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Relationship Between Leptin G2548A and Leptin Receptor Q223R Gene Polymorphisms and Obesity and Metabolic Syndrome Risk in Tunisian Volunteers

Imen Boumaiza,[1] Asma Omezzine,[1] Jihène Rejeb,[1] Lamia Rebhi,[1] Amani Ouedrani,[1] Nabila Ben Rejeb,[1] Naoufel Nabli,[1] Ahmed Ben Abdelaziz,[2] and Ali Bouslama[1]

Leptin is a key hormone of weight regulation that modulates food intake. Since the elaboration of the leptin action mechanism, several studies tried to establish the relationship between obesity and the common polymorphisms of leptin (LEP) and leptin receptor (LEPR) genes, but results were controversial. We studied the association of G2548A of the *LEP* gene and Q223R of *LEPR* gene polymorphisms with obesity and metabolic syndrome (MetS). We recruited 169 nonobese volunteers (body mass index [BMI] $<30\,kg/m^2$) and 160 obese ones (BMI $\geq 30\,kg/m^2$). Glucose, insulin, and lipids were measured. BMI, homeostasis model assessment-insulin resistance (HOMA-IR), and daily energy intake were calculated. After adjustment to confounders parameters, 2548AA was found to increase the MetS ($p=0.043$) and obesity risk ($p=0.019$) in the studied population. After stratification according to the degree of obesity, the odds ratio [OR] of 2548AA was associated with moderate obesity ($p=0.048$) and morbid obesity ($p=0.048$). The *LEPR* 223RR genotype was associated with obesity in the studied population (OR = 1.74, $p=0.037$) and only in the overweight (OR = 1.8, $p=0.049$). Subjects with 2548AA had significantly higher BMI, daily energy intake, total cholesterol (TC), waist circumference (WC), insulinemia, and low high-density lipoprotein-cholesterol (HDL-C) levels. With regard to 223RR, we noted a significantly higher daily energy intake, BMI, TC, glycemia, insulinemia, HOMA-IR index, and low HDL-C levels. Haplotype model AR (2548A + 223R) and AQ (2548A + 223Q) increased the risk of obesity (OR = 3.36, $p<0.001$; OR = 2.56, $p=0.010$, respectively). When we added daily energy intake in adjustment, these significant associations disappeared. In addition, the AR and AQ increased the MetS risk. This significant association persisted after we had added daily energy intake in adjustment. This study showed that *LEP* G2548A and *LEPR* Q223R polymorphisms and haplotype combination were associated with MetS and obesity risk in Tunisian volunteers.

Introduction

Obesity, one of the most challenging health problems of the last century with a tremendous increase in incidence, is considered an important risk factor for type 2 diabetes and cardiovascular diseases (Walley *et al.*, 2006). Obesity has a complex pathogenesis that results from interactions between genetic and environmental factors which lead to malfunctioning of several signaling peptides, which are involved in body energy balance and nutritional status (Cummings and Schwartz, 2003).

Adipose tissue plays a crucial role in the regulation of energy homeostasis, insulin sensitivity and lipid and carbohydrate metabolism. These mechanisms are mediated by the actions of numerous hormones. There are three main adipokines: leptin, acylation stimulating protein, and adiponectin (Lu *et al.*, 2007).

Leptin is a metabolic and neuroendocrine hormone that is produced and released mainly by adipocytes (Considine *et al.*, 1996). It has several systemic effects such as body mass control, reproduction, angiogenesis, immunity, wound healing, bone remodeling, and cardiovascular function (Considine *et al.*, 1996; Fruhbeck, 2006). Plasma leptin concentration is proportional to body adiposity and is markedly increased in obese individuals (Considine *et al.*, 1996; Paracchini *et al.*, 2005).

The leptin (*LEP*) and leptin receptor (*LEPR*) genes have been investigated in the search for gene variants that are potentially related to the pathophysiology of obesity, diabetes, and associated complications (Heo *et al.*, 2002; Otero *et al.*,

[1]Department of Biochemistry, UR MSP 28/04, Sahloul University Hospital, Sousse, Tunisia.
[2]Information System Direction, Sahloul University Hospital, Sousse, Tunisia.

