

Evaluation of Four Obesity-Related Genes Polymorphism in Obese Women Residing in East Azerbaijan, Iran

Abolfazl Barzegari¹, Alireza Ostadrahimi², Vahideh Ebrahimzadeh Attari^{3*}, Abolfazl Gorbani⁴, Mohammad Asghari Jafarabadi⁵

1. Research Center for Pharmaceutical Nanotechnology; Tabriz University of Medical Science; Tabriz, Iran
2. Nutrition Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
3. Kidney Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
4. Department of Animal Science, Islamic Azad University, Shabestar Branch, Shabestar, Iran
5. Road Traffic Injury Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Abstract

In view of the fundamental role of genetics in development of obesity, the present study aimed to investigate the single nucleotide polymorphism of some obesity-related genes among a subset of obese women living in Tabriz, Iran. For this purpose, 70 eligible obese women (aged 18-45 years) were genotyped for the *uncoupling protein-1 (UCP-1)* -3826A>G, *β3-adrenergic receptor (β3ADR)* Trp64Arg, *leptin* G-2548A and *adiponectin* +45 T>G polymorphisms. Accordingly, genomic DNA was isolated from whole blood samples using the conventional phenol chloroform extraction method and the single-nucleotide polymorphism of *leptin*, *adiponectin (ADIPOQ)*, *UCP-1* and *β3ADR* genes were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The frequencies of GG, GA, and AA genotypes for *leptin* polymorphism G-2548A were found 20.0%, 51.4% and 28.6%, respectively. The genotype frequencies of TT, TG, and GG for *adiponectin* polymorphism +45 T>G were found 67.1%, 27.1% and 5.7%, respectively. The genotype frequencies of AA, AG, and GG for *UCP-1* polymorphism A-3826G were found 48.6%, 32.9% and 18.6%, respectively. Finally, the genotype frequencies of Trp64Trp, Trp64Arg, and Arg64Arg for *β3ADR* gene were found 84.3%, 15.7%, and 0%, respectively. The genotype-allelic frequencies of *ADIPOQ* and *β3ADR* genes were almost the same in different populations, while *leptin* and *UCP-1* genes had different genotype distributions. Since the aforementioned genetic variations have important roles in development of obesity and also response to treatments like weight lowering diet, it is worth studying the genotype of every population before conducting any interventional program for the obesity management.

Key words: Obesity; Polymorphism; *UCP-1* gene; *β3ADR* gene; *leptin* gene; *adiponectin* gene

Introduction

Obesity is a multifactorial disease which results from the complex interaction of different factors, including genetic, environmental, behavioral, and cultural factors (1). To date, several epidemiological and genetic studies have revealed the contribution of several candidate genes and their single nucleotide polymorphisms (SNPs) to the obesity phenotypes (2,3). In this regard, uncoupling protein-1 (*UCP-1*) and

β3-adrenergic receptor (β3ADR) genes are of important candidate genes for obesity, because of their major roles in regulation of metabolism, thermogenesis, and adipose tissue lipolysis (4-7). Among different identified polymorphisms of these genes, the -3826A>G common variation in the promoter region of the *UCP-1* gene has been associated with higher fat accumulation, reduced postprandial thermogenesis and lipid/lipoprotein metabolism (5,8). Likewise, the missense single nucleotide mutation in the *β3ADR* gene (Trp64Arg polymorphism) has been related to increasing weight gain, abdominal obesity, difficulty in weight loss, lower basal metabolic rate (BMR) and insulin resistance (7,9,10).

Moreover, the genetic variations of adipokines

* Vahideh Ebrahimzadeh Attari, PhD

Kidney Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Email: ebrahimzadehv@tbzmed.ac.ir

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like adiponectin and leptin maybe related to development of obesity. Adiponectin, the most abundant adipokine, exerts both insulin-sensitizing and anti-atherogenic effects and its concentration increases with weight loss (11,12). A silent T to G substitution in exon 2 of the *adiponectin* gene (*ADIPOQ*), which is one of the most commonly studied SNPs at *ADIPOQ* (+45 T>G), has been associated with serum levels of adiponectin, obesity, insulin resistance, and risk of type 2 diabetes (13-15).

The other adipokine, leptin, has a primary role in the regulation of body weight through controlling food intake and energy expenditure. It also has pro-inflammatory properties and has been implicated in the pathogenesis of insulin resistance and atherosclerosis (16,17). Interestingly, leptin associates with UCP-1 and B3ADR to increase thermogenesis in brown adipose tissue (18). Up to now, numerous SNPs have been identified in the *leptin* gene. Among them, a common variation within the 5' promoter region of the *leptin* gene (LEP G-2548A) has been related to high body mass index (BMI) and leptin levels in some populations (19-21).

So far, several studies have been conducted to determine the common genetic variations in different populations. The aforementioned single nucleotide polymorphisms (SNPs) were also examined by a few studies in the Iranian population. However, to our knowledge, the -3826A>G polymorphism of *UCP-1* has not yet been assessed among Iranians. Therefore, the present study aimed to investigate the frequencies of the SNP of *leptin*, *adiponectine*, *UCP-1* and

β3ADR genes in a group of obese women living in Tabriz, Iran.

