

Vitamin D Receptor Gene Haplotype and Late-Onset Obesity in Iranian Azeri Turkish Women

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ABSTRACT

Introduction: A large body of literature has revealed the association between vitamin D3-VDR complex and obesity. The aim of the present study was to survey the rate of the VDR polymorphisms in obese women and to determine whether there may be an association between VDR BsmI and Tru9I haplotypes and obesity in Iranian Azeri Turkish women.

Material and methods: 65 Iranian Azeri Turkish women were enrolled in the study and PCR amplification and direct sequencing of PCR products were used for genotypings.

Results: The findings of this study showed that VDR BsmI G/G genotype, VDR BsmI A/A genotype, Tru9I A allele and Tru9I A/A genotype were more frequent in obese women compared to controls. The frequency of VDR BsmI G/Tru9I A (GA), VDR BsmI G/Tru9I G (GG), VDR BsmI A/Tru9I G (AG), and VDR BsmI A/Tru9I A (AA) haplotypes were 19.74%, 42.11%, 38.16% and 0% in cases, and 11.11%, 40.74%, 42.59 and 5.56% in controls. Statistically significant differences were found between cases and controls regarding the VDR AA haplotype ($P=0.03$).

Conclusions: Our findings demonstrated that the VDR AA haplotype frequency was significantly lower in subjects with obesity compared with normal controls. This study shows that the VDR AA haplotype is significantly associated with a decreased risk of obesity in the tested group. This report is the first of its kind in the West Azerbaijani population.

Keywords: VDR, haplotype, obesity, women

INTRODUCTION

Obesity is known as unwarranted fat amassing that exposes public health to numerous risks (1). In a population, the body mass index (BMI) is used to measure the se-

verity of obesity. BMI is calculated via dividing the weight in kilograms by the square of the height in meters. A person with a BMI of 30 or more is defined as obese (1). Globally, in 2015, about 2.3 billion people (age 15+) and over 700 million adults (age 18+) were obese (2). Worldwide, the prevalence of obesity increased con-

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siderably in the last decades from 4.2% in 1990 to 6.7% in 2010, and it is estimated to reach 9.1% in 2020 (3). In Iran, similarly to the other countries, the prevalence of obesity has been increasing (3). In 2013, the prevalence of overweight and obesity among people in Tehran aged 20–84 was 34.1% (95% CI 32.3–35.9) and 15.4% (95% CI 14.0–16.8), respectively (4). The prevalence of overweight in urban population is expected to be about 22% and 40% in 15–39 and 40–69 year olds, respectively. Relevant values in Iranian females seem to be higher (5). In Tehran, Iran, 40% and 23.1% of the adult study group were overweight (BMI, 25 to 29.9 kg/m²) and obese (BMI $\geq$ 30 kg/m²), respectively. Frequency of overweight and obesity was 42.6% versus 38.1%, and 14.4 versus 29.5% in males versus females, respectively (6). This study shows the impact of gender on increasing incidence of obesity and overweight in Iranian population (6).

Obesity is one of the most important health problems leading to several diseases such as diabetes mellitus (7), hypertension (8), hyperlipidemia (9), depression (10, 11), and death (12). Obesity influences the long-term dialysis (13) as well as the function and survival of renal allograft following transplantation (14). In women, obesity results in irregular menstrual cycles and oligo-anovulation as well as infertility (15–17). Obese women have poor reproductive outcomes in assisted conceptions such as induction of ovulation in polycystic ovarian syndrome, *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI), and oocyte donation cycles (18). In men, obesity is associated with reduced levels of testosterone and spermatogenesis and subsequent infertility (18). The prevalence of obesity is regularly increasing in the world because of several factors such as lifestyle, diet behavior and physical activity (19). Environmental and genetic factors are the subject of studies in different ethnic groups (20). Recent findings suggest that vitamin D and vitamin D (1,25-dihydroxyvitamin D₃) receptor (VDR) polymorphisms play a role in obesity via various mechanisms (21–24).

