Research Article

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

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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, *ß3-adrenergic receptor* (*ß3ADR*) 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 *ß3ADR* 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 *ß3ADR* gene were found 84.3%, 15.7%, and 0%, respectively. The genotype-allelic frequencies of *ADIPOQ* and *ß3ADR* 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; *ß3ADR* 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 ß3-adrenergic receptor (*ß3ADR*) 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 *ß3ADR* 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

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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 *ß3ADR* 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), *ß3ADR* (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*, *ß3ADR*, *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
Leptin	G -2548A	rs7799039	F:TTTCCTGTAATTTTCCCGTGAG 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
Adiponectin	+45 T>G (T+45G)	rs2241766	F: GAAGTAGACTCTGCTGAGATGG R: TATCAGTGTAGGAGGTCTGTGATG	bp 372	95°C:5min, 95°C:30s, 50°C:50s, 72°C:1min, **32× (stage 2-4)**, 72°C:5min	14
UCP-1	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
B3ADR	Trp64Arg	rs4994	F: CGCCCAATACCGCCAACAC 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
Leptin	*HhaI*	5′... G C G|C…3′ 3′... C|G C G…5′	37°C	8 h	20
Adiponectin	*SmaI*	5′ . C C C|G G G…3′.. 3′... G G G|C C C…5′	30°C	12 h	14
UCP-1	*BclI*	5′…T|G A T C A …3′ 3′…A C T A G|T…5′	55°C	5-6 h	8
B3ADR	*MvaI (BstNI)*	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 yeilded 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 and153-bp as mutant GG genotype.

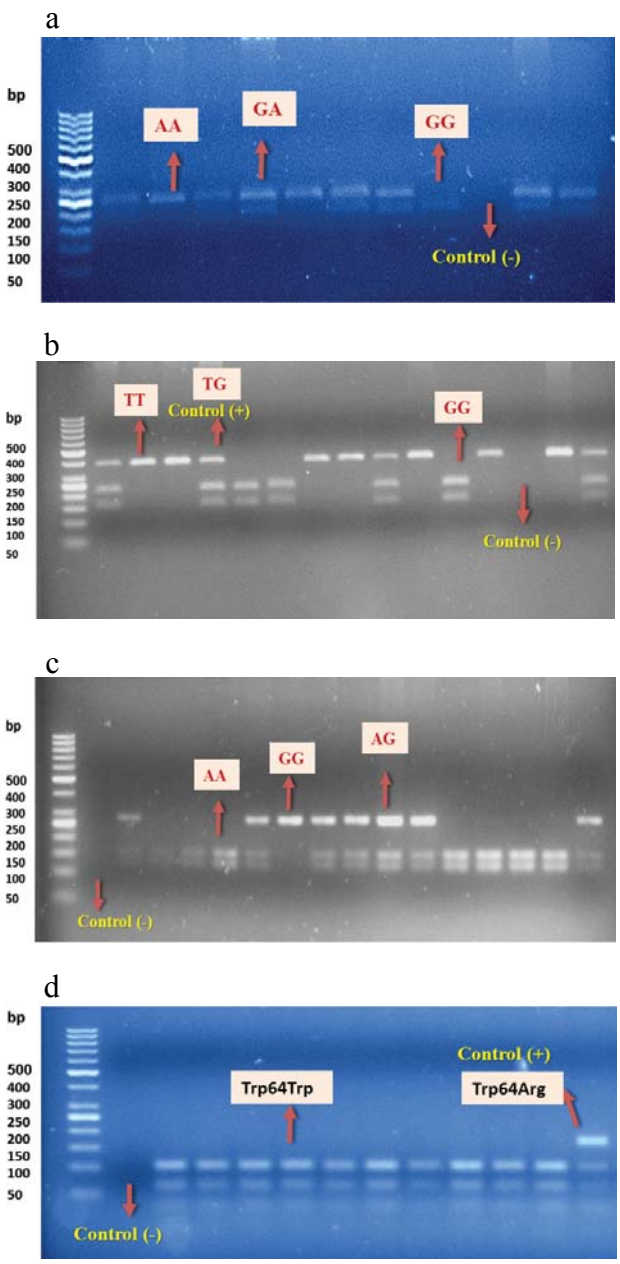

Figure 1: PCR-based restriction fragment length polymorphism analysis of (a): *leptin*, (b): *adiponectin*, (c): *UCP-1* and (d): *β3ADR*: 50 bp DNA ladder