2005; Paracchini *et al.*, 2005). *LEP* G2548A (rs7799039) is an *LEP* gene single nucleotide polymorphism (SNP) consisting of G to A substitution at nucleotide (nt) −2548 upstream of the ATG start site in the *LEP* gene promoter. The association between G2548A and increased leptinemia is controversial (Le Stunff *et al.*, 2000; Mammès *et al.*, 2000; Hoffstedt *et al.*, 2002; Mattevi *et al.*, 2002; Portolés *et al.*, 2006; Ben Ali *et al.*, 2009). Interestingly, with regard to the *LEPR* gene polymorphism, the A to G transition in exon 6 at nt 668 from the start codon 223 *LEPR* Q223R (rs1137101) was associated with impaired leptin-binding activity (Yiannakouris *et al.*, 2001). The *LEPR* Q223R polymorphism has been associated with decreased body mass index (BMI), fat mass, leptin levels, and systolic and diastolic blood pressure (Gotoda *et al.*, 1997), but other studies are in conflict with this (Rosmond *et al.*, 2000; Takahashi-Yasuno *et al.*, 2003). There are a few studies dealing with the association between *LEP* G2548A and *LEPR* Q223R polymorphisms, obesity status, and daily energy intake.

The aim of our study was to investigate whether two SNPs in the *LEP* (G2548A) gene and its receptor (*LEPR* Q223R) gene are related to obesity and metabolic variability in Tunisian volunteers.

Materials and Methods

Study subjects

This study was performed on two groups previously described in Boumaiza *et al.* (2011). Briefly, one group was composed of 160 Tunisian unrelated obese subjects, on the basis of BMI (kg/m^2) $\geq 30\,kg/m^2$, who are volunteers from external consultations from the Sahloul University Hospital (Sousse, Tunisia). The mean age was 47.86 ± 11.17 years, and their mean BMI was $36 \pm 4.78\,kg/m^2$. The second group was composed of 169 nonobese unrelated subjects, who are personnel volunteers of the hospital (BMI $< 30\,kg/m^2$) (mean age 42.16 ± 14.26 years; mean BMI $24.51 \pm 3.69\,kg/m^2$). In both groups, we excluded the patients taking lipid-lowering drugs and all subjects having renal failure, hepatic pathology, or/and dysthyroidia.

All the study participants were individually interviewed by a structured questionnaire, for information on sociodemographic characteristics, details of personal history, presence of diseases, drug intake, if any, and smoking habits. The participants underwent physical examinations and laboratory tests. The examiners undertook training in the questionnaire collections and measures.

The study was approved by the ethics committee of the Medical Hospital, and informed consent was obtained from all study subjects.

Assessment of dietary intake

Subjects were privately interviewed, face to face; trained interviewers using pretested questionnaires conducted the interviews. Dietary intake assessment was undertaken with 3 day recalls. Two days were randomly selected from weekdays, and the third one was selected from the weekend (Moshfegh *et al.*, 2008). Standard reference tables were used to convert household portions to grams for computerization. After coding of diaries, the dietary recall form was linked to a nutrient database (Dietetik® designed for Tunisian foods and Nutrilog®), and daily energy intake for each individual was determined.

Anthropometric parameters and blood pressure measurements

Weight and height were measured on the subjects who were barefooted and lightly clothed. BMI was calculated as body weight (kg)/height^2 (m^2), and obesity was defined as BMI $\geq 30\,kg/m^2$ (WHO, 1995). Waist circumference (WC) was measured by a trained examiner from the narrowest point between the lower borders of the rib cage and the iliac crest.

Blood pressure was read thrice from the left arm of seated subjects with a blood pressure monitor after 20 min of rest. The average of the two last measurements was recorded for each subject.

Biochemical measurements

Blood samples were collected from subjects after a 12 h overnight fast. Serum total cholesterol (TC) and triglycerides (TG) were determined by standard assays. High-density lipoprotein cholesterol (HDL-C) was measured by direct assay. Low-density lipoprotein-cholesterol (LDL-C) concentrations were calculated with the Friedwald formula (Friedewald *et al.*, 1972) if TG < 4 mM. If not, LDL-C concentrations were measured by direct assay. Fasting glucose was measured by the glucose oxidase method.

All biochemical parameters were performed on a Synckrom CX7 Clinical System (Beckman, Fullerton, CA). The TC/HDL-C ratio was calculated.

Insulin concentration was measured by microparticule immunoassay on an AxSym® Abbott (Abbott laboratories, Abbott Parck, IL).

