

## POLYMORPHIC VARIATIONS IN VDR GENE IN SAUDI WOMEN WITH AND WITHOUT POLYCYSTIC OVARY SYNDROME (PCOS) AND SIGNIFICANT INFLUENCE OF SEVEN POLYMORPHIC SITES ON ANTHROPOMETRIC AND HORMONAL PARAMETERS

POLIMORFNE VARIJACIJE VDR GENA KOĐ SAUDIJSKIH ŽENA SA I BEZ SINDROMA POLICISTIČNIH OVARIJUMA (PCOS) I ZNAČAJ UTICAJA SEDAM POLIMORFNIH MESTA NA ANTROPOMETRIJSKE I HORMONSKE PARAMETRE

Arwa Al Thomali<sup>1,4</sup>, Maha H. Daghestani<sup>1,4</sup>, Mazin H. Daghestani<sup>3</sup>, Namik Kaya<sup>2</sup>, Arjumand Warsy<sup>4</sup>

<sup>1</sup>Department of Zoology, College of Science, King Saud University, Saudi Arabia

<sup>2</sup>Department of Genetics, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia

<sup>3</sup>Department of Obstetrics and Gynecology, Umm-Al-Qura University, Makkah, Saudi Arabia

<sup>4</sup>Central Laboratory, Center for Science and Medical Studies for Girls, King Saud University, Saudi Arabia

### Summary

**Background:** This study was designed to evaluate the associations between vitamin D receptor (VDR) gene polymorphisms and biochemical characteristics of Saudi women with polycystic ovary syndrome (PCOS).

**Methods:** Serum levels of LH, FSH, and Vitamin D were measured in 33 women: 16 patients and 17 normal controls (18 to 36 years). DNA was extracted and used for sequencing of the exons of VDR gene using ABI PRISM 3730xi Genetic Analyzer.

**Results:** Weight, BMI, Vit D, LH and FSH levels were higher in the PCOS patients compared to control group, where Vit D level correlated positively and significantly with FSH, in the control, but showed a negative and non-significant correlation in the PCOS patients. Sequencing results showed extensive polymorphisms in both groups, but the differences in the frequencies were not significant. Demographic and hormonal parameters were compared in the different genotypes of the SNPs. Significant differences were observed in the values of the studied parameters in rs11168276,

### Kratik sadržaj

**Uvod:** Ovo izučavanje je planirano da proceni vezu između genskog polimorfizma vitamin D receptora (VDR) i biokemijskih karakteristika saudijskih žena sa sindromom policističnih ovarijuma (PCOS).

**Metode:** Nivoi serumskog LH, FSH i vitamina D mereni su kod 33 žene: 16 pacijenata i 17 zdravih žena (između 18 i 36 godina). DNK je izolovan i korišćen za sekvenciranje eksona VDR gena primenom AQBI PRISM 3730xi Genetic Analyser-a.

**Rezultati:** Težina, BMI, nivoi vitamina D, LH i FSH bili su viši kod pacijenata sa PCOS nego kod kontrolne grupe, dok su nivoi vitamina D pozitivno i značajno korelirali sa FSH kod kontrolne grupe, dok je nađena negativna korelacija kod pacijenata sa PCOS. Rezultati sekvenciranja su pokazali ekstenzivni polimorfizam u obe grupe, a razlike u frekvencama u obe grupe nisu bile značajne. Demografski i hormonski parametri su poređeni kod različitih genotipova SNPs. Značajne razlike su nađene kod vrednosti proučavanih parametara u rs11168276, rs2228570, rs11168266,

Address for correspondence:

Dr. Maha Daghestani  
Department of Zoology,  
College of Sciences, King Saud University, Riyadh,  
11495, Kingdom of Saudi Arabia  
Tel: +966-1-464-7272 x39612, +966-1-442-4585  
e-mail: mdaghestani@ksu.edu.sa

