TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2016) 46: 1335-1340
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doi:10.3906/sag-1502-138

The association of vaspin rs2236242 and leptin rs7799039 polymorphism with metabolic syndrome in Egyptian women

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Received: 23.02.2015 • **Accepted/Published Online:** 06.12.2015 • **Final Version:** 17.11.2016

Background/aim: Genetic predisposition is implicated in the etiology of metabolic syndrome. This study aimed to investigate the association of vaspin rs2236242 and leptin rs7799039 polymorphisms with their serum levels and with the risk of developing metabolic syndrome in Egyptian women.

Materials and methods: This case control study included 100 Egyptian women with metabolic syndrome and 100 without metabolic syndrome. The genotypes of vaspin rs2236242 and leptin rs7799039 were detected by a methodology based on PCR-RFLP. Serum vaspin and leptin levels were determined by ELISA.

Results: The metabolic syndrome group was associated with higher serum vaspin and leptin levels when compared to the nonmetabolic syndrome group. The AA genotype of leptin rs7799039 was associated with metabolic syndrome and with higher serum leptin levels, while the different genotypes of vaspin rs2236242 were not associated with metabolic syndrome or different serum vaspin levels.

Conclusion: The AA genotype of leptin rs7799039 was associated with metabolic syndrome and higher serum leptin levels. Serum leptin and vaspin can be used as diagnostic markers of metabolic syndrome.

Key words: Gene polymorphism, leptin rs7799039, metabolic syndrome, vaspin rs2236242

1. Introduction

Metabolic syndrome (MeS) became a major health problem after the worldwide prevalence of obesity, primarily central obesity (1). This is because it enhances the possibility of developing cardiovascular diseases and type 2 diabetes (2). The central adipose tissues secrete a large number of adipokines, which is considered a major factor in disease development (3).

Vaspin, first identified by Hida et al. (4) in a rat model of type 2 diabetes, is a serine protease inhibitor derived from visceral adipose tissue. The vaspin level correlates with the body mass index (BMI), as its expression is specific to adipocytes in visceral adipose tissues. Vaspin improves glucose tolerance and has insulin-sensitizing effects on adipocyte (5). This may be due to its antiinflammatory effect (6) or inhibition of proteases that degrade antihyperglycemic and antiorexigenic molecules (7). A single nucleotide polymorphism in intron 4 of a vaspin gene (its approved code by the HUGO Gene Nomenclature Committee is SERPINA12), vaspin rs2236242, was found to be strongly associated with diabetes (8).

Leptin, the other adipokine, is almost exclusively expressed by differentiated adipocytes mainly of subcutaneous fat, so its level in the circulation is directly correlated with subcutaneous fat (9,10). Leptin decreases food intake and increases energy expenditure through various mechanisms. Centrally, it acts mainly on hypothalamic cells, inducing anorexigenic factors and inhibiting orexigenic neuropeptides (11). It may also regulate feeding behavior by affecting the cortex and limbic areas (12). Peripherally, it has an autocrine or paracrine-related role in lipid metabolism, inhibiting fatty acids synthesis and stimulating lipolysis.

One of the several polymorphisms identified in this gene is leptin rs7799039, a G-2548A leptin promoter variant (located upstream of the LEP gene), which

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may have a role in the pathophysiology of obesity and, consequently, MeS (13).

The target of this study is to appraise the correlation between vaspin rs2236242 and leptin rs7799039 polymorphic forms and the serum level of their corresponding adipokines, vaspin and leptin, and to evaluate the impact of these polymorphisms on the risk of MeS in a sample of Egyptian women.

2. Materials and methods

2.1. Subjects

Women with and without MeS (n = 100 each) were recruited in this case control study from Kasr Alaini University Hospital in Cairo after having given their informed consent. Ethical approval was obtained from the Local Ethics Committee of the National Institute for Research, Cairo, Egypt.