Materials and Methods

Subjects

A total of 70 eligible healthy obese women, aged 18-45 years, with BMI values 30-40 kg/m², participated voluntarily in the present cross-sectional study, through a general call schedule across the city of Tabriz, Iran. The subjects were excluded in case of clinically diagnosed diabetes mellitus, cardiovascular disease, gallstone, hypo- or hyperthyroidism, deep depression, pregnancy, breast feeding, or menopause. The study was approved by the Ethics Committee of Tabriz University of Medical Sciences (vide reference number 92154). All subjects were made aware of the content of the study, and signed a written consent form at the beginning of the study.

Genotyping

Genomic DNA was isolated from the blood samples which had been kept in EDTA tubes at -70°C, using the conventional phenol chloroform extraction method as previously described in detail (22). The single-nucleotide polymorphisms of *UCP-1* (-3826A>G), *β3ADR* (Trp64Arg), *leptin* (-2548G>A), and *adiponectin* (+45 T>G) genes were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Briefly, the identified fragment of each gene was PCR amplified using specific primers and programs in a final volume of 25 μL, as shown in Table 1.

The PCR products of *UCP-1*, *β3ADR*, *leptin*

Table 1: Specific primers for selected SNPs and their PCR thermocycler program*

Gene	SNP	rs number	forward and reverse primers (5'→3')	fragment lengths	Thermocycler program	ref
<i>Leptin</i>	G -2548A	rs7799039	F:TTTCCTGTAATTTCCCGTGAG R: AAAGCAAAGACAGGCATAAA	bp 241	95°C:5min, 95°C:30s, 50.6°C:50s, 72°C:1min, 32× (stage 2-4), 72°C:5min	20
<i>Adiponectin</i>	+45 T>G (T+45G)	rs2241766	F: GAAGTAGACTCTGCTGAGATGG R: TATCAGTGTAGGAGGCTGTGTATG	bp 372	95°C:5min, 95°C:30s, 50°C:50s, 72°C:1min, 32× (stage 2-4), 72°C:5min	14
<i>UCP-1</i>	A -3826G	rs1800592	F: CCAGTGGTGGCTAATGAGAGAA R: GCACAAAGAAGAAGCAGAGAGG	bp 279	95°C:5min, 95°C:30s, 60°C:50s, 72°C:1min, 32× (stage 2-4), 72°C:5min	8
<i>B3ADR</i>	Trp64Arg	rs4994	F: CGCCAATACCGCCAACAC R: CCACCAGGAGTCCCATCACC	bp 210	95°C:5min, 95°C:30s, 59°C:50s, 72°C:1min, 32× (stage 2-4), 72°C:5min	10

* The identified fragment of each gene was PCR amplified using their specific primers and programs in a final volume of 25 μL

and *adiponectin* genes were then digested respectively with *BclI*, *BstNI*, *HhaI*, and *SmaI* restriction enzymes (Thermo Scientific, Lithuania, EU) according to the enzymes instructions, as shown in Table 2.

Table 2: Characteristics of the restriction enzymes*

Gene	Enzyme	cutting position (5'→3')	Incubation temperature	Incubation time	ref
<i>Leptin</i>	<i>HhaI</i>	5'...G C G C...3' 3'...C G C G...5'	37°C	8 h	20
<i>Adiponectin</i>	<i>SmaI</i>	5'...C C C G G G...3'... 3'...G G G C C C...5'	30°C	12 h	14
<i>UCP-1</i>	<i>BclI</i>	5'...T G A T C A...3' 3'...A C T A G T...5'	55°C	5-6 h	8
<i>β3ADR</i>	<i>MvaI (BstNI)</i>	5'...C C W G G...3'... 3'...G G W C C...5'	37°C	8 h	10

* The PCR product of each gene were digested according to its relative enzyme instruction

Then, the digestion products were separated on a 3% agarose gel and visualized using SYBR green I safe staining (Invitrogen, USA) under UV excitation. Moreover, one PCR product from each gene (a heterozygote genotype) was sent to the MacroGen Corp., Seoul, South Korea, for sequencing in order to re-confirm the results of genotyping.

Statistical analyses

Data were analyzed using SPSS software, version 21.0 (IBM Corp., Armonk, NY, USA). The genotype and allele frequencies of all genes were calculated. All genotype distributions were tested for deviation from the Hardy-Weinberg equilibrium (HWE) using the PopGene.S2 software based on the chi-square test ($P > 0.05$).

Results

The enzymatic digestion products of each gene after electrophoresis are shown in Figures 1a-d. The *leptin* polymorphism genotyping yielded 181-bp and 61-bp fragments for the GG homozygotes; 242-bp, 181-bp, and 61-bp products for the GA heterozygotes; and a single 242-bp product for the AA homozygotes. Moreover, genetic analysis of *adiponectin* polymorphism yielded the 372-bp as wild type TT genotype, 372-bp, 219-bp, and 153-bp fragments as TG heterozygotes and the fragments of 219-bp and 153-bp as mutant GG genotype.