List of abbreviations: BMI, Body mass index; CI, 2.5–95% confidence interval; ELISA, Enzyme Linked Immuno Sorbent Assay; FSH, Follicle stimulating hormone; GnRH, Gonadotropin-releasing hormone; LH, Luteinizing hormone; IRB, Institutional Review Board; N, Number; OR, Odds ratio; PCOS, Polycystic ovary syndrome; PCR, Polymerase Chain Reaction; p, P value-Statistical significance; r, Correlation coefficient; s, Second; SNP, Single nucleotide polymorphisms; UTR, Untranslated region; Vit D, Vitamin D; VDR, Vitamin D receptor; VDR, Vitamin D receptor gene;  $\chi^2$ , Chi-square

rs2228570, rs11168266, rs3858733, rs121909790, rs11168265 and rs731236. Vitamin D level was influenced significantly by the genotypes of rs11168265 (AA) ( $p=0.008$ ), rs11168276 (AA;  $p=0.018$ ) and rs731236 (CC;  $p=0.024$ ).

**Conclusion:** Vitamin D deficiency does not associate with PCOS in Saudi females. Several SNPs are identified in the VDR gene, in normal and PCOS females, but there is no difference in their frequencies between the two groups. The results show that polymorphism in VDR gene influences certain anthropometric and hormonal parameters in PCOS patients. Further detailed studies are required to confirm the associations between VDR and PCOS.

**Keywords:** vitamin D receptor; polycystic ovarian syndrome, SNPs, vitamin D

## Introduction

Vitamin D (Vit D) is involved in a broad variety of biological processes (1) and plays a major role in many hormonal and metabolic disorders associated with the female reproductive system, such as Polycystic Ovary Syndrome (PCOS) (2). Most of the processes brought about by Vit D are mediated through its binding to the Vit D receptor (VDR) (3). The VDR is a member of the nuclear receptor superfamily and is spread across different tissues, including intestine, kidney, parathyroid gland, pancreatic beta cells, and bones, all involved in maintenance of calcium homeostasis. It is also expressed in human ovarian tissue and in the human endometrium (4–5), and is shown to have a role in steroidogenesis of sex hormones (6). Recently, considerable interest has focused on the pathogenesis of PCOS, since it is one of the most common endocrine disorders in women of childbearing age and has a strong genetic predisposition (7). Some studies have implicated an involvement of mutant VDR gene in the pathogenesis of PCOS. Other studies have shown an association between PCOS and Vit D deficiency and insulin resistance (8–11). A higher prevalence of Vit D deficiency has been reported in women with PCOS (12, 13) and since the physiological effect of Vit D is dependent on the VDR, several investigations have been directed to study the genetic mutations and polymorphisms in VDR in PCOS patients (14–17).

The VDR Gene is located on chromosome 12q13.11 and spans about 75kb with 11 exons. Many allelic variants due to single nucleotide polymorphisms (SNP) have been reported in the VDR gene and differences are obvious in different racial groups (1, 18–20). Wehr and coworkers (21) reported no significant influence of genetic variations and PCOS susceptibility, but VDR and Vit D level-related variants were shown to be associated with some dis-

rs3858733, rs121909790, rs11168265, i rs731236. Na vitamin D značajno su uticali genotipovi rs11168265 (AA) ( $p = 0,008$ ), rs11168276 (AA;  $p = 0,018$ ) i rs731236 (CC;  $p = 0,024$ ).

**Zaključak:** Deficit vitamina D nije povezan sa PCOS kod saudijskih žena. Nekoliko SNPs je identifikovano kod VDR gena kako kod zdravih žena tako i onih sa PCOS, i nije bilo razlike u njihovim frekvencijama u obe grupe. Rezultati su pokazali da polimorfizam VDR gena utiče na izvesne antropometrijske i hormonske parametre u PCOS pacijenta. Neophodna su dalja izučavanja kako bi se potvrdile veze između VDR i PCOS.