2.2. Anthropometric measures

The height, weight, hip circumference (HC), and waist circumference (WC) were measured for each woman. BMI was calculated by dividing weight in kg by squared height in m^2. WC was divided by HC to calculate waist/hip ratio (WHR).

2.3. Diagnosis of metabolic syndrome

MeS was diagnosed according to the International Diabetes Federation criteria, which include WC ≥ 80 cm (or BMI ≥ 30 kg/m^2) in addition to any two of the following: triglycerides (TG) ≥ 150 mg/dL, high density lipoprotein cholesterol (HDL-C) < 50 mg/dL, fasting plasma glucose ≥ 100 mg/dL, and blood pressure ≥ 130/85 mmHg, or treatment of any of the above abnormalities (14).

2.4. Blood sampling and laboratory assays

After overnight fasting, blood samples were drawn from consenting participants and were analyzed using standard laboratory methods to determine plasma TG, glucose, total cholesterol, HDL-C, and low density lipoprotein cholesterol (LDL-C). Serum insulin concentration was determined using the Immulite 1000 Automated Analyzer (Diagnostic Products Corporation). The calculation of insulin resistance (HOMA-IR) was done as fasting insulin (mU/L) × fasting glucose (mg/dL) / 405 (15). A portion of serum was kept frozen at –70 °C until the analysis for serum vaspin and leptin levels was completed. The analysis was determined using ELISA kits supplied by BioVender GmbH-Laboratorni Medicina (Brno, Czech Republic) and BioSource Europe S.A. (Nivelles, Belgium). Another portion of blood was collected in EDTA-containing vacutainer tubes and stored at –70 °C for genotyping analysis.

2.5. Molecular analysis

2.5.1. DNA extraction

DNA extraction was done from EDTA-anticoagulated whole blood using a DNA extraction kit provided by QIAGEN (Hilden, Germany) according to the manufacturer's instructions.

2.5.2. Genotyping of vaspin rs2236242 and leptin rs7799039

Gene amplification was done by a tetra amplification refractory mutation system polymerase chain reaction (T-ARMS-PCR) for vaspin rs2236242 (16) and by a conventional polymerase chain reaction (PCR) for leptin rs7799039 (17) using the following primers: the primers for vaspin rs2236242 were forward inner (T allele) 5'AAGACGCCGCTTCTGTGCACT3', reverse inner (A allele) 5'CACAGGGACCCAGGATAACTTGCT3', forward outer 5'GGAGGCAGACCAGGCACTAGAAA3' and reverse outer 5'ACCATCTCTCTGGCT TCAGGCTTC3'. The primers for leptin rs7799039 were forward 5'TTTCCTGTAATTTCCCGTGAG3' and reverse 5'AAAGCAAAGACAGGCATAAAAA3'.

For vaspin rs2236242 amplification**,** an initial denaturation of 5 min at 95 °C was followed by 30 amplification cycles each of 90 s with 30 s at 95 °C, 30 s at 62 °C, and 30 s at 72 °C, and then a final step for 10 min at 72 °C was performed. The amplification gives products of 174 bp for the T allele, 248 bp for the A allele, and 378 bp for the control band. For leptin rs7799039 amplification, an initial denaturation at 95 °C for 5 min was followed by 30 amplification cycles each of 3 min with 1 min at 94 °C, 1 min at 58.5 °C, and 1 min at 72 °C, followed by a final extension for 7 min at 72 °C. The amplification products (242 bp fragments) were digested at 37 °C for 60 min by 1.0 U/15 µL restriction enzyme, CofI Promega (Madison, WI, USA), generating fragments of 61 bp and 181 bp when the cleavage site was present. The PCR products of the vaspin rs2236242 and the restriction fragment products of the leptin rs7799039 were visualized by 2% agarose gel electrophoresis stained with ethidium bromide.

