cases, not in overweight ones; and leptin receptor levels were higher in normal subjects, not in overweight or obese ones.

Obesity is a polygenic disorder with several candidate genes playing a role in determining the final severity of obesity. Leptin and leptin receptor genes are 2 of those candidate genes to be evaluated. There are several different studies focusing not only their levels, but also on possible effects of their polymorphic genes on the severity of weight gain.

We studied not only the levels of leptin and leptin receptors, but also their polymorphisms, leptin G2548A and leptin receptor Q223R. In a previous study in an Asian population, it was found that the leptin G2548A variant was significantly associated with extreme obesity (≥35 kg/m^2), but not with moderate obesity (≥27 kg/m^2), (8). In another study, no relationship was found between the same leptin polymorphism and obesity-related variables such as BMI and waist circumference, but a positive correlation was found between GG genotype and leptin receptor levels (13).

In a study of a Turkish population, no relationship was found between the leptin receptor Q223R polymorphism

Table 4. The frequencies of haplotypes of leptin G-2548A and leptin receptor Q223R gene polymorphisms in subjects.

Haplotype association		Leptin receptor genotypes		
		QR n (%)	RR n (%)	QQ n (%)
Leptin genotypes	AA	18 (56.3)	3 (9.4)	11 (34.4)
	AG	32 (39.5)	10 (12.3)	39 (48.1)
	GG	9 (9.6)	4 (2.8)	9 (9.6)

Data are presented as number of subjects, with frequency in parentheses.

and BMI, which was in parallel with our results (14). In a previous study in a Romanian population, it was found that the leptin receptor Q223R polymorphism was not associated with obesity, but the R allele was concluded to be a possible predisposing factor for obesity (13). In our study, leptin levels were higher in homozygote leptin AA subjects than in those with AG or GG genotypes, in contrast to the decreased leptin receptor levels between the same groups. Leptin levels were lower in subjects with the G allele than in those without it. However, no relationship between leptin or leptin receptor levels and leptin receptor genotypes could be determined. No statistically significant relationship was observed between any leptin–leptin receptor genotype distributions either.

Mendez-Sanchez et al. determined that the leptin receptor Q223R polymorphism was more frequent in subjects with higher BMI (15), but we did not determine such a leptin receptor genotype or allele difference between normal (<25 kg/m^2) and overweight (≥25 kg/m^2) subjects. Our results were in parallel with those of a previous study in a Japanese population (16).

Ours was a rare study evaluating both levels and common polymorphisms of leptin and leptin receptors. Obesity is an important disease predisposing to more serious problems. Therefore, it has to be investigated in further detail. We obtained interesting results that warrant further elaboration. Therefore, in subsequent studies we are planning to increase the numbers of subjects in the case and control groups and to investigate the relationships between those parameters and chronic diseases related to obesity.

References

1. Huang XD, Fan Y, Zhang H, Wang P, Yuan JP, Li MJ, Zhang MJ. Serum leptin and soluble leptin receptor in non-alcoholic fatty liver disease. World J Gastroenterol 2008; 14: 2888–2893.
2. Lahlou N, Clement K, Carel JC, Vaisse C, Lotton C, Le Bihan Y, Basdevant A, Lebouc Y, Froguel P, Roger M et al. Soluble leptin receptor in serum of subjects with complete resistance to leptin: relation to fat mass. Diabetes 2000; 49: 1347–1352.
3. Sun Q, van Dam RM, Meigs JB, Franco OH, Mantzoros CS, Hu FB. Leptin and soluble leptin receptor levels in plasma and risk of type 2 diabetes in U.S. women: a prospective study. Diabetes 2010; 59: 611–618.
4. Hamnvik OP, Liu X, Petrou M, Gong H, Chamberland JP, Kim EH, Christophi CA, Kales SN, Christiani DC, Mantzoros CS. Soluble leptin receptor and leptin are associated with baseline adiposity and metabolic risk factors, and predict adiposity, metabolic syndrome, and glucose levels at 2-year follow-up: the Cyprus Metabolism Prospective Cohort Study. Metabolism 2011; 60: 987–993.
5. Guran T, Turan S, Bereket A, Akcay T, Unluguzel G, Bas F, Gunoz H, Saka N, Bundak R, Darendeliler F et al. The role of leptin, soluble leptin receptor, resistin, and insulin secretory dynamics in the pathogenesis of hypothalamic obesity in children. Eur J Pediatr 2009; 168: 1043–1048.
6. Mutch DM, Clément K. Genetics of human obesity. Best Pract Res Clin Endocrinol Metab 2006; 20: 647–664.
7. Crabbe P, Goemaere S, Zmierczak H, Van Pottelbergh I, De Bacquer D, Kaufman JM. Are serum leptin and the Gln223Arg polymorphism of the leptin receptor determinants of bone homeostasis in elderly men? Eur J Endocrinol 2006; 154: 707–714.
8. Wang TN, Huang MC, Chang WT, Ko AM, Tsai EM, Liu CS, Lee CH, Ko YC. G-2548A polymorphism of the leptin gene is correlated with extreme obesity in Taiwanese aborigines. Obesity (Silver Spring) 2006; 14: 183–187.
9. Franek E, Nowak J, Safranow K, Adler G, Bińczak-Kuleta A, Ciechanowicz A, Wiecek A. G(-2548)A leptin gene polymorphism in obese subjects is associated with serum leptin concentration and bone mass. Pol Arch Med Wewn 2010; 120: 175–180.
10. Miller SA, Dykes DD, Polesky HS. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Res 1988; 16: 1215.
11. Anderlová K, Kremen J, Dolezalová R, Housová J, Haluzíková D, Kunesová M, Haluzik M. The influence of very-low-calorie-diet on serum leptin, soluble leptin receptor, adiponectin and resistin levels in obese women. Physiol Res 2006; 55: 277–283.
12. Cohen SE, Kokkotou E, Biddinger SB, Kondo T, Gebhardt R, Kratzsch J, Mantzoros CS, Kahn CR. High circulating leptin receptors with normal leptin sensitivity in liver-specific insulin receptor knock-out (LIRKO) mice. J Biol Chem 2007; 282: 23672–23678.
13. Constantin A, Costache G, Sima AV, Glavce CS, Vladica M, Popov DL. Leptin G-2548A and leptin receptor Q223R gene polymorphisms are not associated with obesity in Romanian subjects. Biochem Biophys Res Commun 2010; 391: 282–286.
14. Mergen H, Karaaslan C, Mergen M, Deniz Ozsoy E, Ozata M. LEPR, ADBR3, IRS-1 and 5-HTT genes polymorphisms do not associate with obesity. Endocr J 2007; 54: 89–94.
15. Méndez-Sánchez N, Bermejo-Martínez L, Chávez-Tapia NC, Zamora-Valdés D, Sánchez-Lara K, Uribe-Ramos MH, Ponciano-Rodríguez G, Baptista-González HA, Ramos MH, Uribe M. Obesity-related leptin receptor polymorphisms and gallstones disease. Ann Hepatol 2006; 5: 97–102.
16. Matsuoka N, Ogawa Y, Hosoda K, Matsuda J, Masuzaki H, Miyawaki T, Azuma N, Natsui K, Nishimura H, Yoshimasa Y et al. Human leptin receptor gene in obese Japanese subjects: evidence against either obesity-causing mutations or association of sequence variants with obesity. Diabetologia 1997; 40: 1204–1210.