Biochemical and Biophysical Research Communications 391 (2010) 282–286

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Leptin G-2548A and leptin receptor Q223R gene polymorphisms are not associated with obesity in Romanian subjects

Alina Constantin [a,*], Gabriela Costache [a], Anca V. Sima [b], Cristiana S. Glavce [c], Maria Vladica [d], Doina L. Popov [a]

[a] Department of Vascular Dysfunctions in Diabetes and Obesity, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania
[b] Department of Lipoproteins and Atherosclerosis, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania
[c] Department of Anthropology, Institute of Anthropology "Francisc I. Rainer", Bucharest, Romania
[d] Laboratory Medicine, Institute of Diabetes, Nutrition and Metabolic Diseases "Nicolae Paulescu", Bucharest, Romania

ARTICLE INFO

Article history:
Received 27 October 2009
Available online 12 November 2009

Keywords:
Obesity
Leptin
Leptin receptor
Polymorphism

ABSTRACT

We aimed to investigate whether polymorphisms *LEP* G-2548A and *LEPR* Q223R in the human leptin (*LEP*), and leptin receptor (*LEPR*) genes are associated with obesity and metabolic traits in a sample of Romanian population. Two hundred and two subjects divided in obese (body mass index, BMI $\geqslant$ 30 kg/m^2), and non-obese were included in this study. The polymorphisms were genotyped using polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) analysis. The results showed no significant differences in *LEP* and *LEPR* genotype and allele frequencies between obese and non-obese subjects. Logistic regression analysis showed that *LEP* -2548GG genotype presented an increased risk of obesity ($p = 0.013$, OR = 1.003, 95% CI = 1.000–1.007), after adjusting for age and gender. The association analysis with metabolic syndrome quantitative traits showed that homozygous for *LEP* -2548G allele had significantly higher leptin levels (17.2 ± 6.6 ng/ml vs. 13.2 ± 4.9 ng/ml, $p = 0.011$), and carriers of R allele had higher levels of triglycerides ($p = 0.017$) and glucose ($p = 0.040$), and enhanced systolic ($p = 0.015$) and diastolic blood pressure ($p = 0.026$), after adjustment for age, gender, and BMI. These results indicate that LEP G-2548A and LEPR Q223R SNPs may not be considered as genetic risk factors for obesity in a sample of Romanian population. However, LEP -2548GG genotype appear to be important in regulating leptin levels, whereas the LEPR 223R allele might predispose healthy subjects to develop metabolic disturbances.

Obesity arises from a complex interaction between genetic variance, environment, and lifestyle changes [1]. Obesity is one of the most challenging health problems of the last century with a tremendous increase in the prevalence, considered to be an important risk factor for type 2 diabetes and cardiovascular diseases [2].

Leptin, the *obese* (*ob*) gene product [3] acts to reduce food intake and to increase energy expenditure [4], and plays an important role both in the development of obesity and in insulin secretion [5]. Leptin exerts its pleiotropic actions directly through distinct receptors (Ob-R) encoded by the diabetes (*db*) gene [6]. In humans, *LEP* and *LEPR* have been mapped to 7q31.3 [7] and 1p31 [6], respectively. The long form of leptin receptor OB-Rb is thought to be essential in leptin's weight-reducing effects through the hypothalamus [7,8].

Serum leptin level is significantly increased in obese persons and is proportionally with body adiposity [9]. In type 2 diabetes leptin levels have been reported to be either unchanged [10] or reduced [11].

The leptin (*LEP*) and leptin receptor (*LEPR*) genes have been investigated in the search for gene variants potentially related to the pathophysiology of obesity, diabetes and its associated complications [12]. A leptin gene SNP consisting in G to A substitution at nucleotide (nt) -2548 upstream of the ATG start site in the *LEP* gene promoter, *LEP* G-2548A, has been associated with adipocytes increased leptin production and secretion [13–15]. Interestingly, as for *LEPR* gene polymorphism, the A to G transition in exon 6 at nt 668 from the start codon 223 (Q223R) was associated with impaired leptin-binding activity [16]. The *LEPR* Q223R polymorphism has been associated with body mass index (BMI), fat mass, leptin levels, and systolic and diastolic blood pressure [17]. There are few studies dealing with the association between *LEP* G-2548A and *LEPR* Q223R polymorphisms, type 2 diabetes, and obesity status. Thus, in the Chinese population, *LEP* -G2548A showed a positive correlation with incidence of type 2 diabetes [18]. Recently,