2.6. Statistical analysis

SPSS 18.0 (SPSS Inc., Chicago, IL, USA) was used for analysis. Continuous variables were expressed as mean ± standard deviation (SD) and categorical variables as percentage and frequency. The frequency of different alleles and genotypes in different groups were compared using the chi-square test. The odds ratio (OR) and 95% confidence intervals (CIs) of genetic risk in MeS were estimated by logistic regression analysis. To compare means, we used the independent Student t-test and ANOVA. To evaluate the diagnostic value of serum vaspin and leptin and to identify the cutoff values, receiver operating characteristics analysis was used. $P < 0.05$ was regarded as significant.

3. Results

All observed subjects, both in the MeS group and the group without metabolic syndrome, were in Hardy–Weinberg equilibrium. All biochemical and anthropometric

parameters of the participants in the study are presented in Table 1. Serum vaspin levels showed significant increase in cases of MeS (3.34 ± 0.52 ng/mL) when compared to non-MeS cases (1.87 ± 0.54 ng/mL), P < 0.001. Serum leptin levels were also significantly higher in cases of MeS (36.7 ± 10.19 ng/mL) when compared to non-MeS cases (11.44 ± 5.88 ng/ml), P < 0.001.

Table 2 shows the cutoff values for both vaspin (≥2.80 ng/mL) and leptin (≥19.00 ng/mL) serum levels at which the highest sensitivity and specificity were obtained to distinguish the patients with MeS from those without MeS.

Table 3 shows no significant association between the A allele of vaspin rs2236242 and MeS when compared to the T allele, P = 0.08. The AT and AA genotypes also showed no association with MeS when compared to the TT genotype (wild type), with P = 0.08 for AT and P = 0.09 for the AA genotype.

On the contrary, the A allele of leptin rs7799039 is associated with MeS when compared to the G allele, P < 0.001. Only the AA genotype of leptin rs7799039 is associated with MeS and the risk of developing the disease is 4 times that with the GG genotype, while the GA genotype is not associated with the disease. For the AA genotype, P < 0.001 and for the GA genotype, P = 0.94 (Table 3).

There was no significant difference in the serum vaspin level in different polymorphic forms of vaspin rs2236242, P = 0.33. On the contrary, serum leptin was significantly different in different polymorphic forms of leptin rs7799039, P = 0.011. Upon doing the post hoc test, serum leptin levels were significantly higher in the AA genotype (28.63 ± 13.45 ng/mL) when compared to the GG genotype (19.70 ± 15.82 ng/mL), P = 0.008 (Table 4).

4. Discussion

Genetic predisposition and environmental factors have been implicated in the development of MeS (18). Some of the polymorphic genes known to be associated with MeS are those encoding fat mass and obesity-associated protein, cholesteryl ester transfer protein, apolipoprotein E, and apolipoprotein C3 (19).

In our study, we investigated the possible association between vaspin rs2236242 and leptin rs7799039 gene polymorphism with the corresponding serum levels and with the risk of developing MeS in a sample of Egyptian women. To the best of our knowledge, this is the first time such an association has been investigated in Egyptian women.

Our findings showed significantly higher serum levels of vaspin in patients with MeS when compared

Table 1. Characteristics of all participants in the study (values expressed as mean ± standard deviation).

	MeS (n = 100)	Non-MeS (n = 100)	P
Age (years)	50.96 ± 6.38	45.44 ± 14.26	<0.001*
Height (cm)	157.00 ± 5.46	154.00 ± 6.89	0.07
Weight (kg)	93.54 ± 13.88	68.55 ± 22.38	<0.001*
BMI (kg/m^2)	37.93 ± 5.24	28.60 ± 8.74	<0.001*
Waist circumference (cm)	112.38 ± 10.60	93.44 ± 19.80	<0.001*
Hip circumference (cm)	120.80 ±11.20	106.30 ± 15.90	<0.001*
Systolic blood pressure (mmHg)	135.58 ±14.92	126.67 ± 20.73	0.03*
Diastolic blood pressure (mmHg)	87.31 ± 13.21	68.55 ± 22.38	0.02*
Fasting plasma glucose (mg/dL)	96.91 ± 17.72	108.16 ± 22.35	0.46
Insulin	22.42 ± 5.82	16.44 ± 3.06	<0.001*
Insulin resistance	5.39 ± 1.87	4.37 ± 1.12	<0.001*
Triglyceride (mg/dL)	182.02 ± 112.81	139.65 ± 69.22	<0.001*
HDL-C[a] (mg/dL)	38.51 ± 13.99	43.05 ± 13.33	0.02*
LDL-C[b] (mg/dL)	122.20 ± 29.14	95.70 ± 30.14	<0.001*
Vaspin (ng/mL)	3.34 ± 0.52	1.87 ± 0.54	<0.001*
Leptin (ng/mL)	36.70 ±10.19	11.44 ± 5.88	<0.001*