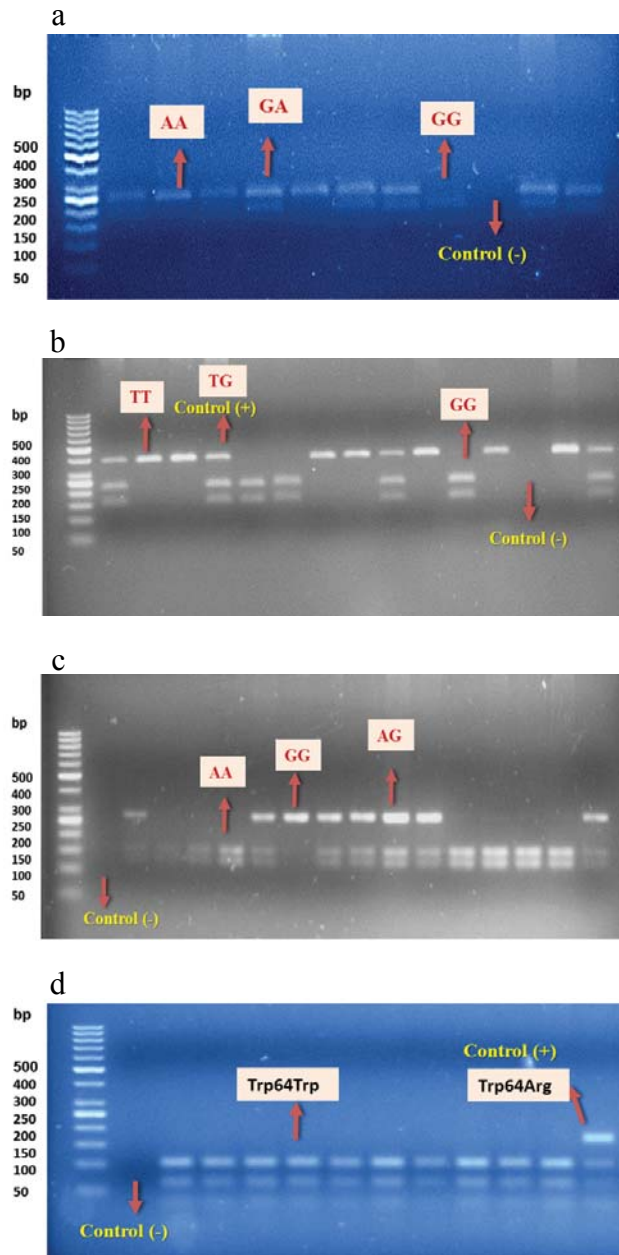


Figure 1: PCR-based restriction fragment length polymorphism analysis of (a): *leptin*, (b): *adiponectin*, (c): *UCP-1* and (d): *β3ADR*: 50 bp DNA ladder

The *UCP-1* polymorphism genotyping resulted in 157-bp and 122-bp fragments for the AA homozygotes; 279-bp, 157-bp, and 122-bp products for the AG heterozygotes; and a single 279-bp product for the GG homozygotes. Moreover, genetic analysis of *β3ADR* polymorphism yielded 97-bp, 61-bp, and 31-bp fragments as Trp64Trp carriers and the fragments of 158-bp, 97-bp, 61-bp, and 31-bp as Trp64Arg heterozygotes. However, we did not find the Arg64Arg

polymorphism in our study population. In addition, we analyzed the results of sequencing (Fig-

ures 2-4) using Chromas software version 2.1.1 (Technelysium Pty. Ltd) in order to confirm our

