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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

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ABSTRACT

Introduction: *In this study we evaluate the involvement of Vitamin D Receptor (VDR) FokI (rs10735810) Exon 2 (C/T) and BsmI (rs1544410) Intron 8 (A/G) gene variations in genetic susceptibility to polycystic ovary syndrome (PCOS) in Iranian Azeri Turkish women.*

Materials and methods: *The RFLP-PCR method was performed on peripheral blood lymphocyte for a total of 46 females with PCOS and 46 controls.*

Outcomes: *VDR FokI (rs10735810) CC,CT,TT,C and T genotypic/allelic frequencies were 22(47.83), 20(43.48), 4(8.696), 64(69.57) and 28(30.43) in cases and 29(63.04), 15(32.61), 2(4.348), 73(79.35) and 19(20.65) in controls, respectively. The frequencies of VDR FokI C and T alleles were 0.7 and 0.3 in cases, and 0.79 and 0.21 in controls, respectively. VDR BsmI (rs1544410) Intron 8 (A/G) AA,AG,GG,A*

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and G genotypic/allelic frequencies were 15(32.6), 27(58.7), 4(8.7), 57(62), and 35(38) in cases and 20(43.5), 24(52.2), 2(4.35), 64(69.6), and 28(30.4) in controls, respectively. The frequencies of VDR BsmI (rs1544410) Intron 8 A and G alleles were 0.7 and 0.3 in cases, and 0.62 and 0.38 in controls, respectively. Statistical analysis showed that the differences in genotypic/allelic frequencies between the cases and controls were not statistically significant regarding of VDR FokI(rs10735810) Exon 2 (C/T) and VDR BsmI (rs1544410) Intron 8 (A>G) (p >0.05).

Conclusions: *It can be concluded that FokI (rs10735810) Exon 2 (C/T) and VDR BsmI (rs1544410) Intron 8 (A>G) were not associated with PCOS susceptibility in studied group. Present investigation is the first study in its own kind in Iranian Azeri Turkish women.*

Keywords: Vitamin D receptor, FokI, BsmI, Polycystic Ovary Syndrome

INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is known as one of the most usual heterogeneous hormonal disease among females in the reproductive age (1). The mechanism and molecular etiology of PCOS is still poorly understood (2). Diagnosis of PCOS was carried out based on abnormal findings in medical checkups regarding hyperandrogenic, polycystic ovarian morphology on ultrasound, ovulatory dysfunction (oligo-anovulation), and amenorrhea as well as exclusion of other related disease such as androgen excess (3,4). It has been shown that not only each female with PCOS suffers from wide range of the symptoms and abnormalities but also those are not the same among affected cases (3,4). PCOS resulting in several disorders such as infertility (5), myocardial infarction (6), dysfunctional uterine bleeding (7), cardiovascular risk (7), endometrial carcinoma (7), coronary artery disease (8,9), insulin resistance (IR) (10-12), diabetes mellitus (11-13), hyperandrogenism (hirsutism, acne, male-pattern hair loss), oligo-anovulation, and polycystic ovaries on ultrasound (14), dyslipidemia (15), amenorrhea (16) and hypertension (17) as well as associated with obesity (18) and high levels of cholesterol (19). PCOS incidence became increased ranging from 5-10% and aging 12 to 45 years old in females (20). The study of PCOS aetiopathogenesis has been suggested that several genes as well as environmental factors have been associated with PCOS in different ethnic groups (21-23). Vitamin D Receptor (VDR) locus variations seem to have important impact on pathogenesis and insulin resistance in PCOS women (24). These findings confirms the influence of VDR genetic variations on intestinal calcium absorption (24). Vitamin D and calcium repletion predict reproductive success following fertilization (25). Calcium is one of the main regulators of process including egg activation, oocyte maturation and follicular development and resulting in embryo development (26). Ranjzad et al (2010) reported that there was significant association between VDR BsmI GG genotype and decreased levels of sex hormone binding globulin (SHBG) in PCOS women (27). The results of several investigations imply that SHBG level became decreased in insulin resistance and actually is known as a very good marker for diagnosis of insulin resistance and PCOS (27-30). Presence of the "F" allele and "FF" genotype of the FokI genetic variation are more important than the "f" allele and "Ff and ff" genotypes (31) and have been associated with increased risk for IR in PCOS women (32). Also, Mahmoudi (2009) reported that "bb" genotype (presence of restriction sites for ApaI and BsmI) has been associated with higher levels of insulin and IR in comparison to ''Ff/ff''and "BB and Bb" genotypes (32). Present investigation is the first to study the role of theVDR locus variations (FokI and BsmI) in genetic susceptibility to PCOS in Iranian Azeri Turkish women. ❑