[a] High density lipoprotein cholesterol. [b] Low density lipoprotein cholesterol.
* Indicates significance at P < 0.05.

Table 2. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for serum vaspin and leptin.

	Serum vaspin	Serum leptin
Cutoff value (ng/mL)	≥2.80	≥19.00
Sensitivity (%)	84.00	97.00
Specificity (%)	92.00	90.00
PPV (%)	95.50	95.10
NPV (%)	74.20	93.80

to the control group. Choi et al. reported similar results in a population of male patients with MeS (20). Some reports have related the abnormalities in the circulating levels of vaspin to individual components of MeS such as BMI and insulin sensitivity. However, these results are contradictory. Some researchers reported no significant difference in serum vaspin levels between morbidly obese and controls (21), while others reported significantly higher serum vaspin in obese groups (22,23). Some researchers found no significant difference in serum vaspin levels between type 2 diabetes patients and controls (22), whereas others found a positive correlation of serum vaspin with insulin resistance (23).

Regarding the relationship between different polymorphic forms of vaspin rs2236242 and serum vaspin levels, this study showed no significant difference in the vaspin level between different polymorphic forms of vaspin rs2236242. In addition, no significant difference in the allele and genotype frequency of vaspin rs2236242 polymorphism between the MeS and non-MeS groups was detected. These results were contradictory to the results obtained by Hashemi et al. (16), who reported that genotypes carrying the A allele (TA and AA) diminished the risk of MeS in comparison to the wild TT genotype in all tested inheritance models. Kempf et al. (8) found that the AA genotype of vaspin rs2236242 confers an increased risk of type 2 diabetes when compared with the TT genotype.

Regarding leptin, this study showed a significant increase in the serum leptin level in the MeS group in comparison to the non-MeS group. This is in line with previous reports that correlated serum leptin levels with MeS in different populations and supposed that it can be useful as a diagnostic marker of the disease (24–27). The highest sensitivity and specificity for the diagnosis of metabolic syndrome were obtained at a cutoff value of ≥19 ng/mL. The AA genotype of leptin rs7799039 was associated with a higher serum leptin level when compared to the wild GG genotype. The results of previous studies were controversial. Some reports showed an association of higher serum leptin levels with genotypes carrying the A allele as shown by the results of this study (17,28,29).

Table 3. Association of the different polymorphic forms of vaspin rs2236242 and leptin rs7799039 with metabolic syndrome (MeS).

Vaspin rs2236242	MeS (n = 100)	Non MeS (n = 100)	Odd ratio (OR) (95% CI)	P
Alleles				
T	127 (63.5%)	109 (54.5%)		
A	73 (36.5%)	91 (45.5%)	0.69 (0.46 to 1.03)	0.08
Genotypes				
TT	38 (38%)	25 (25%)		
TA	51 (51 %)	59 (59%)	0.57 (–1.79 to 0.08)	0.08
AA	11 (11%)	16 (16%)	0.45 (–1.28 to 0.08)	0.09
Leptin rs7799039				
Alleles				
G	86 (43%)	121 (60.5%)		
A	114 (57%)	79 (39.5%)	2.03 (1.36 to 3.02)	<0.001*
Genotypes				
GG	23 (23%)	32 (32%)		
GA	40 (40%)	57 (57%)	0.97 (–0.68 to 0.67)	0.94
AA	37 (37%)	11 (11%)	4.66 (0.73 to 2.55)	<0.001*

* Indicates significance at $P < 0.05$.