The aim of the present study was to survey the rate of the VDR polymorphisms in obese women and to determine whether there was an association between the VDR BsmI and Tru9I alleles/genotypes/haplotypes and obesity in Iranian Azeri Turkish women.

MATERIALS AND METHODS

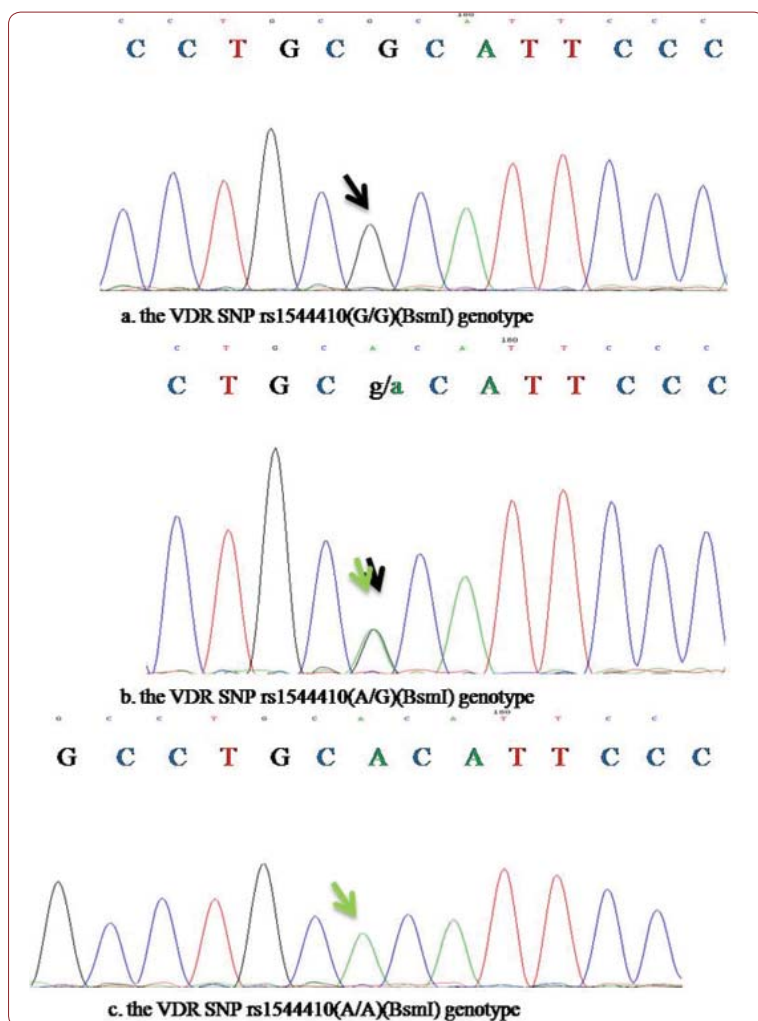
This research project was a case-control study and conducted at the Maternal and Childhood Obesity Research Center, Urmia University of Medical Sciences, Urmia, Iran. The ethics committee of Urmia University of Medical Sciences approved this research project prior to the initial enrollment of any subject (irumsu.rec.1393.47). After a full clarification of this investigation, every individual was informed about the contents and goals of the research project. Individuals who signed the written informed consent were enrolled in investigation. Sixty five Iranian Azeri Turkish women were enrolled in the study; 38 of them were obese and 27 non-obese (control group). All subjects were genetically unrelated and matched for ethnicity, geographical area and age in case and control groups. All subjects were examined in Motahari Teaching Hospital (Urmia, Iran), which is an obstetrics and gynecology referral center. Medical history, physical tests, and clinical evaluations were performed by the same specialist for all individuals. Diagnosis of obesity was based on the finding of the criteria as proposed by Pi-Sunyer (2000) (25). Participants with a history of any known disorders including obesity after pregnancy, endocrine abnormalities (such as Cushing syndrome, hypothyroidism, hyperthyroidism, parathyroid disease, etc), and chronic kidney disease were excluded from the study as well as those who were taking vitamin D₃ or drugs which are known to affect calcium metabolism and lipid profile (25). The salting out method was used to extract genomic DNA from 3–4 mL whole blood collected with EDTA (26).