Insulin resistance was evaluated by the homeostasis model assessment (HOMA) using the following equation: homeostasis model assessment-insulin resistance (HOMA-IR) = {(Fasting insulin (μU/mL) × Fasting glucose (mM)}/22.5 (Matthews *et al.*, 1985).

Definitions of risk factors

Diabetes mellitus was defined as fasting glucose more than 7 mM or currently receiving antidiabetic medication (Alberti and Zimmet, 1998). Hypertension was defined as greater than 140/90 mmHg of current or antihypertensive medication (Chobanian *et al.*, 2003). Dyslipidemia was defined as an LDL-C concentration more or equal to 4.1 mM and/or an HDL-C concentration less or equal to 1 mM and/or a TG concentration more or equal to 1.71 mM (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001). Metabolic syndrome (MetS) was defined according to the International Diabetes Federation definition 2005 (Alberti *et al.*, 2005).

DNA analysis

Genomic DNA was isolated from peripheral blood leucocytes by the salting-out method (Miller *et al.*, 1988).

Genotyping of *LEP* G2548A and *LEPR* Q223R was carried out using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. DNA was amplified using the following flanking primers previously described: F: 5′-AACTCAACGACACTCTCCTT-3′ and R: 5′-TGAACTGAC ATTAGAGGTGAC-3′ for the *LEP* gene. F: 5′-GCCTAATCC AGTATTTTCATATCTG-3′ and R: 5′-GCCACTCTTAATACC CCCAGTAC-3′ for *LEPR* gene polymorphism (Constantin

Table 1. Clinical and Biochemical Characteristics of the Study Population

Variables	Population: Nonobese (n = 169)	Population: Obese (n = 160)	p
Age (years)	43.25 ± 13.12	48.41 ± 10.92	< 0.001
Sex-ratio (men/women)	0.594	0.221	0.001
Smoking *n* (%)	29 (17.2)	11 (6.9)	0.004
Weight (kg)	66.94 ± 11.29	93.60 ± 12.97	< 0.001
BMI (kg/m^2)	24.73 ± 3.50	36.6 ± 4.8	< 0.001
WC (cm)	89.09 ± 13.67	117.85 ± 12.61	< 0.001
Hypertension *n* (%)	23 (13.6)	72 (45.3)	< 0.001
Diabetes *n* (%)	32 (18.9)	54 (34)	0.002
Cardiovascular disease *n* (%)	0 (0)	22 (14)	< 0.001
Dyslipidemia *n* (%)	82 (48.5)	78 (49.1)	0.923
MetS *n* (%)	20 (12)	77 (48.1)	< 0.001
Fasting insulin (μU/mL)	6.43 ± 4.05	10.62 ± 9.31	< 0.001
HOMA-IR	1.35 ± 0.800	3.69 ± 4.92	< 0.001
TC (mM)	4.75 ± 1.1	5.17 ± 1.25	< 0.001
TG (mM)	0.98 ± 0.61	1.33 ± 0.69	< 0.001
HDL-C (mM)	1.31 ± 0.49	1.13 ± 0.34	0.009
LDL-C (mM)	3.27 ± 0.94	3.24 ± 0.99	0.262
Coffee consumption *n* (%)	125 (74.2)	98 (61.5)	0.009
Daily energy intake (kcal)	3092 ± 1541	3204 ± 1333	0.636

Mean ± standard deviation or *n* (%).
BMI, body mass index; HOMA-IR, homeostasis assessment model insulin resistance; TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein-cholesterol; LDL, low density lipoprotein-cholesterol; MetS, metabolic syndrome; WC, waist circumference.

et al., 2010). PCR products (262 bp for *LEP* and 416 bp for *LEPR*) were detected on 2% agarose gels containing ethidium bromide. Aliquots of the PCR products were digested for 3 h at 37°C with 3 U *Hha*I or *Msp*I restriction enzymes for the *LEP* G2548A and *LEPR* Q223R polymorphisms, respectively. The restricted fragments were separated on 2% agarose gel electrophoresis. The digestion resulted in 181 and 71 bp fragments for the A allele and 252 bp for the G allele. With regard to *LEPR* Q223R, the digestion resulted in 229 and 187 bp fragments for the R allele and 416 bp fragments for the Q allele.