**Ključne reči:** receptor vitamina D, sindrom policističnih ovarijuma, SNPs, vitamin D

orders in PCOS including Vit D levels. Mahmoudi and coworkers (22) reported that the Apal (rs7975232) genotype »AA« could be considered as a marker of decreased susceptibility for PCOS, while the »aa« genotype was associated with an increased risk for PCOS. Mcgrath et al. (23) showed that there was a significant relationship between rs10735810 (merged with rs2228570) and levels of Vit D, and the T allele was associated with higher concentrations of Vit D. In contrast, Bagheri et al. (24) reported no statistically significant association with PCOS susceptibility. Wehr et al. (21), found that Apal (rs7975232) variants are associated with testosterone levels in PCOS patients. A study from Iran reported an association between VDR TaqI (rs731236) (CC) genotype, and serum levels of LH (25). This finding was confirmed by Bagheri and coworkers (26).

Since there are several contradictory reports of association between SNPs in VDR and PCOS and associated hormonal abnormalities, we designed this study to investigate the levels of Vit D and polymorphism in VDR gene in Saudi females with and without PCOS and to correlate the findings to demographic and hormonal values in the two groups.

## Materials and Methods

### Study population

The study was approved by the Institutional Ethical Committee, and an IRB was obtained. Each female who volunteered to join in the study, was explained the nature of the study and a signed informed consent was obtained. The study group included 33 women with age ranging from 18-36 years (16 women suffering from PCOS and 17 healthy women as normal controls). The PCOS patients were attending clinics at the An Noor Hospital in Makkah Al Mukarama and were diag-

**Table I** Primers used for amplification of different fragments of the VDR Gene.

Exone	Forward Primer (F) or Reverse Primer (R)	Sequence	MT	Annealing Temp. (°C)
1	F	GTGGCCTATAGGGTGGTTGA	79	54
	R	CATACCTGGGCCCTGTAAGA	79	
2	F	TGTGCTGATTGTCCCATTG	77	60
	R	CAGTGGTTACAGGCTGACTCTG	80	
3	F	CTTCCCACTGCTCCTGCTAC	80	60
	R	ATCCTGGGTGGTATCCCTTC	79	
4	F	CTGAGCTCCCTGGTGGTG	79	60
	R	GGAGCTGAGAGGAGGGAAAA	80	
5	F	GGCCTCATGTCTTCTGTTGG	79	60
	R	GCTGGCAGCTACAGAGGAAG	80	
6	F	CGAGTGTTAAAGCCCCTCCT	81	58
	R	CTGCCCTCTGTCCCTACTCC	79	
7	F	TGAACAGAAGTGGGGTAGGG	79	54
	R	TTGGGTAAGTACCCCTTTC	78	
8	F	GAAGGGTGACCTGTGGAGTC	79	54
	R	AGGTTGCAGTGAGCCGAGAT	78	
9	F	AGGAACACTCTGTCCCTTCC	79	54
	R	TCCCTGTTGGTGCCTAACTC	79	
10	F	TCTTTTTCAGCTCCCAGATTC	77	60
	R	GCTCTGCAAACCAGCAAAGT	78	
11	F	TGGTATCACCGTCAGCAGT	80	58
	R	GTGAGGAGGGCTGCTGAGTA	79	
UTR	F	CGAAGTGTTTGGCAATGAGA	77	54
	R	GAGAGGGAGACCCCACTAGG	81	
UTR	F	GGACAGAGACACCTGCACCT	80	56
	R	AGGGCTTCCACCTCAACC	79	
UTR	F	GTCCCTGTCACCAAGCTCAC	80	56
	R	CTCTTAGCCCTGTGGGTGAA	79	
UTR	F	GGGTCTGGAGAAGCAGTGAG	77	60
	R	CATTCCCCAACTCAAGCA	80	
UTR	F	AGAAAGGCGTTCTTCGAGGT	78	60
	R	TCAAACAAGGGTCTCTCCCTA	78	
UTR	F	GGGGAGAAGTACATTGTGAAA	77	60
	R	CGATCTCAGCTCACTGCAAC	79	
UTR	F	AGGCTGAGGCAGAAGAATTG	78	60
	R	TGAGGCAACAGCATTATCCA	77	
UTR	F	CCAACCCATCAGAAGGAGAA	78	60
	R	TTCCAGTTAGCCAGCCAGAG	79	
UTR	F	GAGAAGGTGCCCAAAATG	78	60
	R	AATGATTCATCTCCATAAGGTC	77	