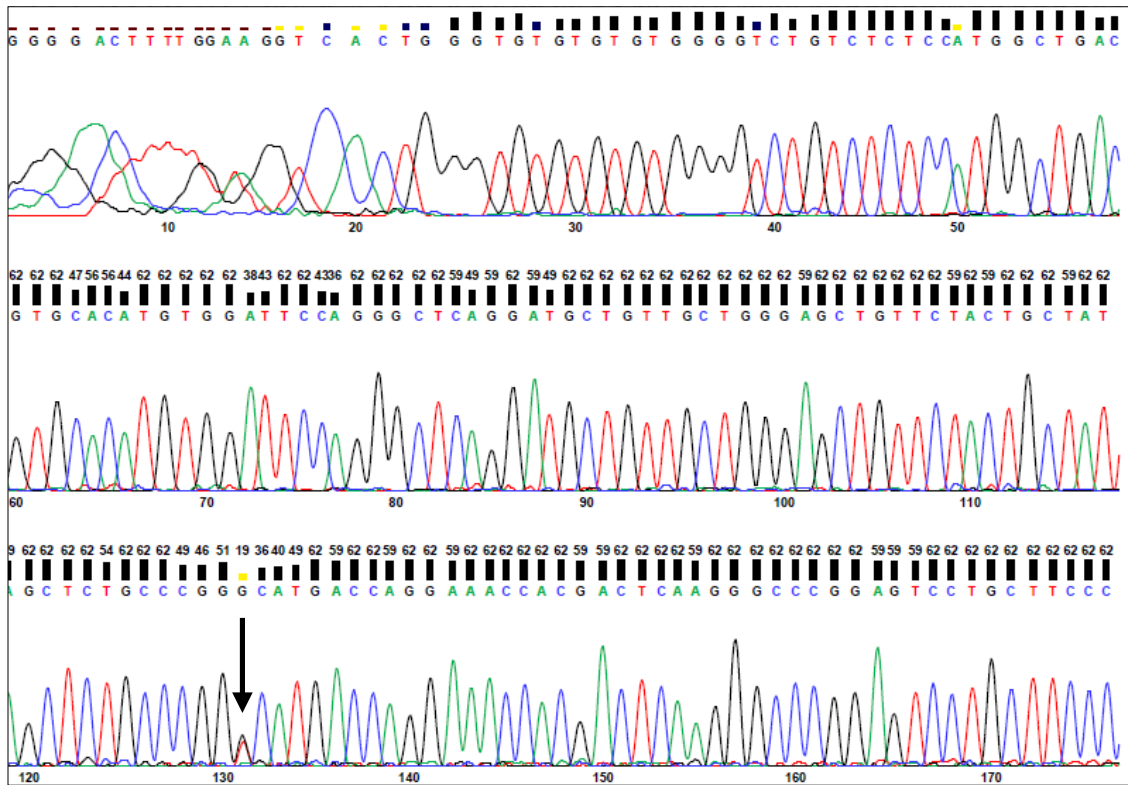


Figure 2: Sequencing data for *Adiponectin* +45 T>G polymorphism (TG genotype)

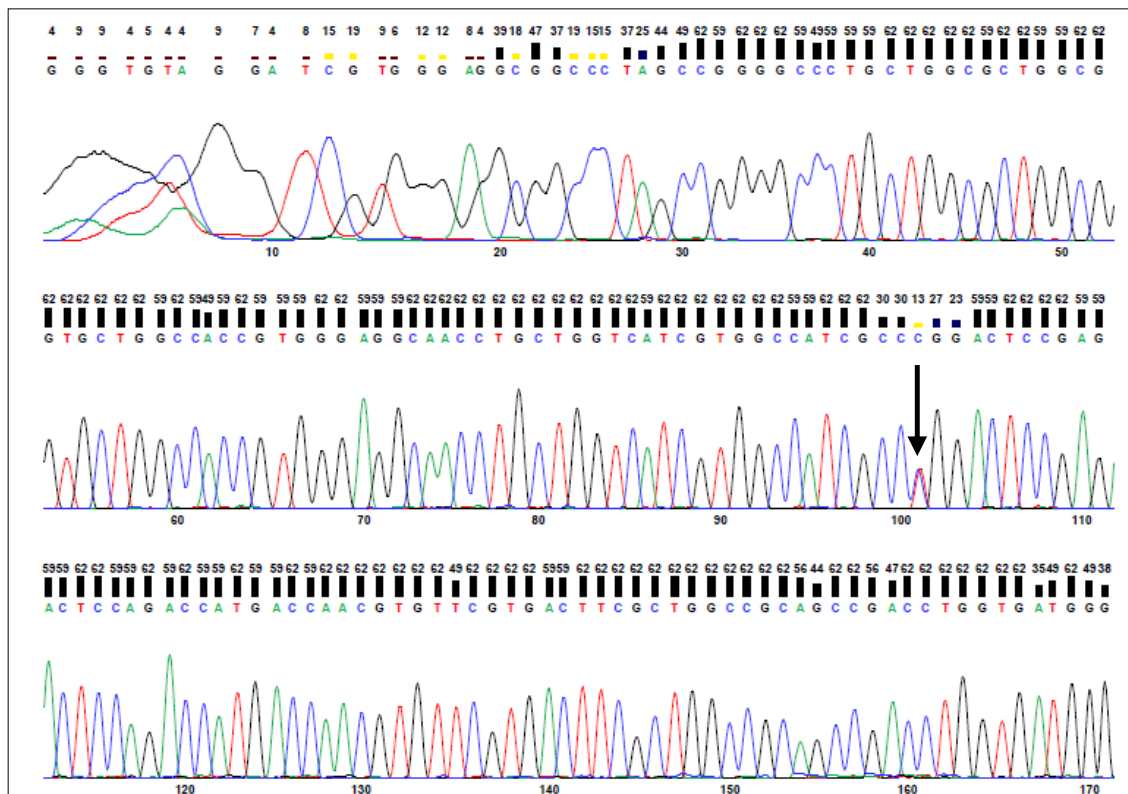


Figure 3: Sequencing data for $\beta 3 A D R$ Trp64Arg polymorphism (Trp64Arg genotype)