Statistical analysis

Statistical analyses were performed by SPSS 17.0. The biological parameters values were reported as means ± standard deviation and were compared by the student's *t* test. Categorical variables were analyzed by the chi-square test or by Fisher's Exact Test for small numbers. We used the SNP analyzer 2 program to test the genotype frequencies for the Hardy–Weinberg equilibrium and to determine haplotypes frequencies (Lowontin, 1984; Yoo *et al.*, 2005). Odds ratios (ORs), two-tailed *p*-values, and 95% confidence interval (CI) were calculated as a measure of the association of the SNPs with the presence of obesity. ORs were adjusted to confounder parameters (all parameters that show a $p < 0.25$ between the two groups) by logistic binary regression. A *p*-value of < 0.05 was considered statistically significant for all tests.

Results

Patient characteristics

The clinical and biological characteristics of these subjects were summarized in Table 1.

There were no significant differences in dyslipidemia frequency, daily energy intake, and LDL-C concentrations between the two groups.

The prevalence of hypertension, diabetes, MetS, and personal cardiovascular disease history were higher in the obese than in the nonobese group ($p < 0.001$).

Compared with the nonobese, the obese subjects had higher weight, WC, TG, TC, HOMA-IR, and insulin levels and but lower HDL-C concentration and coffee consumption.

Genotype frequencies

The genotype distributions for the *LEP* G2548A, *LEPR* Q223R polymorphisms and haplotypes combination in the total studied population, obese and nonobese subjects are presented in Table 2. The AA genotype at the 2548 *LEP* gene was more frequent in the obese (28.12%) than in the nonobese group (13.6%) $p = 0.001$. The GG genotype was more frequent in the nonobese (49.12%) than in the obese group (29.38%) $p = 0.0002$. The frequencies of Q, R alleles of *LEPR* Q223R

Table 2. Genotype Frequencies

SNPs	Genotypes	Study population % (n) (329)	Obese % (n) (160)	Nonobese % (n) (169)	p^a	p^b
G2548A	GG genotype	39.51 (130)	29.38 (47)	49.12 (83)	0.0002	< 0.001
	GA genotype	39.81 (131)	42.5 (68)	37.28 (63)	0.337	
	AA genotype	20.68 (68)	28.12 (45)	13.6 (23)	0.001	
Q223R	QQ genotype	41.34 (136)	36.25 (58)	46.16 (78)	0.068	0.176
	QR genotype	40.12 (132)	41.87 (67)	38.46 (65)	0.527	
	RR genotype	18.54 (61)	21.88 (35)	15.38 (26)	0.130	
Haplotype G2548A/Q223R	GQ allele	0.336 (114)	0.273 (44)	0.413 (70)	0.008	0.0005
	AQ allele	0.273 (89)	0.295 (47)	0.248 (42)	0.355	
	GR allele	0.228 (75)	0.236 (38)	0.221 (37)	0.890	
	AR allele	0.161 (51)	0.194 (31)	0.116 (20)	0.028	

[a]Comparison between obese and nonobese subjects for each genotype.
[b]Comparison between obese and nonobese subjects for overall genotypes.
SNPs, single nucleotide polymorphisms.

Table 3. Characteristics of the Study Population According to *LEP* G2548A and *LEPR* Q223R Genotypes

	G2548A			*Q223R*			
SNPs Parameters	*GG*	*GA*	*AA*	*QQ*	*QR*	*RR*	*p*
BMI (kg/m^2)	30.66 ± 7.38	31.17 ± 7.47	34.14 ± 8.10	30.57 ± 7. 9	31.07 ± 7.19	33.93 ± 8.0	0.007[a] 0.047[b]
Daily energy intake (kcal)	2853 ± 1215	2889 ± 1277	3431 ± 1609	2871 ± 1318	2941 ± 1256	3513 ± 1596	0.048[a] 0.001[b]
TC (mM)	4.46 ± 0.96	4.92 ± 1.20	4.69 ± 1.09	4.5 ± 0.96	4.7 ± 1.12	4.9 ± 1.26	0.005[a] 0.013[b]
HDL-C (mM)	1.23 ± 0.39	1.18 ± 0.41	1.08 ± 0.36	1.3 ± 0.41	1.17 ± 0.42	1.09 ± 0.32	0.014[a] 0.003[b]
TG (mM)	1.08 ± 0.53	1.15 ± 0.53	1.14 ± 0.64	1.03 ± 0.54	1.19 ± 0.58	1.16 ± 0.51	0.671[a] 0.063[b]
Glycemia (mM)	6.78 ± 3.62	6.32 ± 2.28	6.29 ± 2.74	6.01 ± 2.13	6.52 ± 3.09	7.34 ± 3.75	0.401[a] 0.015[b]
Insulinemia (μU/mL)	6.77 ± 5.16	7.72 ± 5.89	8.86 ± 7.07	6.45 ± 4.41	8.95 ± 7.67	8.18 ± 5.2	0.046[a] 0.010[b]
WC (cm)	101.27 ± 18.66	107.69 ± 20.79	112.17 ± 21.72	104.25 ± 21.37	106.78 ± 19.30	109.39 ± 21.52	0.001[a] 0.260[b]
HOMA-IR	1.85 ± 1.60	2.07 ± 1.71	2.16 ± 2.65	1.57 ± 1.41	1.88 ± 1.66	2.89 ± 2.4	0.612[a] < 0.001[b]