MT = Melting Temperature

F= Forward Primer

R= Reverse Primer

nosed using the Rotterdam criteria (27). A women was diagnosed as PCOS if she had at least 2 of the following criteria [menstrual abnormalities either amenorrhea (absence of periods for more than 6 months) or oligomenorrhea (fewer than six menstrual periods in the preceding year) and the appearance of oversized ovaries with the presence of more than 12 ovarian follicles by ultrasound scanning (21). In addition, all 16 women with PCOS were Saudis and were genetically unrelated. The controls were normal healthy females with regular menstrual cycle and no indications of sign and symptoms of PCOS or any other gynecological problem. History was taken and standard anthropometric data [height (cm), weight (Kg) and body mass index (BMI) ( $\text{kg}/\text{m}^2$ )] were obtained from each patient and control.

#### Sample collection

Fasting blood samples (10 mL) were collected by venipuncture in tubes with no anti-coagulant, for analyses of the hormones, and in EDTA tubes for DNA extraction. The samples were immediately centrifuged to obtain the serum which was stored at  $-20^\circ\text{C}$  until required for analysis.

#### Biochemical analysis

The serum was used for the analysis of luteinizing hormone (LH), follicle stimulating hormone (FSH) and Vit D. The measurements of LH and FSH were made using Enzyme Linked Immuno Sorbent Assay (ELISA) from (Phoenix Pharmaceuticals, Inc., Belmont, CA, USA) and the Vit D level was assayed by ELISA, using kits from EagleBio (Inc, Nashua).

#### DNA extraction

Genomic DNA was extracted from blood using DNA Extraction kits (QIAGEN) (Catalog Number: 158359), for each PCOS patient and the control sample.

#### DNA sequencing of the VDR gene

The VDR gene was divided into the exons. Primers were designed for each fragment using PRIMER 3 program. Each exon and the UTR fragments were amplified by the Polymerase Chain Reaction (PCR) using the primers presented in *Table I* on the Applied Biosystems thermocycler. The PCR cycles consisted of three major steps of PCR, which were repeated for 35 cycles. Denaturation at  $94^\circ\text{C}$  for 40 s; annealing at  $54^\circ\text{C}$  40s, and extension step at  $72^\circ\text{C}$  for 40s. The PCR products were separated on 1.8% agarose gels to access the purity, and bands were visualized using ethidium bromide for imagining

under ultraviolet light by gel documentation instrument. Each, clean PCR product obtained by amplification of the patients and control samples, was subjected to DNA sequencing. Sequencing of the VDR was carried out using Sanger Sequencing at the Core facility at King Faisal Specialist Hospital and Research Center using ABI PRISM 3730xi Genetic Analyzer (Cat#3730S, Applied Biosystems, Foster city, CA 94404, USA). The resulting sequence was matched with the reference sequence, and genotypes were constructed for each patient and control.

#### Statistical analyses

All data were analyzed using SAS (version 9.3). Data are presented as mean  $\pm$  SD or as percentages. Correlation studies were conducted, and Pearson Correlation coefficient ( $r$ ) and  $p$  values were obtained. The genotypes were obtained manually, by comparison, using DNASTar and the genotype and allele frequencies were calculated separately for the PCOS and control group. The patients and control data were compared, and odds ratio (OR), 2.5–95% confidence interval (CI), chi-square ( $\chi^2$ ) and the  $p$  values were obtained. Allele frequencies were calculated and compared. Statistical significance was defined as  $p < 0.05$ .