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

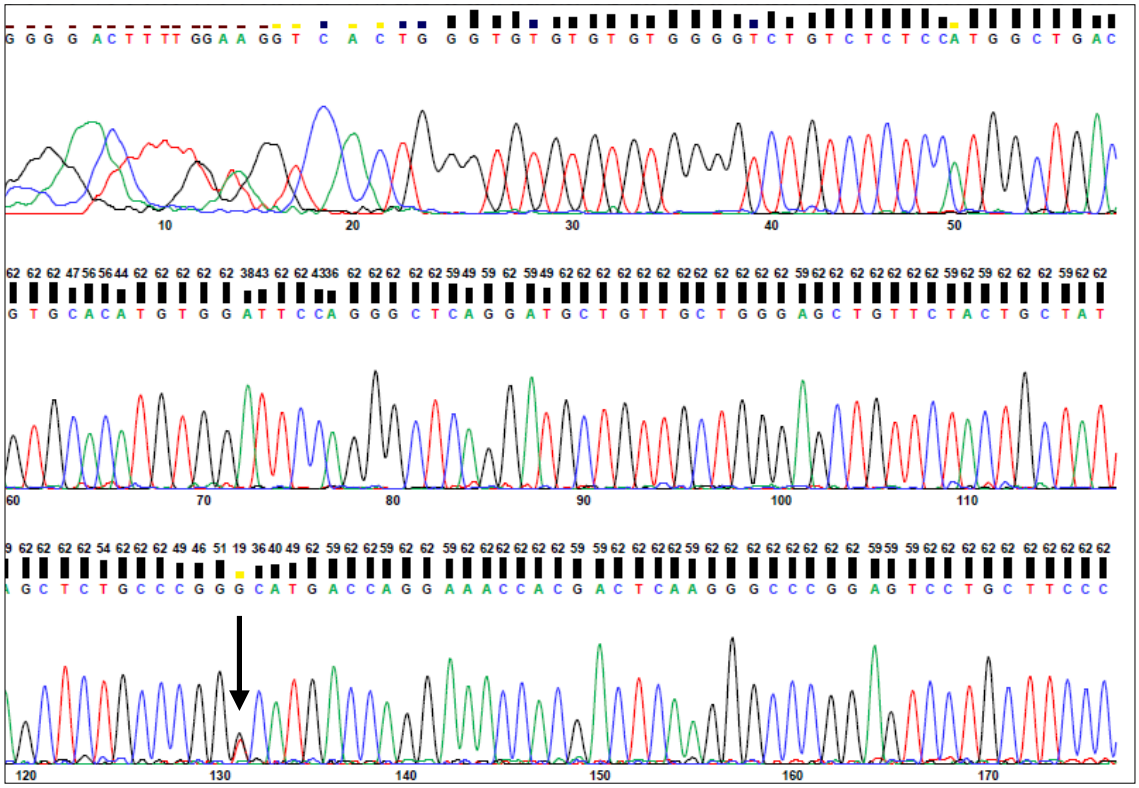

Figure 2: Sequencing data for *Adiponectin* +45 T>G polymorphism (TG genpotype)

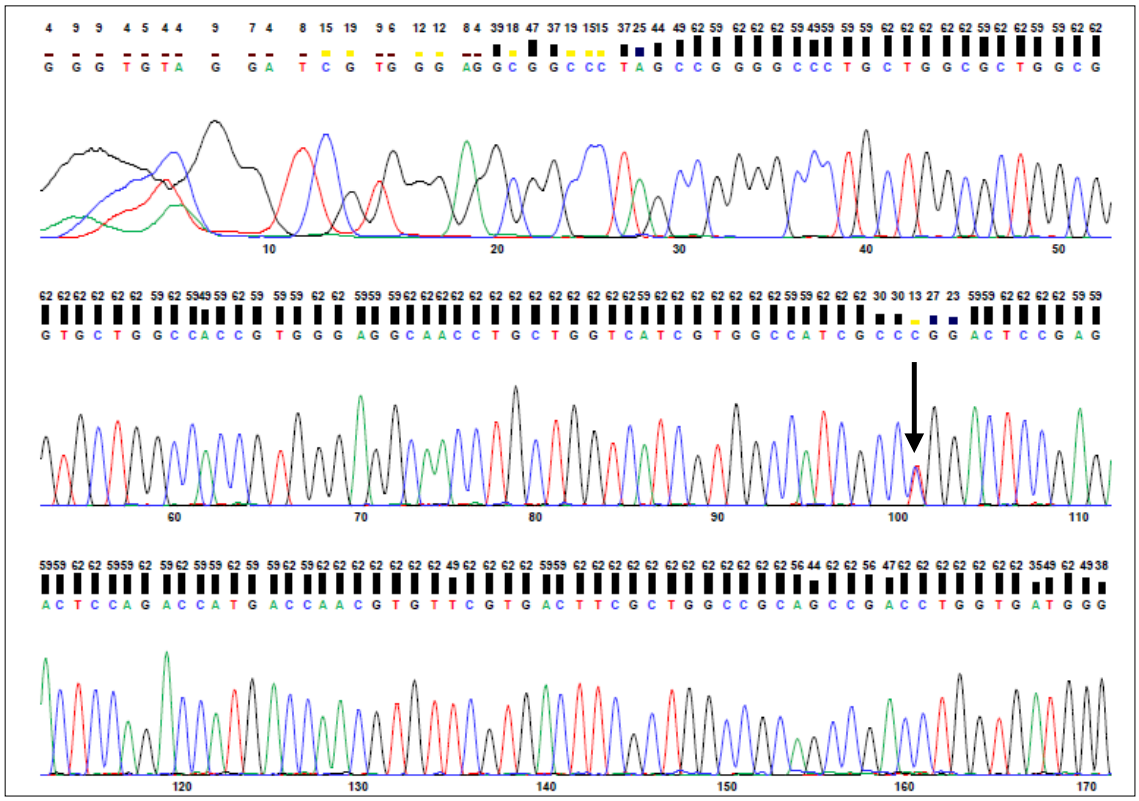

Figure 3: Sequencing data for *β3ADR* Trp64Arg polymorphism (Trp64Arg genpotype)