* Corresponding author. Address: Department of Vascular Dysfunctions in Diabetes and Obesity, Institute of Cellular Biology and Pathology "Nicolae Simionescu", 8, B.P. Hasdeu Street, P.O. Box 35-14, Bucharest 050568, Romania. Fax: +40 21 319 45 19.
E-mail address: alina.constantin@icbp.ro (A. Constantin).

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.11.050

it was reported that *LEPR* Q223R polymorphism has been associated with impaired glucose tolerance and conversion to type 2 diabetes [19], and insulin resistance [20]. The aim of this study was to investigate whether two common single nucleotide polymorphisms (SNPs) in the leptin (*LEP* G-2548A) gene and its receptor (*LEPR* Q223R) gene are related to obesity in a sample of urban Romanian population. Also, the influence of the *LEP* G-2548A and the *LEPR* Q223R polymorphisms on the variability of metabolic phenotypes (anthropometric variables, glucose, insulin and leptin concentration, and lipid profile) was investigated.

Subjects and methods

Subjects. We studied 202 unrelated Romanian subjects divided in obese ($n = 108$) and non-obese ($n = 94$), according to their body mass index (BMI). Obesity was defined as BMI $\geqslant 30$ kg/m^2 based on criteria of the World Health Organization (WHO) classification [21]. All participants lived in the urban area of Bucharest and belonging to the same ethnic group. Anthropometric measurements (weight, height, waist circumference, WHR waist-to-hip ratio, fat mass) were taken for each individual. Diabetes mellitus was defined according to the WHO criteria [21]. Type 2 diabetes patients were under treatment (with oral hypoglycemic agents or insulin treatment) at the outpatient clinic of the Institute of Diabetes, Nutrition and Metabolic Diseases "Nicolae Paulescu". Hypertension was defined as systolic blood pressure (SBP) > 130 mm Hg and/or diastolic blood pressure (DBP) > 85 mm Hg. Hypertensive subjects were under antihypertensive medication. Written informed consent was obtained from each subject. The research protocol was approved by the Ethical Committee of the Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania.

Biochemical analysis. Venous blood was obtained after overnight fasting. Fasting glucose, insulin, total cholesterol, high density lipoprotein (HDL) cholesterol, and triglycerides levels were determined enzymatically using commercially available kits. HOMA-IR index (homeostatic model assessment for insulin resistance) was calculated using the formula: HOMA-IR = (Io $*$ Go)/22.5, where Go is fasting glucose (expressed as mg/dl) and Io is fasting insulin (expressed in μU/ml). The blood pressure readings were recorded with the participants sitting, after 5 min resting. Serum leptin concentrations (expressed as ng/ml) were measured by ELISA (Assay Design, Inc., USA). The sensitivity of the method was 0.8 ng/ml, and the intra-assay CV was less than 6%.

Genotyping. Genomic DNA was isolated from peripheral blood leukocyte using standard methods. Genotyping of *LEP* G-2548A and *LEPR* Q223R was carried out using PCR-RFLP assay. DNA was amplified using following flanking primers described previously—F: 5′-AACTCAACGACACTCTCCTT-3′ and R: 5′- TGAACTGACATTAG AGGTGAC-3′ for the *LEP* gene [22], F: 5′-AACTCAACGACACTCT CCTT-3′ and R: 5′-TGAACTGACATTAGAGGTGAC-3′ for *LEPR* gene polymorphism [23]. The protocol for PCR amplification was similar for both polymorphisms, and was carried out in 25 μl reaction mixture by using 100–200 ng DNA, 0.3 μM of each primer, 200 μM dNTPs, 1.5 mM $MgCl_2$, and 0.5 U of Taq DNA polymerase (Go Taq DNA Polymerase, Invitrogen), and 10 mM Tris–HCl, pH 7.4. The PCR consisted of an initial denaturation for 5 min at 94 °C, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 45 s, and extension at 72 °C for 60 s, and with a final extension at 72 °C for 5 min (Hybaid Thermal Cycler, UK). PCR products (262 bp for *LEP* and 80 bp for *LEPR*) were detected on 2% agarose gel containing ethidium bromide. Aliquots of the PCR products were digested for 3 h at 37 °C with 5 U HhaI or MspI restriction enzymes for the *LEP* G-2548A, and for the *LEPR* Q223R polymorphisms, respectively. The restricted fragments were separated either on 2% agarose gel electrophoresis for the *LEP* variants, or on 8% polyacrylamide gel electrophoresis for *LEPR* polymorphism.