PCR and sequencing

Optimized primer pairs of 5'-ggcaacctgaaggga-gacgta-3' and 5'-ctcttggacatcaccgac-3' were used for PCR amplification and direct sequencing of PCR products regarding VDR SNPs rs1544410(A/G) (BsmI) and rs757343 (G/A)(Tru9I) (27). PCR reactions were carried out in 50 μ L solution including 50 ng of DNA, 1x reaction buffer 5 pmol of each primer, 200 μ mol of each dNTPs, 0.3 unit of Taq DNA polymerase, and 1.5 mmol MgCl₂. PCR program was 93°C for 45 s, 66°C for 30 s, and 72°C for 45 s (35 cycles) (27). PCR products were evaluated by electrophoresis on 2% agarose gel stained with CinnaGen DNA safe Stain (CinnaGen Co. Tehran,

Marker	Allele/Genotype/ Haplotype	Cases F (% F)	Controls F (% F)	OR (95% CI)	P value
rs1544410	G	48(63.16)	29(53.7)	1.478(0.727-3.004)	0.279
	A	28(36.84)	25(46.3)	0.677(0.333-1.376)	0.279
	GG	14(36.84)	5(18.52)	2.567(0.794-8.3)	0.109
	GA	18(47.37)	19(70.37)	0.379(0.134-1.075)	0.064
	AA	6(15.79)	3(11.11)	1.5(0.34-6.613)	0.590
rs757343	G	60(78.95)	45(83.33)	0.75(0.304-1.851)	0.531
	A	16(21.05)	9(16.67)	1.333(0.54-3.291)	0.531
	GG	25(65.79)	18(66.67)	0.962(0.339-2.731)	0.941
rs1544410/ rs757343	GA	10(26.32)	9(33.33)	0.714(0.243-2.099)	0.539
	AA	3(7.89)	0(0)	-	0.134
	GA	15(19.74)	6(11.11)	1.967(0.71-5.453)	0.187
	GG	32(42.11)	22(40.74)	1.058(0.521-2.149)	0.876
	AG	29(38.16)	23(42.59)	0.832(0.409-1.693)	0.611
	AA	0(0)	3(5.56)	-	0.037
	AA	0(0)	3(5.56)	-	0.037

TABLE 1. Markers, alleles, genotypes and haplotypes in studied groups and analysis of the VDR polymorphisms data



Iran). Presence or absence of a 461(bp) fragment was monitored by UV transilluminator. Subsequently, direct sequencing of the PCR products was carried out in an ABI 730XL DNA analyzer (Applied Biosystems). Chromas Lite version 2.1.1 (2012) was used for chromatogram visualization of sequenced DNA fragments (Chromas Lite version 2.1 (2012), Technelysium Pty Ltd, South Brisbane, Queensland, Australia). VDR SNPs rs1544410 (A/G) (BsmI) and rs757343 (G/A)(Tru9I) alleles, genotypes, and haplotypes were found regarding BsmI and Tru9I sites on chromatograms.

Statistical analysis

Descriptive statistics were used to report the frequency of the VDR polymorphisms. P value, odds ratio (OR), and 95% confidence interval (CI) have been computed for detection of statistically significant differences between cases and controls regarding the frequencies in studied markers. The frequencies of our data were compared using the chi-square test or the Fisher's exact test

FIGURE 1. Schematic representation of chromatography and the VDR SNP rs1544410 (G/A) (BsmI) polymorphisms that is located in the intron 8 at position 10,583,292 of the chromosome 12q12-q14 in a sample. Black color arrow represents the VDR BsmI (rs1544410) G allele and green color arrow represents the VDR BsmI (rs1544410) A allele in suspected DNA locus. The VDR SNP rs1544410 (A/G)(BsmI) genotypes: a: g/g; b: g/a; c: a/a.

in the case and control groups. A p -value ≤ 0.05 was considered as statistically significant difference between tested groups.