METHODS AND MATERIALS

The research project was approved by the Ethics Committee of Urmia University of Medical Sciences and all subjects signed informed consents. This investigation was performed in Urmia University of Medical Sciences in the city of Urmia, Iran. Between 2011 and 2012, a total number of 92 females with age ranging from 18 to 40 years (46 patients with PCOS and 46 healthy women as normal

controls) entered the study. Cases and controls were genetically unrelated and matched for ethnicity, and geografical region. All participants were clinically examed in ART Reproductive Center and Infertility Clinic by ART and infertility specialists. Familial and medical history, physical evaluations, and clinical tests were carried out by the same physicians for all individuals. All diagnosis was based on the finding of three or more of the criteria proposed by the Rotterdam criteria (33) and on the basis of the NICHD criteria (34). Participants with a history of any known cause of oligomenorrhea, amenorrea, hyper-androgenism including non-classic congenital adrenal hyperplasia, hyperprolactinaemia and other confounding factors as well as individuals taking drugs that affect calcium homeostasis were excluded from the study (27). A 3-4 mL of whole blood was collected with EDTA-containing tubes for extraction of DNA by standard "salting out" method (35). Genotypes of VDR BsmI (rs1544410) Intron 8 (G/A) and FokI (rs10735810) Exon 2 (C/T) were determined using RFLP-PCR method. Optimized primer pairs of the VDR gene (Fok-I and BsmI) were used as reported earlier (27). Type of SNPs, site of SNPs, PCR Conditions (primers and programs), un-cut PCR products, restriction enzymes, incubation temperature, and allele size (bp) are summerized in Table 1. PCR reaction was performed in a 20 μl solution including 100 ng of DNA, 1x reaction buffer 10 pmol of each primer, 200 μmol of each dNTPs, 0.5 unit of Taq DNA polymerase, and 1.5 mmol MgCl2. Following the production of PCR products, PCR fragments were digested with restriction enzymes (Fermentas, Stockholm, Sweden). Digested PCR products were analyzed by electrophoresis on 2.5% agarose gel containing ethidium bromide stain, and presence or absence of fragments were monitored by UV transilluminator.VDR BsmI (rs1544410) Intron 8 (G/A) and FokI (rs10735810) Exon 2 (C/T) genotypic and allelic frequencies were counted directly. The chi-square (χ^2) test was performed to compare VDR BsmI (rs1544410) Intron 8 (G/A) and FokI (rs10735810) Exon 2 (C/T) genotypic and allelic distributions between patients and healthy control group. The data were analyzed for deviations from Hardy-Weinberg equilibrium (HWE) at each locus. A minimum sample size of 38 individuals in cases group had a statistical power of about 90% (two-tailed, α = 5%). Calculation of χ^2 value, the odds ratio (OR), and 95% confidence interval (CI) as well as analysis of independent T-Test for detection of differences between cases and controls regarding clinical characteristics were performed by SPSS ver. 16.0 software and Microsoft Office Excel 2007. A p-value of less than 0.05 was considered statistically significant difference between tested groups. ❑