Statistical analysis. The clinical and biochemical parameters were presented as mean values ± SD (standard deviation) and as percentage for categorical variables. Variables that were not distributed normally were log-transformed. Comparisons between obese and non-obese subjects were made using unpaired *t*-test. A goodness-of-fit test χ^2 test was used for evaluation of the Hardy–Weinberg equilibrium. Also, χ^2 tests were used to compare genotypes distribution and allele frequencies and other qualitative data. Analysis of covariance using the univariate general linear model (GLM) tool was performed to correct for the effect of covariates (age, gender, and BMI) in obese and non-obese groups. Univariate and multivariate logistic regression (backward stepwise criteria for selection of independent variables) was performed to evaluate the association of the *LEP* and *LEPR* genotypes and metabolic risk factors with obesity in our groups. Differences in anthropometric and metabolic characteristics between the genotypes (*LEP* -2548GG vs. *LEP* -2548GG+AA and *LEPR* 223QQ vs. *LEPR* 223QR+RR) were analyzed by unpaired *t*-tests. Differences were considered significant when $p < 0.05$. Statistical analyses were performed with SPSS for Window Version (10.1.1).

Results

The anthropometric and metabolic characteristics of the obese and non-obese subjects are presented in Table 1. The prevalence of diabetes and hypertension was higher in obese patients than in control group ($p < 0.001$). Obese individuals had higher levels of total cholesterol, LDL, and triglycerides, fasting glucose, fasting insulin, and an increased HOMA-IR index. Also, lower levels of HDL were observed in obese as compared to non-obese subjects ($p < 0.0001$). Serum leptin levels in obese were significantly higher than those in non-obese individuals ($p < 0.0001$).

The genotypes distribution and allele frequencies for the *LEP* G-2548A and *LEPR* Q223R polymorphisms in obese and non-obese subjects are presented in Table 2. Both polymorphisms are in the Hardy–Weinberg equilibrium. The frequencies of alleles G and A for *LEP* G-2548A polymorphism were: 0.63 and 0.37 in the obese group, and 0.57 and 0.43, respectively, in the non-obese group; *LEPR* Q223R polymorphism showed Q and R allelic frequencies of 0.54 and 0.46 in the obese group, and 0.63 and 0.37, respectively,

Table 1
Clinical and biochemical characteristics of the obese and non-obese subjects.

Characteristics	Obese ($n = 108$)	Non-obese ($n = 94$)	p
Gender (m/f, %)	30/78	28/66	0.752
Age (years)	51 ± 7	42 ± 11	<0.0001
BMI (kg/m^2)	37.3 ± 5.7	24.3 ± 3.6	<0.0001
Fat mass (kg)	41.8 ± 13.3	17.9 ± 5.7	<0.0001
Waist circumference (cm)	108.8 ± 12.0	82.6 ± 12.9	<0.0001
WHR	0.90 ± 0.01	0.83 ± 0.01	<0.0001
Leptin* (ng/ml)	18.3 ± 5.9	10.7 ± 2.3	<0.0001
Cholesterol (mg/dl)	222.9 ± 50.9	203.4 ± 47.8	0.006
HDL (mg/dl)	43.8 ± 12.2	53.1 ± 14.9	<0.0001
LDL (mg/dl)	140.9 ± 36.2	112.7 ± 79.8	0.008
Triglycerides* (mg/dl)	149.8 ± 83.5	88.82 ± 67.92	0.001
Fasting glucose (mg/dl)	248.61 ± 206.45	90.67 ± 16.39	<0.0001
Fasting insulin* (μU/ml)	9.71 ± 4.74	6.30 ± 1.55	<0.0001
HOMA-IR*	4.86 ± 2.89	1.38 ± 0.39	<0.0001
Diabetes (%)	51	4	<0.0001
Hypertension (%)	39	10	<0.0001

Number of individuals in parentheses. Continuous variables are presented as mean ± SD and were compared by *t*-test. Categorical variables were compared by χ^2. $p < 0.05$ was considered as significant.
* Natural logarithmic transformations were performed before analysis.