RESULTS

The investigation was performed on 38 obese women (mean age: 31.5 ± 1.9) and 27 healthy controls (mean age: 28.2 ± 5.2). Our cases were obese women (Grade I) (BMI > 30 kg/m²). Significant difference was found between cases and controls regarding the BMI (kg/m²) ($p < 0.05$). VDR SNPs rs1544410 (A/G) (BsmI) and rs757343 (G/A) (Tru9I) were detected for all subjects. The findings of this study are shown in Table 1 and Figures 1-3. Allele/genotype/haplotype association with obesity was examined by verifying the distribution of VDR allele/genotype/haplotype in obese vs. controls. In obese women, results showed that the VDR BsmI (rs1544410) G allele, VDR BsmI (rs1544410) G/G genotype, VDR BsmI (rs1544410) A/A genotype, Tru9I (rs757343) A allele and Tru9I (rs757343) A/A genotype were more frequent compared with controls. But the differences between cases and controls were not significant (P value > 0.05). The distribution of VDR haplotypes in obese vs. controls were determined. Results indicated that the frequency of VDR BsmI (rs1544410) G/ Tru9I (rs757343) A (GA), VDR BsmI (rs1544410) G/ Tru9I (rs757343) G (GG), VDR BsmI (rs1544410) A/Tru9I (rs757343) G (AG), and VDR BsmI (rs1544410) A/Tru9I (rs757343) A (AA) haplotypes were 19.74%, 42.11%, 38.16% and 0% in cases and 11.11%, 40.74%, 42.59, and 5.56% in controls respectively. Statistically significant differences were found between cases and controls, regarding VDR BsmI (rs1544410) A/Tru9I (rs757343) A (AA) haplotype (P value = 0.03) and maybe suggesting a "protective" role. The presence of the "protective" VDR AA haplotype was associated with a reduced risk of obesity in our cases.

DISCUSSION

Obesity affects adults and children (28), and gene-lifestyle interactions have an important role in adiposity (28). It has been demonstrated that more than 40 genetic variants had been associated with obesity (28), and one of these genetic variants was VDR (24). The VDR gene spans more than 100 kb (chromosome