[a]Comparison for G2548A.
[b]Comparison for Q223R.

polymorphisms were, respectively, 0.57 and 0.43 in the obese group and 0.65 and 0.35 in the nonobese group.

Haplotypes containing the two mutated alleles A2548 and R223 were more frequent in the obese than in the nonobese group ($p = 0.028$).

Characteristics of the study population according to LEP *G2548A and* LEPR *Q223R genotypes*

An analysis of metabolic characteristics across genotypes is provided in Table 3. The 2548AA genotype had significantly higher BMI ($p = 0.007$), daily energy intake ($p = 0.048$), TC ($p = 0.005$), WC ($p = 0.001$), and insulinemia ($p = 0.046$) and low HDL-C levels ($p = 0.014$). With regard to the 223 RR genotype, we noted a significantly higher BMI ($p = 0.047$), daily energy intake ($p = 0.001$), TC ($p = 0.013$), glycemia ($p = 0.015$), insulinemia ($p = 0.010$), and HOMA-IR index ($p = 0.001$) and low HDL-C levels ($p = 0.003$) compared with QQ and QR genotypes.

Association between LEP *polymorphisms and MetS risk*

Table 4 shows the association of ORs and 95% CI of *LEP* SNP G2548A and *LEPR* SNP Q223R to MetS. It shows that SNP Q223R has no association with MetS risk. With regard to G2548A, the OR of MetS associated with 2548AA was 1.63 [1.026–2.6] $p = 0.035$. This significant association between G2548A and MetS risk persists after adjustment to age, smoking, gender, coffee consumption, and daily energy intake.

Association between LEP *polymorphisms and obesity risk*

In the total studied population, analysis of *LEP* G2548A polymorphism showed that the OR of obesity associated with 2548AG and 2548AA was 1.81 [1.10–2.98] $p = 0.019$ and 3.405 [1.78–6.48] $p < 0.001$ respectively. Concerning *LEPR* Q223R, RR genotype was significantly associated with obesity (OR = 1.74, CI [1.136–3.236], $p = 0.037$). When we stratified the studied population according to the degree of obesity, we found this significant for the group's majority except the overweight group. With regard to Q223R, we lost this significance after stratification except for the overweight group.

All previously described significant associations persist after adjustment to age, smoking, gender, and coffee consumption. However, when we add daily energy intake in adjustment, this significant association disappears (Table 5).

Table 4. Association Between Leptin Polymorphisms and Metabolic Syndrome Risk

SNPs	*Genotypes*	*OR*	*CI*	*p*
G2548A	GA/GG	1.23	0.936–1.637	0.132
		1.56[a]	0.85–2.85	0.147
		1.42[b]	0.556–2.87	0.575
	AA/GG	1.63	1.026–2.60	0.035
		1.51[a]	1.03–2.27	0.034
		1.47[b]	1.09–2.42	0.031
Q223R	QR/QQ	1.76	1.01–3.07	0.052
		1.62[a]	0.883–2.97	0.119
		2.05[b]	0.991–4.24	0.053
	RR/QQ	1.72	0.888–3.34	0.120
		1.29[a]	0.767–1.661	0.540
		0.970[b]	0.621–1.51	0.893

[a]After adjustments for age, smoking, gender, and coffee consumption.
[b]After adjustments for age, smoking, gender, coffee consumption, and daily energy intake.
OR, odds ratio; CI, confidence interval.