OUTCOMES

The studied group consisted a total number of 92 females (46 PCOS women (mean±SD age, 26.58±3.33) and 46 healthy women as normal controls (mean±SD age, 28.24±5.25). Statistically significant difference between cases and controls was not found regarding age (p-value >0.05). But in the case of BMI (kg/m^2) statistically significant difference between cases and controls was found (p-value <0.05). The prevalence of hirsutism and obesity (BMI >27 kg/m2) in our tested cases were about 100 % and 61.53%, respectively. Our cases (χ^2 = 0.03 <3.84, p value with degree of freedom 2 = 0.98 >0.05) and controls (χ^2 = 0.001 <3.84, p value with degree of freedom 2 = 0.99 >0.05) were consistent with HWE regarding VDR FokI (rs10735810) Exon 2 (C/T). VDR FokI (rs10735810) CC,CT,TT,C and T genotypic/allelic frequencies were 22(47.83), 20 (43.48), 4(8.696), 64(69.57) and 28(30.43) in cases and 29(63.04), 15(32.61), 2(4.348), 73 (79.35) and

Gene/ SNP(SNP ID)	Location	PCR detailes
VDR/FokI (rs10735810)	Exon 2 (C/T)	5′-agctggccctggcactgactctgctct-3′ 5′-atggaaacaccttgcttcttctccctc-3′ PCR program: x35: 93°C 45 s,66°C30 s, 72°C 45 s un-cut PCR products: 265 bp restriction enzymes,Incubation temperature: FokI, 55°C Alleles: Allele C: 265, Allele T: 169+96 (bp)
VDR/BsmI (rs1544410)	Intron 8 (G/A)	5′-ggcaacctgaagggagacgta-3′ 5′-ctctttggacctcatcaccgac-3′ PCR program: x35: 93°C 45 s,66°C30 s, 72°C 45 s un-cut PCR products: 461(bp) restriction enzymes,Incubation temperature: BsmI, 37°C Alleles: Allele A: 461, Allele G: 258+203 (bp)

TABLE 1. Type of SNPs, site of SNPs, PCR Conditions (primers and programs), un-cut PCR products, restriction enzymes, incubation temperature, and allele size (bp).

19(20.65) in controls, respectively. The frequencies of VDR FokI C and T alleles were 0.7 and 0.3 in cases, and 0.79 and 0.21 in controls, respectively. In the case of VDR BsmI (rs1544410) Intron 8 (A/G),our cases (χ^2 = 2.76 <3.84, p value with degree of freedom 2 = 0.25 >0.05) and controls (χ^2 = 2.47 <3.84, p value with degree of freedom 2 = 0.28 >0.05) were consistent with HWE.VDR BsmI (rs1544410) Intron 8 (A/G) AA,AG,GG,A and G genotypic/allelic frequencies were 15(32.6), 27(58.7), 4(8.7), 57(62), and 35(38) in cases and 20(43.5), 24(52.2), 2(4.35), 64 (69.6), and 28(30.4) in controls, respectively. The frequencies of VDR BsmI (rs1544410) Intron 8 A and G alleles were 0.7 and 0.3 in cases, and 0.62 and 0.38 in controls, respectively. Statistical analysis showed that the differences in genotypic/allelic frequencies between the cases and controls were not statistically significant regarding VDR FokI(rs10735810) Exon 2 (C/T) and VDR BsmI (rs1544410) Intron 8 (A>G) (p >0.05) (see Table 2 and Figure 1). ❑

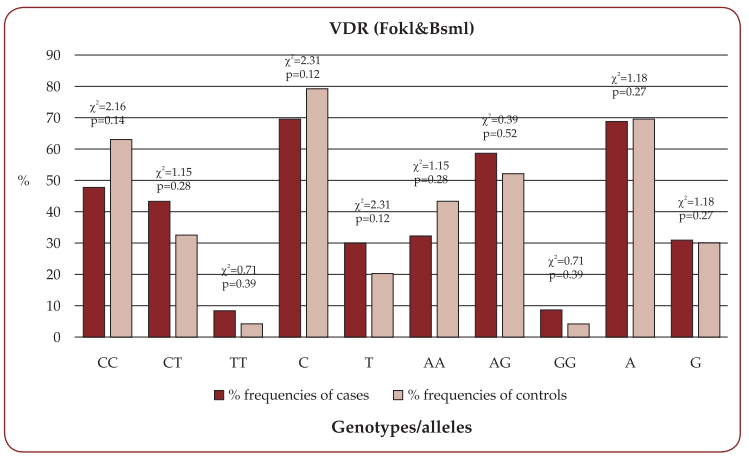

FIGURE 1. FokI (rs10735810): CC,CT,TT,C, and T; BsmI (rs1544410): AA, AG, GG, A, and G.