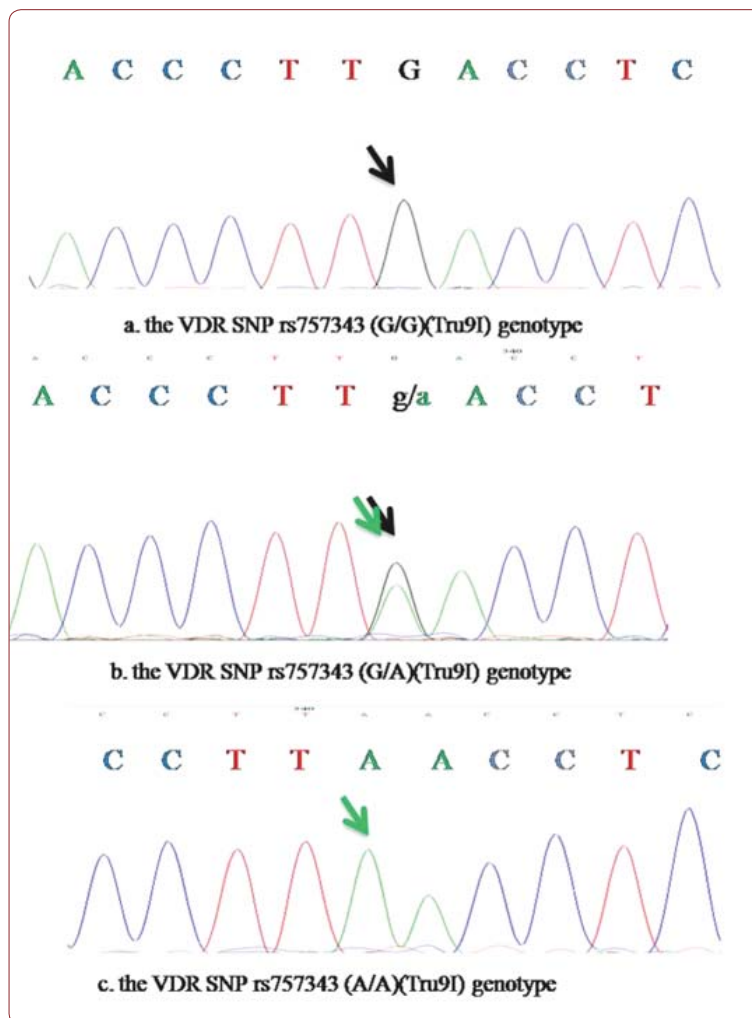


FIGURE 2. Schematic representation of the VDR SNP rs757343 (G/A) (Tru9I) polymorphisms that are located in the intron 8 at position 10,583,292 of the chromosome 12q12-q14 in a sample. Black color arrow represents the VDR Tru9I (rs757343) G allele and green color arrow represents the VDR Tru9I (rs757343) A allele in suspected DNA locus. The VDR SNP rs757343 (G/A)(Tru9I) genotypes: a: g/g ; b: g/a; c: a/a.

12q13.11) and its promoter region generates numerous tissue-specific transcripts (29). The VDR gene contains 11 exons and encodes the nuclear hormone receptor for vitamin D₃ (29). Vitamin D₃ as a neurosteroid mediates its role through the VDR (30). The VDR gene has numerous SNPs in the vicinity of the 3' un-translated region that are recognized by related restriction endonuclease (TaqI, BsmI and ApaI) (30). Several investigations have studied the association between the vitamin D₃-VDR complex and human diseases (30, 31). VDR gene polymorphisms (VDR SNPs rs731236 (G) (TaqI) and rs1544410 (T) (BsmI) minor allele polymorphisms are associated with obesity (31). We studied the associa-