## Results

*Table II* presents the demographic data and hormone values obtained in the patients and control groups and the results of the Student's  $t$  test to show the significance of the difference between the two groups. Weight, Vit D and LH were higher in the PCOS patients, but age and BMI did not differ significantly. The Vit D level showed a positively skewed distribution in both groups. In the control group the minimum and maximum values ranged from 25.0–56.0 ng/mL ( $41.5 \pm 7.5$  ng/mL), while in the PCOS they were 30.0–60.0 ng/mL ( $47.2 \pm 6.9$  ng/mL) and the difference was statistically significant ( $p = 0.0324$ ). The number of women with different levels of Vit D deficiency was calculated in the two groups, and the results are presented in *Table III*. None of the women had severe Vit D deficiency or highly elevated Vit D level. Majority had optimal Vit D level in both groups, but 25% of the PCOS had Vit D level in the upper normal range.

The level of Vit D was correlated with the demographic data and the hormones. The correlation coefficient ( $r$ ), and the significance ( $p$ ) are presented in *Table IV*. Both negative and positive correlations were observed but the only significant correlation was between Vit D and FSH.

A statistically significant positive correlation was seen between the level of Vit D and FSH in the con-

**Table II** Levels of demographic variables and hormonal parameters in PCOS compared to the values in the normal healthy controls.

Variable	Control (N = 17) Mean ± SD	PCOS (N = 16) Mean ± SD	P value
Weight (kg)	58.8 ± 12.1	71.8 ± 11.4	0.0034 *
Height (cm)	162.2 ± 4.1	155.2 ± 8.6	0.0078 *
BMI (kg/m <sup>2</sup> )	35.0 ± 9.1	30.0 ± 5.7	0.0737
Vit D Level (ng/mL)	41.5 ± 7.5	47.2 ± 6.9	0.0324 *
LH (IU/L)	8.6 ± 1.3	15.2 ± 2.3	<.0001 *
FSH (IU/L)	5.8 ± 1.4	6.2 ± 4.3	0.7127

N = Total sample; SD = Standard deviation; (\*) = P –value less than 0.05

**Table III** LVit D Level in PCOS and control.

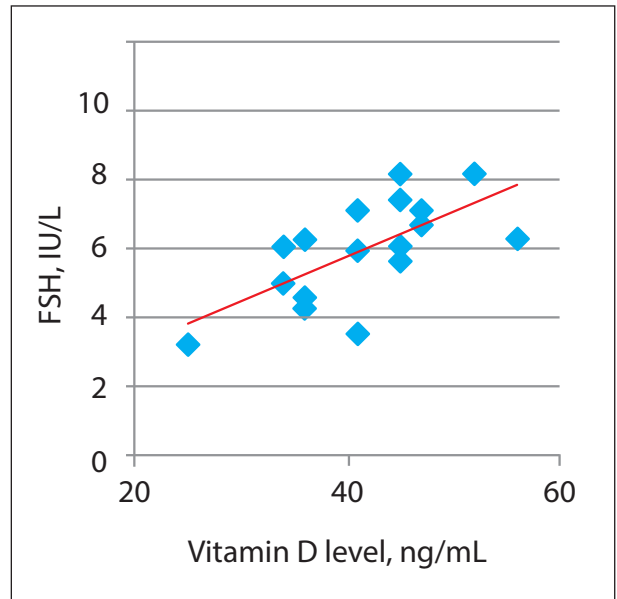
Vit D level	Control (N=17)	PCOS (N=16)
	n (%)	n (%)
Suboptimal Vit D provision (20–30 ng/mL)	1(5.8)	– (–)
Optimal Vit D level (30–50 ng/mL)	14(82.3)	12(75)
Upper normal (50–70 ng/mL)	2(11.7)	4(25)
Total	17(100)	16(100)

N= Total sample, n= Frequency

**Table IV** The Correlation between Vit D Level and the studied variables.

Correlation between Vit. D and:	Control (N = 17)		PCOS (N = 16)	
	R	P value	R	P value
Age (year)	-0.226	0.382	0.417	0.107
Weight (kg)	0.078	0.767	-0.351	0.182
Height (cm)	-0.258	0.318	-0.255	0.340
BMI (kg/m <sup>2</sup> )	0.161	0.536	-0.123	0.649
LH (IU/L)	0.090	0.731	0.023	0.934
FSH (IU/L)	0.665	0.004 *	-0.328	0.214

N = Total Sample (\*) = P – value less than 0.05



**Figure 1** Positive correlation between Vit D and FSH levels in the control group.

control group (p=0.004) (Figure 1), but the correlation was negative and non-significant in the PCOS group. Also, there were no relationships between the level of Vit D and the other variables (age, weight, height, body mass index, LH) in both groups.