Table 4. Serum vaspin and leptin level (ng/mL) in different vaspin rs2236242 and leptin rs7799039 genotypes (values expressed as mean ± standard deviation).

	N	Mean ± SD	F	P
Vaspin rs2236242 genotypes				
AA	21	2.748 ±1.00	1.132	0.33
AT	116	2.657 ± 0.96		
TT	63	2.471 ± 0.75		
Leptin rs7799039 genotypes				
AA	48	28.63 ± 13.45	4.585	0.011*
AG	98	24.24 ± 14.99		
GG	54	19.70 ± 15.82		

* Indicates significance at $P < 0.05$.

On the other hand, studies done on obese and nonobese Greek women (30), obese Tunisian women (31), obese Romanian subjects (32), healthy Spanish adolescents (33), and Brazilian women (34) showed low serum leptin levels in the AA genotype. Nevertheless, other studies reported no significant difference in leptin levels in different leptin rs7799039 genotypes (35,36).

The frequency of the A allele was significantly higher in the MeS group in comparison to the control group, and the leptin rs7799039 AA genotype was associated with MeS. To our knowledge, there are no previous reports on the association between leptin rs7799039 polymorphism and metabolic syndrome, but there are reports on the association of this gene polymorphism with obesity, which is an important component of MeS. Riestret al. (33) and Hinuy et al. (34) reported a significantly lower frequency of the A allele in obese females. Mammes et al. (17) reported this association only in obese males.

In summary, this study demonstrated that serum vaspin and leptin levels are significantly higher in cases of MeS and these levels can be used as diagnostic markers of disease. The different polymorphic forms of vaspin rs2236242 do not affect serum vaspin levels and are not associated with MeS. The AA genotype of leptin rs7799039 is associated with higher serum leptin levels and metabolic syndrome. Consequently, the results of previous research are contradictory. This may be due to the interactions of these polymorphisms with other polymorphisms of the gene; the different ethnicities of the studied groups; the sample sizes of the populations, which possibly affected the statistical power; or the model used in statistical analysis.

It is recommended to test these results in a larger cohort and in several populations in order to demonstrate the validity of our findings. Environmental risk factors such as diet or physical activity can also modify genetic effects, so it is important that these be considered in subsequent studies.

Recently, bioinformatics, such as multifactor dimensionality reduction, has been used for the analysis of gene × gene and gene × environment interactions and has been emphasized as a new alternative for understanding the etiology of common complex traits because the above-mentioned interactions are difficult to detect and characterize using traditional parametric statistical methods.

References

1. Alberti KGM, Zimmet P, Shaw J. The metabolic syndrome—a new worldwide definition. Lancet 2005; 366: 1059-1062.
2. Wannamethee SG, Shaper AG, Lennon L. Reasons for intentional weight loss, unintentional weight loss, and mortality in older men. Arch Intern Med 2005; 165: 1035-1040.
3. Klein S, Allison DB, Heymsfield SB, Kelley DE, Leibel RL, Nonas C, Kahn R. Waist circumference and cardiometabolic risk: a consensus statement from shaping America's health: Association for Weight Management and Obesity Prevention; NAASO, the Obesity Society; the American Society for Nutrition; and the American Diabetes Association. Obesity 2007; 15: 1061-1067.
4. Hida K, Wada J, Eguchi J, Zhang H, Baba M, Seida A, Hashimoto I, Okada T, Yasuhara A, Nakatsuka A. Visceral adipose tissue-derived serine protease inhibitor: a unique insulin-sensitizing adipocytokine in obesity. P Natl Acad Sci USA 2005; 102: 10610-10615.
5. Klöting N, Berndt J, Kralisch S, Kovacs P, Fasshauer M, Schön MR, Stumvoll M, Blüher M. Vaspin gene expression in human adipose tissue: association with obesity and type 2 diabetes. Biochem Bioph Res Co 2006; 339: 430-436.
6. Wada J. Vaspin: a novel serpin with insulin-sensitizing effects. Expert Opin Inv Drug 2008; 17: 327-333.
7. Blüher M. Vaspin in obesity and diabetes: pathophysiological and clinical significance. Endocrine 2012; 41: 176-182.
8. Kempf K, Rose B, Illig T, Rathmann W, Strassburger K, Thorand B, Meisinger C, Wichmann HE, Herder C, Vollmert C. Vaspin (SERPINA12) genotypes and risk of type 2 diabetes: results from the MONICA/KORA studies. Exp Clin Endocr Diab 2010; 118: 184-189.
9. Fain J, Bahouth S, Madan A. TNFα release by the nonfat cells of human adipose tissue. Int J Obesity 2004; 28: 616-622.