Table 2
Genotype distributions and allele frequencies of the *LEP* G-2548A and *LEPR* Q223R polymorphisms in obese and non-obese subjects.

LEP G-2548A polymorphism				*LEPR* Q223R polymorphism			
	Obese (n = 108)	Non-obese (n = 94)	p		Obese (n = 108)	Non-obese (n = 94)	p
GG	47 (43.5%)	34 (36.2%)	0.141	QQ	29 (26.9%)	33 (35.1%)	0.550
GA	42 (38.9%)	40 (42.6%)		QR	59 (54.6%)	52 (55.3%)	
AA	19 (17.6%)	20 (2.3%)		RR	20 (18.51%)	9 (9.6%)	
G allele	136 (63%)	23 (57%)	0.258	Q allele	117 (54%)	118 (63%)	0.08
A allele	80 (37%)	80 (43%)		R allele	99 (46%)	18 (37%)	

Data are presented as number of cases, with frequency in parentheses. Allele frequencies and genotypes distribution of the *LEP* G-2548A and *LEPR* Q223R in obese were compared to those in non-obese subjects.

in the non-obese group. No significant differences in genotype distribution and allele frequencies of the *LEP* G-2548A and *LEPR* Q223R polymorphisms have been found between obese and non-obese subjects.

To evaluate the association between obesity and metabolic and genetic (*LEP* -2548GG and *LEPR* 223 QR+RR genotypes) risk factors we performed univariate logistic regression. The results showed that diabetic subjects with hypertension had higher risk of obesity than diabetic patients ($p < 0.0001$, OR = 12.288, 95% CI = 5.339–28.282). Enhanced levels of leptin, as well as increased concentrations of total cholesterol, LDL, and triglycerides were also associated with obesity in the population under study (Table 3). Subjects bearing *LEP* -2548GG genotype showed an increased risk of obesity ($p = 0.013$, OR = 1.003, 95% CI = 1.000–1.007) than carriers of A allele, after adjusting the variable to the covariates age, and gender. Subsequent to the multivariate logistic regression analysis we found that leptin ($p = 0.011$, OR = 4.465, 95% CI = 1.402–14.218), fasting glucose ($p = 0.020$, OR = 1.034, 95% CI = 1.005–1.064), and DBP ($p = 0.014$, OR = 0.848, 95% CI = 0.745–0.967) were associated with obesity.

The influence of genetic variants *LEP* -2548GG vs. A allele, and *LEPR* 223QQ vs. R allele, respectively, on the phenotype of the subjects studied here was assessed by general linear model, after adjustment for covariate age, gender, and BMI (Table 4). Subjects homozygous for *LEP* -2548GG had higher serum leptin levels, as compared with carriers of A allele (17.2 ± 6.6 ng/ml vs. 13.2 ± 4.9 ng/ml, $p = 0.011$) (Table 4). As for *LEPR* Q223R polymorphism, subjects carrying the R allele showed a non-significant trend toward a higher serum leptin levels than the subjects without this allele (15.7 ± 5.9 ng/ml vs. 14.9 ± 6.2 ng/ml). However, carriers of R allele in the studied group had significantly increased levels of total fasting glucose ($p = 0.040$), triglycerides ($p = 0.017$), and decreased levels of HDL ($p = 0.035$), and raised SBP ($p = 0.015$) and DBP ($p = 0.026$), with respect to homozygous for Q allele.

Table 3
Univariate logistic regression analysis of the variables associated with obesity.