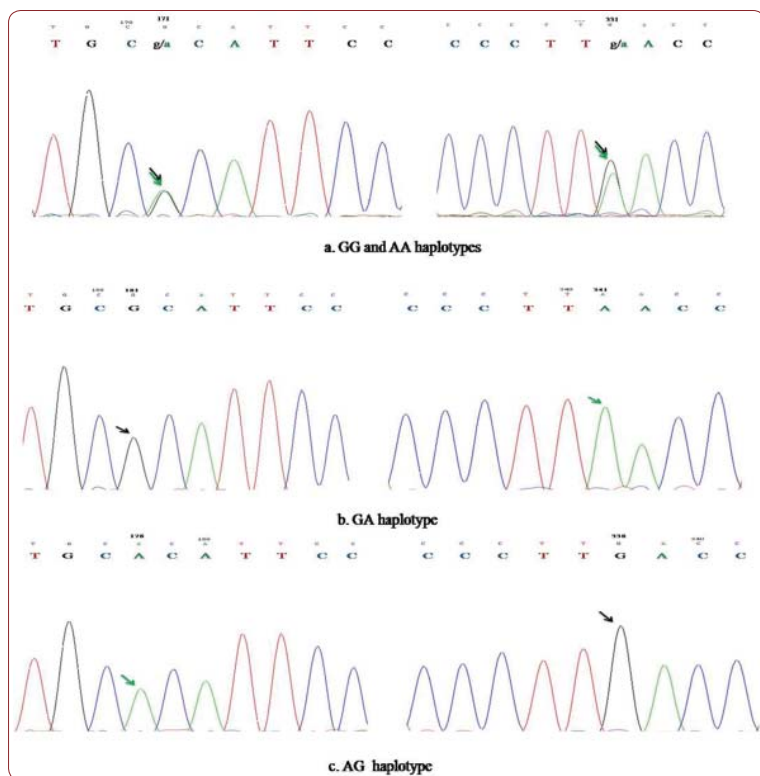


FIGURE 3. Schematic representation of the VDR haplotypes in our tested groups. a) BsmI (rs1544410) G/ Tru9I (rs757343) G (GG) and VDR BsmI (rs1544410) A/ Tru9I (rs757343) A (AA) haplotypes; b) BsmI (rs1544410) G/ Tru9I (rs757343) A (GA) haplotype; c) BsmI (rs1544410) A/ Tru9I (rs757343) G (AG) haplotype.

tions of the two VDR SNPs rs1544410 (A/G) (BsmI) and rs757343 (A/G)(Tru9I) with obesity among Iranian Azeri Turkish women. Consequently, the goal of this study was to observe the rate of the VDR BsmI (rs1544410) G and A alleles, VDR BsmI (rs1544410) G/G, A/G and A/A genotypes, Tru9I (rs757343) G and A alleles, Tru9I (rs757343) G/G, A/G and A/A genotypes as well as the “GA”, “GG”, “AG”, “AA” haplotypes of the VDR gene in obese women regarding BsmI and Tru9I sites.

Our results showed that the frequency of “GA”, “GG”, “AG”, “AA” haplotypes were 19.74%, 42.11%, 38.16% and 0% in cases, and 11.11%, 40.74%, 42.59 and 5.56% in controls, respectively. No statistically significant differences were found between cases and controls, but the only exception was VDR (AA) haplotype (P value = 0.03). The “protective” VDR AA haplotype is thus associated with reduced risk of obesity in our cases. Notably, the VDR GA haplotype had an increased rate in obese women [OR (95% CI) = 1.967 (0.71-5.453)]. New studies address the potential role of

VDR and VDR allelic variation in the mechanism of glucose homeostasis (32, 33). Studies in mice showed that lacking a functional VDR leads to distraction of the VDR signaling pathway and is associated with a prominent destruction in oral glucose tolerance and impaired insulin secretory capacity as well as reduced level of pancreatic insulin mRNA (32). The presence of the GG (bb) genotype of the BsmI SNP is responsible for a difference of approximately 9 kg of body weight and an increase in the incidence of obesity as compared to the other genotypes (33). Associations of VDR genotypes with body size were found in some studies (19). The mechanisms of this association remain unexplained. VDR is expressed in pre-adipocytes and may play an important role in adipocyte differentiation (34). It has been demonstrated that 25-dihydroxyvitamin D3 inhibits uncoupling protein 2 expression in human adipocytes (35) and adipose differentiation of pre-adipocytes (36), and stimulates the terminal adipose differentiation (37) and secretion of lipoprotein lipase in cultured adipocytes (38). There is an inverse association between BMI and serum levels of 25-hydroxyvitamin D (39). These data indicate that the VDR alleles are correlated to different levels of circulating vitamin D.

CONCLUSION

In summary, we evaluated the effect of VDR polymorphisms on the risk of obesity using a small sample size of obese and normal controls. Our analysis demonstrated that the VDR AA haplotype frequency of rs1544410 (A) and rs757343 (A) was significantly lower in obese subjects compared with normal controls. This study shows that the VDR AA haplotype is significantly associated with decreased risk of obesity in Iranian Azeri Turkish women; this effect may result from the associations of VDR alleles with body mass, and obesity may be related to allelic inflection of insulin production. Our findings may reveal evidence for a genetic role in the pathogenesis of obesity. $\square$

Conflicts of interest: none declared.