10. Zha JM, Di WJ, Zhu T, Xie Y, Yu J, Liu J, Chen P, Ding G. Comparison of gene transcription between subcutaneous and visceral adipose tissue in Chinese adults. Endocr J 2009; 56: 935-944.

11. Chan JL, Heist K, DePaoli AM, Veldhuis J.D, Mantzoros CS. The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men. J Clin Invest 2003; 111: 1409-1421.

12. Rosenbaum M, Sy M, Pavlovich K, Leibel RL, Hirsch J. Leptin reverses weight loss–induced changes in regional neural activity responses to visual food stimuli. J Clin Invest 2008; 118: 2583-2591.

13. Otero M, Lago Ro, Lago F, Casanueva FF, Dieguez C, Gómez-Reino JJ, Gualillo O. Leptin, from fat to inflammation: old questions and new insights. Febs Lett 2005; 579: 295-301.

14. Zimmet P, Alberti K, Serrano Ríos M. A new International Diabetes Federation (IDF) worldwide definition of the metabolic syndrome: the rationale and the results. Rev Esp Cardiol (English Edition) 2005; 58: 1371-1375.

15. Matthews D, Hosker J, Rudenski A, Naylor B, Treacher D, Turner R. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412-419.

16. Hashemi M, Rezaei H, Eskandari-Nasab E, Zakeri Z, Taheri M. Association between chemerin rs17173608 and vaspin rs2236242 gene polymorphisms and the metabolic syndrome, a preliminary report. Gene 2012; 510: 113-117.

17. Mammes O, Betoulle D, Aubert R, Herbeth B, Siest G, Fumeron F. Association of the G-2548A polymorphism in the 5′ region of the LEP gene with overweight. Ann Hum Genet 2000; 64: 391-394.

18. Joy T, Lahiry P, Pollex RL, Hegele RA. Genetics of metabolic syndrome. Curr Diabetes Rep 2008; 8: 141-148.

19. Povel C, Boer J, Reiling E, Feskens E. Genetic variants and the metabolic syndrome: a systematic review. Obes Rev 2011; 12: 952-967.

20. Choi SH, Kwak SH, Lee Y, Moon MK, Lim S, Park YJ, Jang HC, Kim MS. Plasma vaspin concentrations are elevated in metabolic syndrome in men and are correlated with coronary atherosclerosis in women. Clin Endocrinol 2011; 75: 628-635.

21. Auguet T, Quintero Y, Riesco D, Morancho B, Terra X, Crescenti A, Broch M, Aguilar C, Olona M, Porras JA. New adipokines vaspin and omentin. Circulating levels and gene expression in adipose tissue from morbidly obese women. Bmc Med Genet 2011; 12: 60.

22. Youn BS, Klöting N, Kratzsch J, Lee N, Park JW, Song ES, Ruschke K, Oberbach A, Fasshauer M, Stumvoll M. Serum vaspin concentrations in human obesity and type 2 diabetes. Diabetes 2008; 57: 372-377.