Variables	p	Odds ratio	95% confidence interval
Age	<0.0001	1.101	1.064–1.140
Leptin	0.036	0.903	0.821–0.993
Cholesterol	0.008	1.008	1.002–1.015
LDL	0.009	1.010	1.003–1.018
HDL	<0.0001	0.951	0.929–0.974
Triglycerides	0.005	1.006	1.002–1.010
Diabetes	<0.0001	11.749	5.583–24.726
Hypertension	<0.0001	12.288	5.339–28.282
LEP -2548GG[#]	0.013	1.003	1.000–1.007
LEPR 223QR+RR[#]	0.054	0.988	0.976–1.002

[#] Adjusted to the covariates age and gender.

Discussion

In this study, we reported no association between the *LEP* G-2548A polymorphism in the leptin gene and *LEPR* Q223R polymorphism in the leptin receptor gene and obesity, in a sample of the Romanian population. Furthermore, we found no association of the *LEP* 2548G/A polymorphism with common obesity-related variables (BMI, fat mass, waist circumference, and WHR), and metabolic traits. However, significant and independent association between the *LEP* -2548GG carrier status and higher leptin levels was identified. Previous studies analyzing the association between the *LEP* G-2548A and obesity or BMI have been controversial. An association of the *LEP* G-2548A polymorphism and increased BMI was reported in overweight Europeans [24] and in a sample of Taiwan-

Table 4
Characteristics of the study population according to *LEP* G-2548A and *LEPR* Q223R genotypes.

Variables	*LEP* G-2548A			*LEPR* Q223R		
	GG (n = 81)	GA+AA (n = 121)	p^*	QQ (n = 62)	QR+RR (n = 140)	p^*
Age (years)	48 ± 9	46 ± 11	NS	47 ± 10	47 ± 10	NS
Cholesterol (mg/dl)	213.9 ± 50.0	213.8 ± 51	NS	219.1 ± 46.0	211.50 ± 52.1	NS
HDL (mg/dl)	45.1 ± 14.1	52.6 ± 15.4	NS	52.5 ± 14.2	46.3 ± 14.0	0.035
LDL (mg/dl)	137.0 ± 41.3	131.2 ± 38.2	NS	138.3 ± 41..5	131.5 ± 38.5	NS
Triglycerides[*] (mg/dl)	135.1 ± 84.9	131.4 ± 83.2	NS	125.3 ± 94.9	135.9 ± 79.1	0.017
Fasting glucose (mg/dl)	143.7 ± 65.4	145 ± 67.8	NS	133.6 ± 67.0	148.7 ± 66.3	0.040
Fasting insulin[*] (μU/ml)	8.6 ± 3.8	8.60 ± 4.0	NS	8.95 ± 3.6	8.5 ± 4.0	NS
HOMA-IR[*]	3.2 ± 2.6	3.29 ± 2.63	NS	3.0 ± 2.3	3.3 ± 2.7	NS
SBP (mm Hg)	141 ± 22	136 ± 24	NS	132 ± 22	141.5 ± 23.3	0.015
DBP (mm Hg)	79 ± 11	78 ± 14	NS	75 ± 14	80 ± 12	0.026
Leptin[*] (ng/ml)	17.2 ± 6.6	13.2 ± 4.9	0.011	14.9 ± 6.2	15.7 ± 5.9	NS

Data are means ± SD for the anthropometric and clinical characteristics of all subjects in this study. Metabolic parameters were adjusted by covariates (age, gender, and BMI); BMI was adjusted for age and gender. The differences between means were analyzed by unpaired t-test. Results were presented for the dominant genetic model (homozygous for *LEP* -2548GG vs. carriers of A allele and homozygous for *LEPR* 223QQ vs. carriers of R allele, respectively). NS, non-significant.
[*] Natural logarithmic transformations were performed before analysis.

ese Aborigines with extreme obesity [25]. On the other hand, numerous studies have failed to demonstrate an association among this polymorphism and increased BMI [26,27]. Our data point to a relationship between *LEP* -2548GG variant and increased serum leptin levels in healthy subjects, and similar results were also described for obese European, Brazilians, and Tunisian individuals [14,28,29], and diabetics from Asian populations [18]. Moreover, the *LEP* G allele was found to be associated with increased free leptin levels through an interaction between fat mass and gender in healthy Greek individuals [30]. Conversely, carriers of *LEP* G allele had lower leptin concentrations adjusted for fat mass in overweight healthy men from French population [24]. These different results may arise from interactions of G-2548A polymorphism with other polymorphisms in leptin and/or leptin receptor genes, sample size of population, or from to the model used in statistical analysis.