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REFERENCES

1. Chan RS, Woo J. Prevention of overweight and obesity: how effective is the current public health approach. *Int J Environ Res Public Health* 2010;3:765-783.
2. World Health Organization Fact sheet: obesity and overweight. Available online: <http://www.who.int/mediacentre/factsheets/fs311/en/> (accessed on October 5, 2009).
3. Nouri Saeidlou S et al. Trend of overweight and obesity, based on population study among school children in North West of Iran: implications for when to intervene. *Maedica J Clin Med* 2015;3:214-220.
4. Moghimi-Dehkordi B et al. Overweight and obesity and related factors in urban Iranian population aged between 20 to 84 years. *Ann Med Health Sci Res* 2013;2:171-176.
5. Rashidi A et al. Prevalence of obesity in Iran. *Obes Res* 2005;3:191-192.
6. Azizi F, Rahmani M, Majidi M, et al. Serum lipid levels in an Iranian population of children and adolescents: Tehran Lipid and Glucose Study. *Eur J Epidemiol* 2001;3:281-288.
7. Tyrovolas S, Koyanagi A, Garin N, et al. Diabetes mellitus and its association with central obesity and disability among older adults: a global perspective. *Exp Gerontol* 2015;64:70-77.
8. Kaur S, Sachdev H, Dwivedi SN, et al. Association of obesity with hypertension amongst school-age children belonging to lower income group and middle income group in national capital territory of Delhi. *Indian J Community Med* 2013;3:175-179.
9. Lai SW, Ng KC, Lin HF, Chen HL. Association between obesity and hyperlipidemia among children. *Yale J Biol Med* 2001;4:205-210.
10. Omrani MD, Bagheri M, Bushehri B, et al. The association of TGF- β 1 codon 10 polymorphism with suicide behavior. *Am J Med Genet B Neuropsychiatr Genet* 2012; 159B (7):772-775.
11. Nemiary D, Shim R, Mattox G, Holden K. The Relationship between Obesity and Depression among Adolescents. *Psychiatr Ann* 2012;8:305-308.
12. Omrani MD, Bushehri B, Bagheri M, et al. Role of IL-10 -1082, IFN- γ +874, and TNF- α -308 genes polymorphisms in suicidal behavior. *Arch Suicide Res* 2009;4:330-339.
13. McDonald SP, Collins JE, Johnson DW. Obesity is associated with worse peritoneal dialysis outcomes in the Australia and New Zealand patient populations. *J Am Soc Nephrol* 2003;11:2894-2901.
14. Gore JL, Pham PT, Danovitch GM, et al. Obesity and outcome following renal transplantation. *Am J Transplant* 2006;2:357-363.
15. Bagheri M, Abdi Rad I, Omrani MD, Nanbakhsh F. Polymorphisms of the angiotensin converting enzyme gene in Iranian Azeri Turkish women with unexplained recurrent pregnancy loss. *Hum Fertil (Camb)* 2010;2:79-82.
16. Bagheri M, Abdi Rad I, Hosseini Jazani N, Nanbakhsh F. Vitamin D Receptor TaqI Gene Variant in Exon 9 and Polycystic Ovary Syndrome Risk. *Int J Fertil Steril* 2013;2:116-121.
17. Bagheri M, Abdi-Rad I, Hosseini-Jazani N, et al. An association study between INSR/InsII (rs2059806) and INSR/PmlI (rs1799817) SNPs in women with polycystic ovary syndrome from West Azerbaijan Province, Iran. *J Reprod Infertil* 2015;2:109-112.
18. Palmer NO, Bakos HW, Fullston T, Lane M. Impact of obesity on male fertility, sperm function and molecular composition. *Spermatogenesis* 2012;4:253-263.
19. Wilborn C, Beckham J, Campbell B, et al. Obesity: prevalence, theories, medical consequences, management, and research directions. *J Int Soc Sports Nutr* 2005;2:4-31.