Table 5. Association Between Leptin Polymorphisms and Obesity Risk

SNPs	*Population*	*Genotypes*	*OR*	*CI*	*p*
G2548A	Study population	GA/GG	1.81	1.10–2.98	0.019
			1.75[a]	1.016–3.032	0.044
			1.69[b]	0.966–2.95	0.066
		AA/GG	0.40	1.78–6.48	< 0.001
			1.87[a]	1.106–2.78	0.028
			1.68[b]	0.93–2.66	0.052
	Overweight (25–30 kg/m^2)	GA/GG	1.06	0.96–1.44	0.302
			0.80[a]	0.208–1.22	0.129
			0.79[b]	0.300–1.30	0.102
		AA/GG	1.81	0.855–3.82	0.118
			2.01[a]	0.901–4.49	0.088
			2.25[b]	0.76–7.52	0.186
	Moderate and severe obesity (30–40 kg/m^2)	GA/GG	1.28	0.68–2.42	0.441
			0.93[a]	0.440–1.99	0.865
			0.84[b]	0.391–1.829	0.670
		AA/GG	2.33	1.012–5.37	0.048
			2.01[a]	1.21–3.36	0.007
			1.60[b]	0.888–2.912	0.117
	Morbid obesity (> 40 kg/m^2)	GA/GG	0.70	0.282–1.736	0.441
			0.66[a]	0.191–2.35	0.531
			0.46[b]	0.07–3.045	0.421
		AA/GG	2.45	0.985–6.779	0.048
			1.78[a]	1.085–3.56	0.042
			1.19[b]	0.527–2.71	0.367
Q223R	Study population	QR/QQ	1.36	0.837–2.236	0.211
			1.22[a]	0.727–2.073	0.442
			0.83[b]	0.436–1.612	0.597
		RR/QQ	1.74	1.136–3.236	0.037
			1.41[a]	1.035–1.854	0.045
			1.15[b]	0.786–1.69	0.461
	Overweight (25–30 kg/m^2)	QR/QQ	1.96	0.886–4.35	0.095
			1.97[a]	0.789–4.95	0.146
			1.44[b]	0.630–16.02	0.230
		RR/QQ	1.8	1.641–5.05	0.049
			2.51[a]	1.08–5.81	0.032
			1.72[b]	0.910–3.28	0.095
	Moderate and severe obesity (30–40 kg/m^2)	QR/QQ	1.92	1.018–3.65	0.043
			1.81[a]	0.876–3.74	0.109
			1.53[b]	0.623–3.75	0.353
		RR/QQ	2.05	0.902–4.69	0.083
			1.46[a]	0.906–2.37	0.119
			1.53[b]	0.623–3.75	0.353
	Morbid obesity (> 40 kg/m^2)	QR/QQ	1.63	0.684–3.916	0.267
			2.67[a]	0.783–9.14	0.117
			1.72[b]	0.406–7.28	0.461
		RR/QQ	2.25	0.783–0.46	0.128
			1.64[a]	0.816–3.33	0.164
			1.79[b]	0.602–5.33	0.295

[a]After adjustments for age, smoking, gender, and coffee consumption.
[b]After adjustments for age, smoking, gender, coffee consumption, and daily energy intake.

Association of LEP haplotypes with obesity and MetS risks

When haplotypes of the two polymorphisms were assessed, we noted that haplotype model AR or AQ possessing 2548A + 223R or 2548A + 223Q seems to increase the risk of obesity OR = 3.36; $p = 0.005$ and OR = 2.56; $p = 0.010$, respectively. After adjustment to age, smoking, gender, and coffee consumption, these significant associations persist. However, significance disappears when we add daily energy intake in adjustment.

Haplotype models AR or AQ seem to increase the risk of MetS. This association persisted after we had adjusted to daily energy intake (Table 6).

Table 6. Association Between Leptin Haplotypes Obesity and Metabolic Syndrome Risk in Study Population

	Obesity risk			*MetS risk*		
Genotypes	*OR*	*CI*	*p*	*OR*	*CI*	*p*
AQ/GQ	2.56	1.245–5.266	0.010	1.35	0.572–3.210	0.526
	2.85[a]	1.307–6.12	0.008	2.81[a]	1.094–8.83	0.047
	1.76[b]	0.675–4.608	0.247	3.30[b]	1.052–11.44	0.039
GR/GQ	1.69	0.817–3.51	0.155	1.41	0.631–3.16	0.438
	1.29[a]	0.869–1.939	0.203	1.102[a]	0.821–1.481	0.518
	0.80[b]	0.471–1.360	0.410	1.058[b]	0.738–1.51	0.759
AR/GQ	3.36	1.71–6.59	< 0.001	4.037	1.73–9.42	0.001
	1.52[a]	1.191–1.943	0.001	1.19[a]	1.35–3.56	0.002
	1.28[b]	0.925–1.773	0.136	3.22[b]	1.37–11.99	0.001

[a]After adjustments for age, smoking, gender, and coffee consumption.
[b]After adjustments for age, smoking, gender, coffee consumption, and daily energy intake.