23. Suleymanoglu S, Tascilar E, Pirgon O, Tapan S, Meral C, Abaci A. Vaspin and its correlation with insulin sensitivity indices in obese children. Diabetes Res Clin Pr 2009; 84: 325-328.

24. Zhuo Q, Wang Z, Fu P, Piao J, Tian Y, Xu J, Yang X. Comparison of adiponectin, leptin and leptin to adiponectin ratio as diagnostic marker for metabolic syndrome in older adults of Chinese major cities. Diabetes Res Clin Pr 2009; 84: 27-33.

25. Özçelik E, Uslu S, Kebapçı N, Kara M, Dokumacıoğlu A, Musmul A. Interrelations of serum leptin levels with adrenocorticotropic hormone, basal cortisol and dehydroepiandrosterone sulphate levels in patients with metabolic syndrome. Diabetes Metab Syndr 2010; 4: 13-17.

26. Esteghamati A, Noshad S, Khalilzadeh O, Morteza A, Nazeri A, Meysamie A, Esteghamati A, Nakhjavani M. Contribution of serum leptin to metabolic syndrome in obese and nonobese subjects. Arch Med Res 2011; 42: 244-251.

27. Chiu FH, Chuang CH, Li WC, Weng YM, Fann WC, Lo HY, Sun C, Wang SH. The association of leptin and C-reactive protein with the cardiovascular risk factors and metabolic syndrome score in Taiwanese adults. Cardiovasc Diabetol 2012; 11: 40.

28. Bienertova-Vasku J, Bienert P, Tomandl J, Forejt M, Vavrina M, Kudelkova J, Vasku A. No association of defined variability in leptin, leptin receptor, adiponectin, proopiomelanocortin and ghrelin gene with food preferences in the Czech population. Nutr Neurosci 2008; 11: 2-8.

29. Hinuy HM, Hirata MH, Forti N, Diament J, Sampaio MF, Armaganijan D, Salazar LA, Hirata RD. Leptin G-2548A promoter polymorphism is associated with increased plasma leptin and BMI in Brazilian women. Arq Bras Endocrinol Metabol 2008; 52: 611-616.

30. Yiannakouris N, Melistas L, Yannakoulia M, Mungal K, Mantzoros CS. 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) 2003; 2: 229-236.

31. Ben Ali S, Kallel A, Ftouhi B, Sediri Y, Feki M, Slimane H, Jemaa R, Kaabachi N. Association of G-2548A LEP polymorphism with plasma leptin levels in Tunisian obese patients. Clin Biochem 2009; 42: 584-588.

32. 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 Bioph Res Co 2010; 391: 282-286.

33. Riestra P, Garcia-Anguita A, Viturro E, Schoppen S, De Oya M, Garcés C. Influence of the leptin G-2548A polymorphism on leptin levels and anthropometric measurements in healthy Spanish adolescents. Ann Hum Genet 2010; 74: 335-339.

34. Hinuy HM, Hirata MH, Sampaio MF, Armaganijan D, Arazi SS, Salazar LA, Hirata RD. Relationship between variants of the leptin gene and obesity and metabolic biomarkers in Brazilian individuals. Arq Bras Endocrinol Metabol 2010; 54: 282-288.

35. Fourati M, Mnif M, Kharrat N, Charfi N, Kammoun M, Fendri N, Sessi S, Abid M, Rebai A, Fakhfakh F. Association between Leptin gene polymorphisms and plasma leptin level in 3 consanguineous families with obesity. Gene 2013; 527: 75-81.

36. Ma D, Feitosa MF, Wilk JB, Laramie JM, Yu K, Leiendecker-Foster C, Myers RH, Province MA, Borecki IB. Leptin is associated with blood pressure and hypertension in women from the National Heart, Lung, and Blood Institute Family Heart Study. Hypertension 2009; 53: 473-479.