Also, conflicting results regarding the association between *LEPR* Q223R polymorphism and obesity have been reported [31–33]. Our results showing a lack of association of *LEPR* Q223R polymorphism with obesity and are in agreement with many other reports indicating that it is difficult to explain human obesity on the basis of the common mutations (*LEPR* Q223R) including in the human leptin receptor gene [17,31]. Also, our findings extend previously reported data on positive associations of the R allele with high leptin levels in healthy populations [34], or in patients with insulin-resistant phenotypes [20], since in the Romanian population studied here subjects carrying the R allele exhibited higher serum leptin than those homozygous for Q allele. Regarding the association of the R allele with metabolic traits found in our samples, there are similar reports on the positive associations of the R allele with obese or insulin-resistant phenotypes in young and healthy populations [16,32,34]. In contrast, some studies found an association between 223QQ genotype and hypertension, HDL-cholesterol levels, and other lipid parameters [35,36].

In conclusion, this study showed that *LEP* G-2548A and *LEPR* Q223R polymorphisms in Romanian subjects were not associated with obesity, whereas the *LEPR* 223R allele might be an important risk factor predisposing healthy subjects to obesity. Moreover, the presence of the 223R allele may influence serum lipid metabolism, providing a link between this *LEPR* gene variant, and obesity. Further research is necessary to ascertain the potential implications of the *LEP* and *LEPR* gene variants for differences in the incidence and etiology of obesity, and to elucidate the pathological processes that link obesity with other features of the metabolic disorder.

Acknowledgments

This work was supported by grants from the Romanian Academy and from a Research Program of the Romanian Ministry of Education and Research (VIASAN, Grant No. 204) awarded to Dr. Anca Sima.

We acknowledge Ioana Andreescu and Floarea Georgescu for their excellent technical assistance.