Discussion

In this study, we reported an association of the *LEP* G2548A polymorphism in the *LEP* gene and *LEPR* Q223R polymorphism in the *LEPR* with MetS and obesity risk and with MetS parameters in Tunisians volunteers.

Previous analyses of the association between the *LEP* variant and obesity or BMI have been controversial. The allele frequency of the G2548A polymorphism observed in our study is in accordance with that of other studies (Le Stunff *et al.*, 2000; Mammès *et al.*, 2000; Walley *et al.*, 2006; Hinuy *et al.*, 2008, 2010; Huuskonen *et al.*, 2010) except for one population from Taiwan, where the G allele frequency was lower than the A allele (Wang *et al.*, 2006).

The *LEP* 2548G > A variant may influence the *LEP* gene expression and the leptin secretion by adipose tissue (Cummings and Schwartz, 2003). The association of the *LEP* G2548A polymorphism and obesity risk found in our study was also reported in overweight Europeans (Mammès *et al.*, 2000), in a sample of Taiwanese Aborigines' with extreme obesity (Wang *et al.*, 2006), in Brazilian women (Hinuy *et al.*, 2008), and in Finnish men (Huuskonen *et al.*, 2010). However, numerous studies have failed to demonstrate an association between this polymorphism and increased BMI or MetS parameters (Mattevi *et al.*, 2002; Portolés *et al.*, 2006; Ben Ali *et al.*, 2009; Gottlieb *et al.*, 2009). These different results may arise from interactions of the G2548A polymorphism with environmental factors or other polymorphisms in *LEP* and/or *LEPR* genes, sample size of a population, or from the model used in statistical analysis.

The G2548A polymorphism is located at the 5′ end of the promoter region of *LEP*, and it has been suggested that this remote region may contain inhibitory elements from transcription in adipocytes (Gong *et al.*, 1996). Even though this polymorphism is close to an SP-1 transcription factor binding site, as well as two repetitive sequences MER11 and Alu that may regulate *LEP* transcription, the effect of the G to A substitution at nt 2548 in *LEP* expression remains to be elucidated.

Conflicting results regarding the association between the *LEPR* Q223R polymorphism, obesity, and MetS parameters have been reported. Genotype and allele frequencies observed in our study were similar to those found in other populations (Duarte *et al.*, 2007; Ben Ali *et al.*, 2009; Constantin *et al.*, 2010) except for the Japanese population, where the R allele frequency was higher than the Q allele (Takahashi-Yasuno *et al.*, 2003). Our results showed an association between the RR genotype, overweight, and obesity risk. Many studies suggested that the Q223R polymorphism is associated with high blood pressure, hypertriglyceridemia, hypoHDLemia, insulin resistance, and obesity risk (Chagnon *et al.*, 2000; Quinton *et al.*, 2001; Wauters *et al.*, 2002; Van der Vleuten *et al.*, 2006). Gottlieb *et al.* (2009) reported that the RR genotype increases the predisposition to MetS. However, Heo *et al.* (2002), in a meta-analysis of 18 studies, indicated that there is no association between the *LEPR* Q223R polymorphism, BMI, and WC in the general population. In contrast, some studies found an association between the 223QQ genotype and hypertension, HDL-C levels, and other lipid parameters (Rosmond *et al.*, 2000; Takahashi-Yasuno *et al.*, 2003).

The functional significance of this allele genotype is not entirely known. However, several lines of evidence suggest that this polymorphism may play a role in the pathogenesis of obesity. The Q223R polymorphism is located in the extracellular region of the *LEPR* within the first cytokine domain (C domain), which represents a leptin binding site. It has been previously suggested that this single amino acid changed from neutral to positive, could affect the functionality of the receptor, and alter its signaling capacity (Chung *et al.*, 1997; Chagnon *et al.*, 1999, 2000).