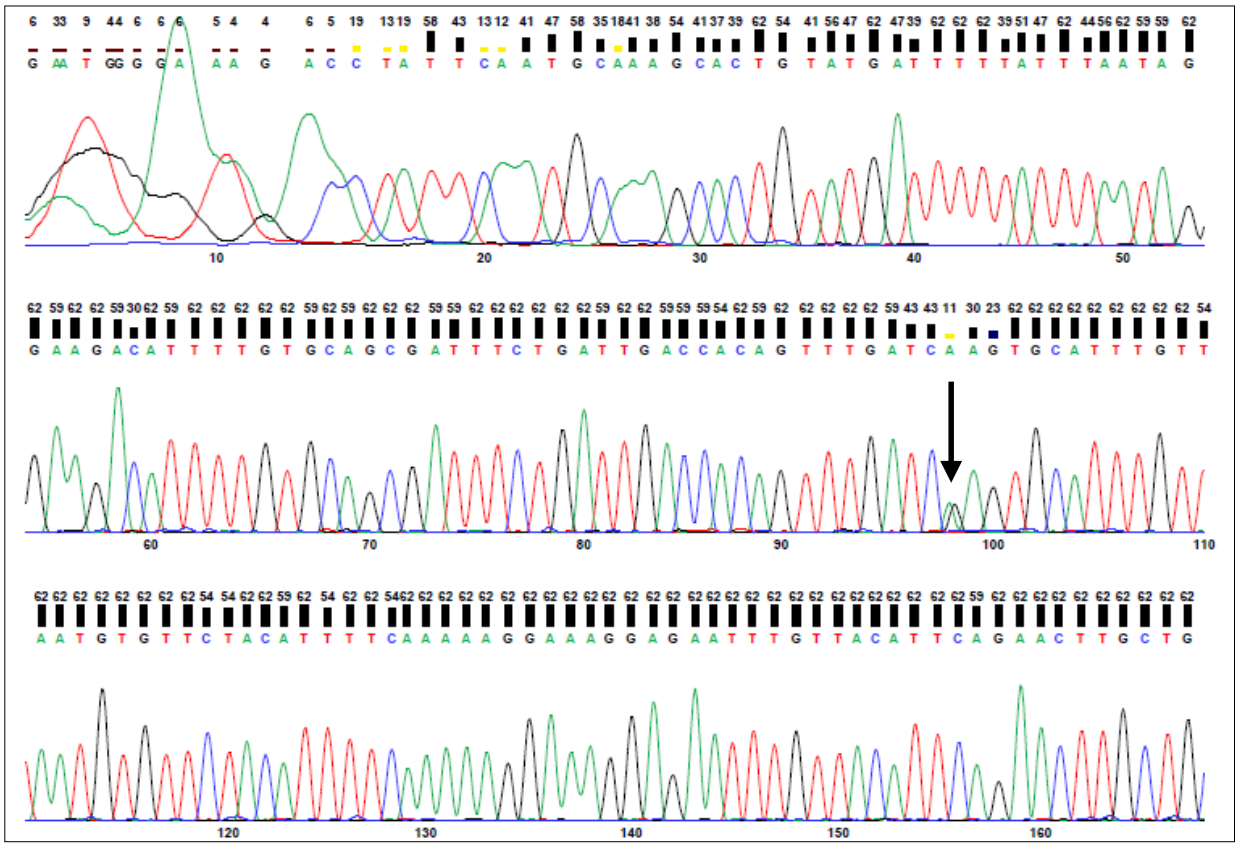

Figure 4: Sequencing data for *UCP-1* -3826A>G polymorphism (AG genpotype)

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%, 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,

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*
Leptin	**GG**	**GA**	**AA**	**G**	**A**	0.519
-2548 G>A	14 (20.0%)	36 (51.4%)	20 (28.6%)	45.7%	54.3%	
Adiponectin	**TT**	**TG**	**GG**	**T**	**G**	0.283
+45 T>G	47 (67.1)	19 (27.1%)	4 (5.7%)	80.7%	19.3%	
UCP-1	**AA**	**AG**	**GG**	**A**	**G**	0.020
-3826A>G	34 (48.6%)	23 (32.8%)	13 (18.6%)	65.0%	35.0%	
β3ADR	**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 *ß3ADR* 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, *adiponectine*, *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*, *adiponectine*, *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 β3ADRs 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 *ß3ADR* 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 *ß3ADR* that is also very rare in other populations. Furthermore, the genotype-allelic frequencies of *ADIPOQ* and *ß3ADR* 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.

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