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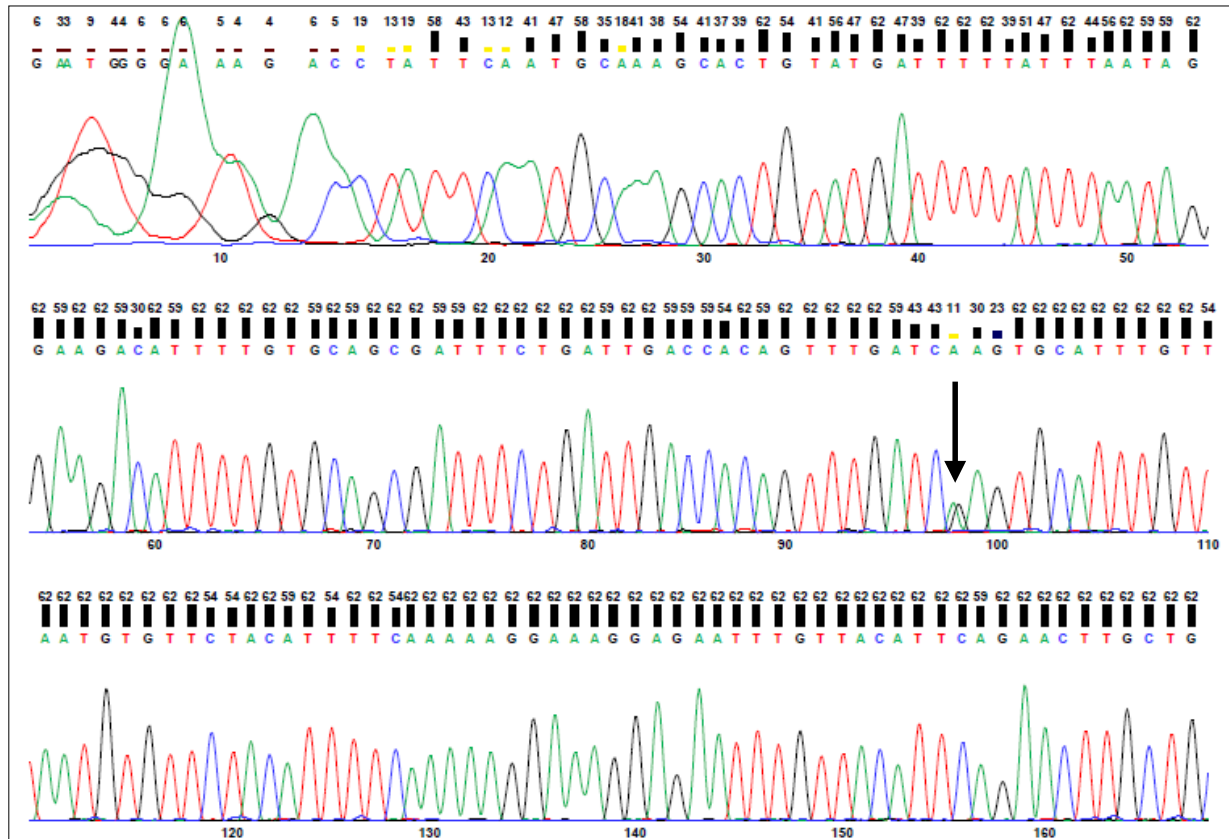


Figure 4: Sequencing data for *UCP-1* -3826A>G polymorphism (AG genotype)

fragments size. It should be noted that there was an error during *leptin* sequencing; therefore, its result is not shown.

The genotype allelic frequencies are shown in Table 3. The frequencies of GG, GA, and AA genotypes for *leptin* polymorphism G-2548A were found 20.0%, 51.4%, and 28.6%, respec-

tively. The genotype frequencies of TT, TG, and GG for *adiponectin* polymorphism +45 T>G were found 67.1%, 27.1%, and 5.7%, respectively. The genotype frequencies of AA, AG, and GG for *UCP-1* polymorphism A-3826G were found 48.6%, 32.9%, and 18.6%, respectively. Finally, the genotype frequencies of Trp64Trp,

Table 3: Genotype allelic frequencies of the study subjects regarding to the single nucleotide polymorphism of *Leptin*, *Adiponectin*, *UCP1* and *β3ADR* genes

SNP	Genotype frequency			Allele frequency		HWE*
<i>Leptin</i>	GG	GA	AA	G	A	0.519
-2548 G>A	14 (20.0%)	36 (51.4%)	20 (28.6%)	45.7%	54.3%	
<i>Adiponectin</i>	TT	TG	GG	T	G	0.283
+45 T>G	47 (67.1)	19 (27.1%)	4 (5.7%)	80.7%	19.3%	
<i>UCP-1</i>	AA	AG	GG	A	G	0.020
-3826A>G	34 (48.6%)	23 (32.8%)	13 (18.6%)	65.0%	35.0%	
<i>β3ADR</i>	Trp64Trp	Trp64Arg	Arg64Arg	Trp 64	Arg 64	0.475
Trp64Arg	59 (84.3%)	11 (15.7%)	0 (0%)	92.1%	7.9%	

*p values are based on the chi-square test. p values >0.05 indicate that there is no deviation from HWE

Trp64Arg, and Arg64Arg for $\beta 3ADR$ gene were found 84.3%, 15.7%, and 0%, respectively. All SNPs were in Hardy-Weinberg equilibrium ($p > 0.05$), except for UCP-1 -3826A>G.