20. Caprio S, Daniels SR, Drewnowski A, et al. Influence of race, ethnicity, and culture on childhood obesity: implications for prevention and treatment: a consensus statement of Shaping America's Health and the Obesity Society. *Diabetes Care* 2008;11:2211-2221.
21. vinh quốc Lu'ong K and Nguyễn LT. The beneficial role of vitamin D in obesity: possible genetic and cell signaling mechanisms. *Nutr J* 2013;12:89.
22. Almesri N, Das NS, Ali ME, et al. Independent associations of polymorphisms in vitamin D binding protein (GC) and vitamin D receptor (VDR) genes with obesity and plasma 25OHD3 levels demonstrate sex dimorphism. *Appl Physiol Nutr Metab* 2016;4:345-353.
23. Nguyen VT, Li X, Elli EF, Ayloo SM, et al. Vitamin D, inflammation, and relations to insulin resistance in premenopausal women with morbid obesity. *Obesity (Silver Spring)* 2015;8:1591-1597.
24. Clemente-Postigo M et al. Serum 25-hydroxyvitamin D and adipose tissue vitamin D receptor gene expression: relationship with obesity and type 2 diabetes. *J Clin Endocrinol Metab* 2015;4:E591-E595.
25. Pi-Sunyer FX - Obesity: criteria and classification. *Proc Nutr Soc* 2000;4:505-509.
26. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;3:1215.
27. Bagheri M et al. Lack of Association of Vitamin D Receptor FokI (rs10735810) (C/T) and BsmI (rs1544410) (A/G) Genetic Variations with Polycystic Ovary Syndrome Risk: a Case-control Study from Iranian Azeri Turkish Women. *Maedica J Clin Med* 2012;4:303-308.
28. Herrera BM, Keildson S, Lindgren CM. Genetics and epigenetics of obesity. *Maturitas* 2011;1:41-49.
29. Uitterlinden et al. Vitamin D receptor gene polymorphisms in relation to Vitamin D related disease states. *J Steroid Biochem Mol Biol* 2004;1-5:187-193.
30. Gezen-Ak D, Dursun E, Bilgiç B, et al. Vitamin D receptor gene haplotype is associated with late-onset Alzheimer's disease. *Tohoku J Exp Med* 2012;3:189-196.
31. Zeitz U, Weber K, Soegiarto DW, et al. Impaired insulin secretory capacity in mice lacking a functional vitamin D receptor. *Faseb J* 2003;3:509-511.
32. Reis AF, Hauache OM, Velho G. Vitamin D endocrine system and the genetic susceptibility to diabetes, obesity and vascular disease. A review of evidence. *Diabetes Metab* 2005;4 Pt 1:318-325.
33. Grundberg E et al. Genetic variation in the human vitamin D receptor is associated with muscle strength, fat mass and body weight in Swedish women. *Eur J Endocrinol* 2004;150:323-328.
34. Shi H, Norman AW, Okamura WH, et al. 1 α , 25-dihydroxyvitamin D₃ inhibits uncoupling protein 2 expression in human adipocytes. *Faseb J* 2002;16:1808-1810.
35. Kawada T, Kamei Y, Sugimoto E. The possibility of active form of vitamins A and D as suppressors on adipocyte development via ligand-dependent transcriptional regulators. *Int J Obes Relat Metab Disord* 1996;20 (suppl.3):S52-S57.
36. Dace A, Martin-el Yazidi C, Bonne J, et al. Calcitriol is a positive effector of adipose differentiation in the OB 17 cell line: relationship with the adipogenic action of triiodothyronine. *Biochem Biophys Res Commun* 1997;3:771-776.
37. Querfeld U, Hoffmann MM, Klaus G, et al. Antagonistic effects of vitamin D and parathyroid hormone on lipoprotein lipase in cultured adipocytes. *J Am Soc Nephrol* 1999;10:2158-2164.
38. Need AG, Morris HA, Horowitz M, Nordin C. Effects of skin thickness, age, body fat, and sunlight on serum 25-hydroxyvitamin D. *Am J Clin Nutr* 1993;6:882-885.
39. Wortsman J, Matsuoka L, Chen TC, et al. Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr* 2000;3:690-693.