References

[1] D.E. Cummings, M.W. Schwartz, Genetics and pathophysiology of human obesity, Annu. Rev. Med. 54 (2003) 453–471.
[2] A.J. Walley, A.I. Blakemore, P. Froguel, Genetics of obesity and the prediction of risk for health, Hum. Mol. Genet. 15 (2006) R124–R130.
[3] Y. Zhang, R. Proenca, M. Maffei, M. Barone, L. Leopold, J.M. Friedman, Positional cloning of the mouse obese gene and its human homologue, Nature 372 (1994) 425–432.
[4] J.L. Halaas, K.S. Gajiwala, M. Maffei, S.L. Cohen, B.T. Chait, D. Rabinowitz, R.L. Lallone, S.K. Burley, J.M. Friedman, Weight-reducing effects of the plasma protein encoded by the obese gene, Science 269 (1995) 543–546.
[5] V. Paracchini, P. Pedotti, E. Taioli, Genetics of leptin and obesity: a HuGE review, Am. J. Epidemiol. 162 (2005) 101–114.
[6] L.A. Tartaglia, The leptin receptor, J. Biol. Chem. 272 (1997) 6093–6096.
[7] N. Isse, Y. Ogawa, N. Tamura, H. Masuzaki, K. Mori, T. Okazaki, N. Satoh, M. Shigemoto, Y. Yoshimasa, S. Nishi, K. Hosoda, J. Inazawa, K. Nakao, Structural organization and chromosomal assignment of the human obese gene, J. Biol. Chem. 270 (1995) 27728–27733.
[8] G. Fruchbeck, Intracellular signalling pathways activated by leptin, Biochem. J. 393 (2006) 7–20.
[9] C.S. Mantzoros, The role of leptin in human obesity and disease: a review of current evidence, Annu. Intern. Med. 130 (1999) 671–680.
[10] A. Hattori, K. Uemura, H. Miura, M. Ueda, N. Tamaya, F. Iwata, M. Muraguchi, Y. Ohmoto, A. Iguchi, Gender-related difference in relationship between insulin resistance and serum leptin level in Japanese type 2 diabetic and non-diabetic subjects, Endocr. J. 47 (2000) 615–621.
[11] M. Roden, C. Ludwig, P. Nowotny, B. Schneider, M. Clodi, H. Vierhapper, A. Roden, W. Waldhäusl, Relative hypoleptinemia in patients with type 1 and type 2 diabetes mellitus, Int. J. Obes. Relat. Metab. Disord. 24 (2000) 976–981.
[12] M. Otero, R. Lago, F. Lago, F.F. Casanueva, C. Dieguez, J.J. Gomez-Reino, O. Gualillo, Leptin, from fat to inflammation: old questions and new insights, FEBS Lett. 579 (2005) 295–301.
[13] O. Mammès, D. Betoulle, R. Aubert, V. Giraud, S. Tuzet, A. Petiet, N. Colas-Linhart, F. Fumeron, Novel polymorphisms in the 5′ region of the LEP gene: association with leptin levels and response to low-calorie diet in human obesity, Diabetes 47 (1998) 487–489.
[14] C. Le Stunff, C. Le Bihan, N.J. Schrk, P. Bougneres, A common promoter variant of the leptin gene is associated with changes in the relationship between serum leptin and fat mass in obese girls, Diabetes 49 (2000) 2196–2200.
[15] J. Hoffstedt, P. Eriksson, S. Mottagui-Tabar, P. Arner, A polymorphism in the leptin promoter region (-2548 G/A) influences gene expression and adipose tissue secretion of leptin, Horm. Metab. Res. 34 (2002) 355–359.
[16] N. Yiannakouris, M. Yannakoulia, L. Relistas, J.L. Chan, D. Klimis-Zacas, C.S. Mantzoros, The Q223R polymorphism of the leptin receptor gene is significantly associated with obesity and predicts a small percentage of body weight and body composition variability, J. Clin. Endocrinol. Metab. 86 (2001) 4434–4439.
[17] T. Gotoda, B.S. Manning, A.P. Goldstone, H. Imrie, A.L. Evans, A.D. Strosberg, P.M. McKeigue, J. Scott, T.J. Aitman, Leptin receptor gene variation and obesity: lack of association in a white British male population, Hum. Mol. Genet. 6 (1997) 869–876.
[18] W. Ren, S.H. Zhang, J. Wu, Y.X. Ni, Polymorphism of the leptin gene promoter in pedigrees of type 2 diabetes mellitus in Chongqing, China, Chin. Med. J. 117 (2004) 558–561.
[19] T. Salopuro, L. Pulkkinen, J. Lindström, J.G. Eriksson, T.T. Valle, H. Hämäläinen, P. Ilanne-Parikka, S. Keinänen-Kiukaanniemi, J. Tuomilehto, M. Laakso, M. Uusitupa, Finnish Diabetes Prevention Study Group, Genetic variation in leptin receptor gene is associated with type 2 diabetes and body weight: the Finnish Diabetes Prevention Study, Int. J. Obes. 29 (2005) 1245–1251.
[20] K.C. Chiu, A. Chu, L.M. Chuang, M.F. Saad, Association of leptin receptor polymorphism with insulin resistance, Eur. J. Endocrinol. 150 (2004) 725–729.
[21] K.G. Alberti, P.Z. Zimmet, Definition, diagnosis, and classification of diabetes mellitus and its complications: Part 1. Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation, Diabet. Med. 15 (1998) 539–553.
[22] A. Nieters, N. Becker, J. Linseisen, Polymorphisms in candidate obesity genes and their interaction with dietary intake of n-6 polyunsaturated fatty acids affect obesity risk in a sub-sample of the EPIC-Heidelberg cohort, Eur. J. Clin. Nutr. 41 (2002) 210–221.
[23] Y.C. Chagnon, J.H. Wilmore, I.B. Borecki, J. Gagnon, L. Pérusse, M. Chagnon, G.R. Collier, A.S. Leon, J.S. Skinner, D.C. Rao, C. Bouchard, Associations between the leptin receptor gene and adiposity in middle-aged Caucasian males from the HERITAGE family study, J. Clin. Endocrinol. Metab. 85 (2000) 29–34.
[24] O. Mammes, D. Betoulle, R. Aubert, B. Herbeth, G. Siest, F. Fumeron, Association of the G-2548A polymorphism in the 5′ region of the LEP gene with overweight, Ann. Hum. Genet. 64 (2000) 391–394.
[25] T.N. Wang, M.C. Huang, W.T. Chang, A.M. Ko, E.M. Tsai, C.S. Liu, C.H. Lee, Y.C. Ko, G-2548A polymorphism of the leptin gene is correlated with extreme obesity in Taiwanese aborigines, Obesity 14 (2006) 183–187.
[26] V.S. Mattevi, V.M. Zembrzuski, M.H. Hutz, Association analysis of genes involved in the leptin-signaling pathway with obesity in Brazil, Int. J. Obes. Relat. Metab. Disord. 26 (2002) 1179–1185.
[27] O. Portoles, J.V. Sorli, F. Frances, O. Coltell, J.I. Gonzalez, C. Saiz, D. Corella, Effect of genetic variation in the leptin gene promoter and the leptin receptor gene on obesity risk in a population-based case-control study in Spain, Eur. J. Epidemiol. 21 (2006) 605–612.
[28] H.M. Hinuy, M.H. Hirata, N. Forti, J. Diament, M.F. Sampaio, D. Armaganijan, L.A. Salazar, R.D. Hirata, Leptin G-2548A promoter polymorphism is associated with increased plasma leptin and BMI in Brazilian women, Arq. Bras. Endocrinol. Metabol. 52 (2008) 611–616.
[29] S. Ben Ali, A. Kallel, B. Ftouhi, Y. Sediri, M. Feki, H. Slimane, R. Jemaa, N. Kaabachi, Association of G-2548A LEP polymorphism with plasma leptin levels in Tunisian obese patients, Clin. Biochem. 42 (2009) 584–588.
[30] N. Yiannakouris, L. Relistas, M. Yannakoulia, K. Mungal, C.S. Mantzoros, The-2548G/A polymorphism in the human leptin gene promoter region is associated with plasma free leptin levels: interaction with adiposity and gender in healthy subjects, Hormones (Athens) 2 (2003) 229–236.
[31] M. Heo, R.L. Leibel, K.R. Fontaine, B.B. Boyer, W.K. Chung, M. Koulu, et al., A meta-analytic investigation of linkage and association of common leptin