Discussion

The common form of obesity is a multi-genetic disorder which is affected by different genes and their variations. Moreover, polymorphism or the common variation of gene plays an important role in development of obesity and obesity-related diseases (1,3,13).

Leptin, *adiponectin*, *UCP-1* and *B3ADR* genes are important candidate genes for obesity and several studies have been conducted to determine their common genetic variations in different populations. Therefore, the present study aimed to investigate the frequency of a single nucleotide polymorphism of *leptin*, *adiponectin*, *UCP-1* and *B3ADR* genes in a group of obese women living in Tabriz, Iran.

Leptin gene (*LEP*), also called the ob gene, has a primary role in body weight regulation through controlling food intake and energy expenditure (16,17). Many SNPs have been identified in *leptin* gene. Among them, a common variation within the 5' promoter region of *leptin* gene (*LEP* G-2548A) has been related to high Body Mass Index (BMI) and leptin levels in some populations (19-21). According to our results, the frequencies of GG, GA, and AA genotypes for *leptin* polymorphism G-2548A were 20.0%, 51.4%, and 28.6%, respectively. The 2548A allele was the most frequent allele with the frequency of 54.3%.

Previously Hassanzadeh et al. (23) reported almost the same genotype-allelic frequencies for *leptin* G-2548A polymorphism in 200 healthy adults living in Hamadan, Iran. Moreover, the results of Portoles et al. (24) among Spanish adults are comparable to our findings. However, our finding is not similar to those reported for North Americans (25), Europeans (26,27), and Brazilians (19), since a smaller frequency of mutant allele A was seen.

Adiponectin gene (*ADIPOQ*) was first identified in 1995. Up to now, sixteen SNPs have been

reported for *ADIPOQ*, but a silent T to G substitution in exon 2 (+45 T>G) is the most commonly studied SNP among these variants (15). It has been associated with serum levels of adiponectin, obesity, insulin resistance, and risk of type 2 diabetes (14,15,28).

Genotype frequencies of TT, TG, and GG for *adiponectin* polymorphism +45 T>G were found 67.1%, 27.1%, and 5.7%, respectively in the present study. This finding is supported by two previous studies in Iran (15,29). Moreover, according to our results, the high frequencies of TT genotype and T allele were also reported in some other populations like Arabs (14), Greeks (13), and Americans (30). In comparison, results of Chinese and Korean populations were different as the frequencies of TT and TG genotypes were similar to each other (31,32).

Uncoupling protein-1 (*UCP-1*) gene has a major role in regulation of metabolism, thermogenesis, and adipose tissue lipolysis. The first identified genetic polymorphism of *UCP-1* (-3826A>G) is an A to G mutation in the promoter region of the gene. There is evidence indicating that the presence of G allele in this locus is associated with increasing risk of obesity, type 2 diabetes, and low expression of UCP-1 (4,7,8). The genotype frequencies of AA, AG, and GG for UCP-1 polymorphism A-3826G were found 48.6%, 32.9%, and 18.6%, respectively, in our participants. To our knowledge, this is the first study that examines the -3826 A>G polymorphism of UCP-1 among Iranians. However, Our findings, are not similar to those reported for African-Americans (33), Japanese (7,8,34) and Europeans (35,36).

B3ADR gene is another important candidate gene for obesity. Since $\beta 3ADRs$ increase the expression of UCP-1, therefore playing a main role in regulation of thermogenesis and body weight. One variant in the *B3ADR* gene is a missense single nucleotide mutation in codon 64 which substitutes tryptophan to arginine in the first cytoplasmic region of receptor (37). It has been related to abdominal obesity, difficulty in weight loss, lower basal metabolic rate (BMR) and insulin resistance (7,9,10). According to our

results, the genotype frequencies of Trp64Trp, Trp64Arg, and Arg64Arg for $\beta 3 ADR$ gene were 84.3%, 15.7%, and 0%, respectively. This finding is in agreement with the results of previous studies from Iran (38), North America (39), Spain (40), and Turkey (28), although Arg64Arg genotype was seen with a higher frequency of about 3%-6% among the Japanese (7,41,42).

The main limitation of our study is however our sample size which is not large enough to represent the association of these genotypes with anthropometric measurements and other biochemical parameters.