In our haplotype analysis, the AR- or AQ-containing A allele at G2548A is most frequent in the obese group. Duarte *et al.* (2007) reported that a haplotype combination of *LEP* and which seems to increase G2548A and *LEPR* Q223R variants was related to a 58% increase in obesity risk. Our study showed that when the 2548A was a variant form, there was a significant association with obesity. All significant associations between polymorphisms and obesity were maintained after adjustment to age, smoking, gender, and coffee consumption but disappear when we added daily energy intake in adjustment.

This loss of significativity between obesity risk and SNPs or haplotypes after adjustment to daily energy intake showed that the statistical effect of polymorphisms is not independent of daily energy intake. With regard to the physiological effect, this loss of association can be explained by the possible effect of polymorphisms on leptinemia, which influences food intake. In fact, our study showed that *LEP* 2548AA was associated to increased daily energy intake, and many studies reported that the AA genotype is associated with decreased leptin levels (Le Stunff *et al.*, 2000; Ren *et al.*, 2004; Hinuy *et al.*, 2008).

Leptin is a key element that is involved in the regulation of food intake. Indeed, it is well established that leptin decreases the food intake while stimulating the appearance of melanocortin, leading to an increase in the hypothalamic hormone: Alpha melanocyte-stimulating hormone (α-MSH). The α-MSH, while binding to its hypothalamic receptor MC4, decreases the food intake. Besides, the leptin inhibits the activity of the orexigen neurons type hypothalamic neuropeptide Y/agouti-related protein, which is responsible for the hunger sensation. The leptin decrease causes some upset in this mechanism, leading to an increase in the food intake, thus obesity (Zhang *et al.*, 1994; Inui, 1999).

The disappearance of the association between Q223R and the obesity risk after adjustment to daily energy intake can be explained by the relation between Q223R and food intake caused by leptin resistance. In fact, Q223R affects the functionality of the receptor and alters its signaling pathways; afterward hunger sensation is altered (Chung *et al.*, 1997; Chagnon *et al.*, 1999, 2000). This may explain why the carriers of mutations at Q223R increase food intake and obesity. In fact, our study showed that QR and RR genotypes were associated with increased daily energy intake and obesity risk.

We reported a significant association between MetS risk, *LEP* G2548A SNP, and the haplotype model containing the A allele of G2548A; this relation persists after adjustment to daily energy intake. This can be explained by the metabolic effect of leptin in addition to food intake. The leptin exercises metabolic actions, which can be related to its effect on the regulation of the weight: The specific loss of fat mass among animals treated to the leptin would be due to the inhibition of the lipogenese and to the stimulation of β-oxidation of fatty acids. Leptin decreases the secretion of insulin by β-cells in the pancreas and increases the sensibility in the insulin. It decreases the action of the insulin on the adipocytes (Seufert, 2004); therefore, it participates in the control of the regulation of the glycemia. In the liver, leptin inhibits the glycogenolysis, which is accompanied by an increase of β-oxidation of fatty acids (Rossetti *et al.*, 1997). With regard to oxidative-type muscle, at rest and contraction, it has been shown that the leptin decreases the stocks of intramuscular TG by increasing their hydrolysis and increases the β-oxidation of fatty acids (Steinberg *et al.*, 2002).

Our study has some limitations. The first is that serum leptin level was not measured and, thus, the pathophysiologic mechanism underling the association could not be further dissected. The second limitation is the small number of our study population. The third limitation is the absence of the measurement of fat mass and body fat distribution besides BMI and hormones such as estrogen that might potentially affect leptin production.

In conclusion, this study showed that *LEP* G2548A and *LEPR* Q223R polymorphisms and a haplotype combination in Tunisian subjects were associated with MetS parameters and with obesity risk. However, the significance between obesity and SNPs disappears after we added daily energy intake in adjustment, suggesting an influence of this polymorphism on the leptin effect on satiety and energy intake. Further research is necessary to ascertain the potential implications of the *LEP* and *LEPR* gene variants that explain the differences in the incidence and etiology of obesity, and to elucidate the pathological processes which link obesity with other features of metabolic disorders.

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Author Disclosure Statement

The authors report no conflicts of interest.

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Address correspondence to:
Prof. Ali Bouslama
Department of Biochemistry
Sahloul University Hospital
4054 Sousse
Tunisia

E-mail: ali.bouslama@rns.tn