receptor (LEPR) polymorphisms with body mass index and waist circumference, Int. J. Obes. Relat. Metab. Disord. 26 (2002) 640–646.
[32] N. Stefan, B. Vozarova, A. Del Parigi, V. Ossowski, D.B. Thompson, R.L. Hanson, The Gln223Arg polymorphism of the leptin receptor in Pima Indians: influence on energy expenditure, physical activity and lipid metabolism, Int. J. Obes. Relat. Metab. Disord. 26 (2002) 1629–1632.
[33] M. Wauters, I. Mertens, T. Rankinen, M. Chagnon, C. Bouchard, L. Van Gaal, Leptin receptor gene polymorphisms are associated with insulin in obese women with impaired glucose tolerance, J. Clin. Endocrinol. Metab. 86 (2001) 3227–3232.
[34] C.T. Van Rossum, B. Hoebee, M.A. Van Baak, M. Mars, W.H. Saris, J.C. Seidell, Genetic variation in the leptin receptor gene, leptin, and weight gain in young Dutch adults, Obes. Res. 11 (2003) 377–386.
[35] R. Rosmond, Y.C. Chagnon, G. Holm, M. Chagnon, L. Perusse, K. Lindell, et al., Hypertension in obesity and the leptin receptor gene locus, J. Clin. Endocrinol. Metab. 85 (2000) 3126–3131.
[36] A. Takahashi-Yasuno, H. Masuzaki, T. Miyawaki, Y. Ogawa, N. Matsuoka, T. Hayashi, et al., Leptin receptor polymorphism is associated with serum lipid levels and impairment of cholesterol lowering effect by simvastatin in Japanese men, Diabetes Res. Clin. Pract. 62 (2003) 169–175.