Conclusion

In conclusion, we found all genotypes of each SNP in our study subjects except for the Arg64Arg genotype of $\beta 3 ADR$ that is also very rare in other populations. Furthermore, the genotype-allelic frequencies of $ADIPOQ$ and $\beta 3 ADR$ genes were similar in various populations, while *leptin* and *UCP-1* genes have different genotype

distributions. The genetic variations among populations may be due to environmental factors, different conditions of study subjects, the synergistic effect of different genes, etc. Regarding the important role of the aforementioned genetic variations in development of obesity and also response to treatments like weight lowering diet, it is recommended to study the genotype of every population before implementing any interventional program.

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Conflict of Interest

The authors have declared that there is no conflict of interest.

References

- Bouchard C. Gene-environment interactions in the etiology of obesity: defining the fundamentals Obesity 2008;16 (Suppl 3):5-10.
- El-Sayed Moustafa JS, Froguel P. From obesity genetics to the future of personalized obesity therapy. Nat Rev Endocrinol 2013;9(7):402-13.
- Waalén J. The genetics of human obesity. Transl Res 2014;164(4):293-301.
- Brondani LA, Assmann TS, Duarte GC, Gross JL, Canani LH, Crispim D. The role of the uncoupling protein 1 (UCP1) on the development of obesity and type 2 diabetes mellitus. Arq Bras Endocrinol Metabol 2012;56(4):215-25.
- Jia JJ, Tian YB, Cao ZH, et al. The polymorphisms of UCP1 genes associated with fat metabolism, obesity and diabetes Mol Biol Rep 2010; 37(3):1513-22.
- Andersson D, Wahrenberg H, Löfgren P. $\beta 3$ -adrenoceptor function and long-term changes in body weight Int J Obes 2009;33(6):662-8.
- Tsunekawa K, Yanagawa Y, Aoki T, et al. Association between accumulation of visceral fat and the combination of $\beta 3$ adrenergic receptor Trp64Arg, $\beta 2$ adrenergic receptor Arg16Gly and uncoupling protein 1 -3826A>G polymorphisms detected by Smart Amplification Process 2. Endocr J 2011;58(12):1079-86.
- Kotani K, Sakane N, Saiga K, et al. Relationship between A-3826G polymorphism in the promoter of the uncoupling protein-1 gene and high-density lipoprotein cholesterol in Japanese individuals: a cross-sectional study. Arch Med Res 2008;39(1):142-6.
- Rudkowska I, Pérusse L. Individualized weight management: what can be learned from nutrigenomics and nutrigenetics? Prog Mol Biol Transl Sci 2012;108:347-82.
- Mirrahimov AE, Kerimkulova AS, Lunegova OS, et al. An association between TRP64ARG polymorphism of the $\beta 3$ adrenoceptor gene and some metabolic disturbances. Cardiovasc Diabetol 2011;10:89.
- Fonseca-Alaniz MH, Takada J, Alonso-Vale MI, Lima FB. Adipose tissue as an endocrine organ: from theory to practice. J Pediatr 2007; 83(5 Suppl):S192-S203.
- Itoh M, Suganami T, Hachiya R, Ogawa Y. Adipose tissue remodeling as homeostatic inflammation. Int J Inflamm 2011;720926.
- Melistas L, Mantzoros CS, Kontogianni M, Antonopoulou S, Ordovas JM, Yiannakouris N. Association of the +45T>G and +276G>T polymorphisms in the adiponectin gene with insulin resistance in non-diabetic Greek women. Eur J Endocrinol 2009;161(6):845-85.
- Al-Daghri NM, Al-Attas OS, Alokail MS, et al. Adiponectin gene polymorphisms (T45G and G276T), adiponectin levels and risk for metabolic diseases in an Arab population. Gene 2012;493(1):142-7.
- Namvaran F, Rahimi-Moghaddam P, Azarpira N, Dabbaghmanesh MH. Polymorphism of adiponectin (45T/G) and adiponectin receptor-2 (795G/A) in an Iranian population: relation with insulin resistance and response to treatment with pioglitazone in patients with type 2 diabetes mellitus. Mol Biol Rep 2012;39(5):5511-8.
- Koleva DI, Orbetzova MM, Atanassova PK. Adipose tissue hormones and appetite and body weight regulators in insulin resistance. Folia Med (Plovdiv) 2013;55(1):25-32.
- Proença AR, Sertié RA, Oliveira AC, et al. New concepts in white adipose tissue physiology. Braz J Med Biol Res 2014;47(3):192-205.
- Scarpace PJ, Matheny M. Leptin induction of UCP1 gene expression is dependent on sympathetic innervation. Am J Physiol 1998;275(2 Pt 1):E259-264.
- Hinuy HM, Hirata MH, Forti N, et al. Leptin G-2548A promoter polymorphism is associated with increased plasma leptin and BMI in Brazilian women. Arq Bras Endocrinol Metabol 2008;52(4):611-6.
- Carrillo JV, Lopez JA, Chimal BV, et al. G-2548A leptin promoter and Q223R leptin receptor polymorphisms in obese Mexican subjects. Am J Agric Biol Sci 2013;8(1):34-43.
- Huuskonen A, Lappalainen J, Tanskanen M, Oksala N, Kyroläinen H, Atalay M. Genetic variations of leptin and leptin receptor are