DISCUSSION

Results of several investigations formed our understanding of VDR genotypes and intestinal calcium absorption in postmenopausal women as well as women's reproductive health (24,25). The aim of present study was to assess the role of VDR FokI (rs10735810) (C/T) and BsmI (rs1544410) (A/G) genetic variation in PCOS for the first time in Iranian Azeri women. PCOS is defined as a syndrome that greatly influences ovarian functions. Research studies indicated that the reason of the ovarian overproduction of testosterone in PCOS women is due by inability of women to mediate insulin effectively (IR or Hyperinsulinemia) (32,36). Hyperandrogenemia and IR are important indicators of PCOS (29). Higher insulin levels and IR is more frequent in PCOS women with F allele and FF genotype of the VDR FokI considering biochemical markers related to PCOS (32). In this condition, level of insulin hormon whithin the blood is too high, therefore the ovaries produce higher level of testosterone (36). SHBG is a carrier protein which regulates the level of un-bound steroids in peripheral blood (28,29). It has been demonstrated that VDR genetic variations have been associated with LH and SHBG levels in PCOS women (27). It has been demonstrated that SHBG expression is reduced in the stromal compartment of endometria of women with polycystic ovary syndrome (30). Increasing of androgens bioavailability result in hyperandrogenemia by hyperinsulinemia in PCOS women with VDR BsmI GG genotypes via lower serum level of SHBG (27). In our study, statistical analysis showed that the differences in genotypic/allelic frequencies between the cases and controls were not statistically significant regarding VDR FokI(rs10735810) Exon 2 (C/T) and VDR BsmI

VDR	Genotype/ Allele	Cases F(F%)	Controls F(F%)	OR(95% CI)	χ^2	P-Value
FokI (rs10735810) Exon 2 (C/T)	CC	22(47.83)	29(63.04)	0.54(0.23-1.24)	2.16	0.142
	TC	20(43.48)	15(32.61)	1.59(0.68-3.71)	1.15	0.283
	TT	4(8.696)	2(4.348)	2.1(0.36-12)	0.71	0.398
	C	64(69.57)	73(79.35)	0.59(0.3-1.17)	2.31	0.128
	T	28(30.43)	19(20.65)	1.68(0.86-3.29)	2.31	0.128
BsmI (rs1544410) Intron 8 (G/A)	AA	15(32.6)	20(43.5)	0.629(0.269-1.469)	1.153	0.283
	AG	27(58.7)	24(52.2)	1.303(0.571-2.97)	0.396	0.529
	GG	4(8.7)	2(4.35)	2.095(0.364-12.05)	0.713	0.398
	A	57(62)	64(69.6)	0.713(0.386-1.314)	1.183	0.277
	G	35(38)	28(30.4)	1.404(0.761-2.588)	1.183	0.277

TABLE 2. Genotype and allele frequencies of FokI (rs10735810) Exon 2 (C/T) and VDR BsmI (rs1544410) Intron 8 in tested groups.

(rs1544410) Intron 8 (A>G) (p >0.05). The exact etiopathogenesis of PCOS are not known regarding vitamin D and IR. Several molecular mechanisms have been suggested to describe the relationship between the VDR locus variations and PCOS in different ethic groups. We had some limitations regarding low sample size, registry data of participants because of poor quality of registration systems. Studies with a large sample size and more information such as other candidate gene variants, haplotypes and genetic linkage assessment are needed for further analysis (37-39). ❑

CONCLUSION

It can be concluded that FokI (rs10735810) Exon 2 (C/T) and VDR BsmI (rs1544410) Intron 8 (A>G) were not associated with PCOS susceptibility in studied group. Present investigation is the first study in its own kind in Iranian Azeri Turkish women.

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