- associated with body composition changes in response to physical training. *Cell Biochem Funct* 2010;28(4):306-12.
22. Samadi Shams S, Zununi Vahed S, Soltanzad F, et al. Highly effective DNA extraction method from fresh, frozen, dried and clotted blood samples. *Bioimpacts* 2011;1(3):183-7.
 23. Hassanzadeh T, Maleki M, Saidijam M, Paoli M. Association between leptin gene G-2548A polymorphism with metabolic syndrome. *J Res Med Sci* 2013;18(8):668-73.
 24. Portolés O, Vicente Sorli J, Francés F, et al. 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* 2006;21:605-12.
 25. Cleveland RJ, Gammon MD, Long CM, et al. Common genetic variations in the LEP and LEPR genes, obesity and breast cancer incidence and survival. *Breast Cancer Res Treat* 2010;120(3):745-52.
 26. 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(1):282-6.
 27. Crescenti A, Solà R, Valls RM, Anguera A, Arola L. Polymorphisms in LEP and NPY genes modify the response to soluble fibre *Plantago ovata* husk intake on cardiovascular risk biomarkers. *Genes Nutr* 2013;8(1):127-36.
 28. Mergen H, Karaaslan C, Mergen M, Deniz Ozsoy E, Ozata M. LEPR, ADRB3, IRS-1 and 5-HTT genes polymorphisms do not associate with obesity. *Endocr J* 2007;54(1):89-94.
 29. Tabatabaei-Malazy O, Hasani-Ranjbar Sh, Amoli MM, et al. Gender-specific differences in the association of adiponectin gene polymorphisms with body mass index. *Rev Diabet Stud* 2010;7(3):241-6.
 30. Kaklamani VG, Sadim M, Hsi A, et al. Variants of the adiponectin and adiponectin receptor 1 genes and breast cancer risk. *Cancer Res* 2008a; 68:3178-84.
 31. Fang WL, Zhou B, Wang YY, Zhang L. Analysis of adiponectin gene polymorphisms in Chinese population with systemic lupus erythematosus. *J Biomed Biotechnol* 2010;2010: 401537.
 32. Chung HK, Chae JS, Hyun YJ, et al. Influence of adiponectin gene polymorphisms on adiponectin level and insulin resistance index in response to dietary intervention in overweight-obese patients with impaired fasting glucose or newly diagnosed type 2 diabetes. *Diabetes Care* 2009;32(4):552-8.
 33. Sale MM, Hsu FC, Palmer ND, et al. The uncoupling protein 1 gene, UCP1, is expressed in mammalian islet cells and associated with acute insulin response to glucose in African American families from the IRAS Family Study. *BMC Endocr Disord* 2007;7:1.
 34. Hamada T, Kotani K, Nagai N, et al. Low-calorie diet-induced reduction in serum HDL cholesterol is ameliorated in obese women with the -3826 G allele in the uncoupling protein-1 gene. *Tohoku J Exp Med* 2009;219(4):337-42.
 35. Labruna G, Pasanisi F, Nardelli C, et al. UCP1 -3826 AG+GG genotypes, adiponectin, and leptin/adiponectin ratio in severe obesity. *J Endocrinol Invest* 2009;32(6):525-9.
 36. Nieters A, Becker N, Linseisen J. 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 Nutr* 2002; 41(5): 210-21.
 37. Malik SG, Saraswati MR, Suastika K, Trimarsanto H, Oktavianthi S, Sudoyo H. Association of beta3-adrenergic receptor (ADRB3) Trp64Arg gene polymorphism with obesity and metabolic syndrome in the Balinese: a pilot study. *BMC Res Notes* 2011;4:167.
 38. Eshraghi P, Hedayati M, Daneshpour MS, Mirmiran P, Azizi F. Association of body mass index and Trp64Arg polymorphism of the b3-adrenoreceptor gene and leptin level in Tehran Lipid and Glucose Study. *Br J Biomed Sci* 2007;64(3):117-20.
 39. Bea JW, Lohman TG, Cussler EC, Going SB, Thompson PA. Lifestyle modifies the relationship between body composition and adrenergic receptor genetic polymorphisms, ADRB2, ADRB3 and ADRA2B: a secondary analysis of a randomized controlled trial of physical activity among postmenopausal women. *Behav Genet* 2010;40(5):649-59.
 40. Piérola J, Barceló A, de la Peña M, et al. Beta3-Adrenergic receptor Trp64Arg polymorphism and increased body mass index in sleep apnoea. *Eur Respir J* 2007; 30(4):743-7.
 41. Lee JS, Kawakubo K, Inoue S, Akabayashi A. Effect of β (3)-adrenergic receptor gene polymorphism on body weight change in middle-aged, overweight women. *Environ Health Prev Med* 2006;11(2):69-74.
 42. Takeuchi S, Katoh T, Yamauchi T, Kuroda Y. ADRB3 polymorphism associated with BMI gain in Japanese men. *Exp Diabetes Res* 2012;2012:973561.