Sequencing of the VDR gene showed extensive polymorphisms in the PCOS patients and controls. Table V presents the SNPs in the VDR gene, identified in both the PCOS and control groups and the genotype and allele frequencies for each SNP. No significant differences were seen between the two groups.

The study groups were assembled according to the genotypes of the different SNPs and the value of demographic and hormonal parameters, were obtained in each of the genotype. Table VI presents the value of only those parameters that showed significant difference in the different genotypes. As shown in Table VI, some of the variables were influenced by the genotype of a VDR SNP, while other showed no difference. Weight was significantly higher in females carrying GG genotype of rs2228570, AA genotype of rs11168266, AC genotype of rs3858733 and GG genotype of rs11168265, but BMI was higher only in the AC genotype of rs3858733. Among the hormonal parameters FSH was higher in the GG genotype of rs11168276 and LH in the GA genotype of rs121909790. Finally, Vit D levels were deficient in the GG genotype of rs11168276, CT genotype of rs731236 and GA genotype of rs11168265.

**Table V** SNPs identified, their location, genotype and allele frequencies in the PCOS and control group and the comparison between the two groups.

SNP(location)	Variation	Control	PCOS	OR	CI	X <sup>2</sup>	P-value
rs1381425375 (prime UTR variant A/C)	TT	16 (94.12)	16(100)	1.00	0.019–53.45	nan	1.000
	TG	1(5.88)	–	0.333	0.013–8.79	0.97	0.324
	GG	–	–	1.000	0.19–53.457	Nan	1.000
	T	33(97.0)	32(100)	2.91	0.114–74.076	0.96	1.312
	G	1(2.9)		0.344	0.013–8.745	0.96	1.359
rs4307775 (Intron variant G/C)	CC	2 (11.76)	–	0.181	0.008–4.268	2.01	0.156
	CG	6 (35.29)	6 (37.50)	5.000	0.199–125.785	1.75	0.185
	GG	9 (52.94)	10 (62.50)	5.526	0.234–130.343	2.01	0.156
	C	10(29.4)	6(18.75)	0.554	0.175–1.757	1.02	0.312
	G	24(70.5)	26(81.25)	1.806	0.569–5726	1.02	0.312
rs4303288 (Intron variant A/C)	TT	3 (17.65)	3 (18.75)	0.500	0.073–3.435	0.50	0.477
	TG	9 (52.94)	3 (18.75)	0.333	0.042–2.631	1.12	0.288
	GG	5 (29.41)	10 (62.50)	2.000	0.291–13.738	0.50	0.477
	T	15(44.1)	9(28.1)	0.496	0.178–1.382	1.82	0.177
	G	19(55.8)	23(71.8)	2.018	0.723–5.627	1.82	0.177
rs111682935 (prime UTR intron variant G/A/T)	CC	8 (47.06)	5 (31.25)	1.250	0.089–17.653	0.03	0.868
	CA	7 (41.18)	10 (62.50)	2.286	0.522–10.011	1.22	0.269
	AA	2 (11.76)	1 (6.25)	0.800	0.057–11.298	0.03	0.868
	C	23(67.6)	20(62.5)	0.797	0.289–2.198	0.19	0.660
	A	11(32.3)	12(37.5)	1.255	0.455–3.459	0.19	0.660
rs11168292 (intron variant C/A/G)	GG	8 (47.06)	5 (31.25)	1.250	0.089–17.653	0.03	0.868
	GC	7 (41.18)	10 (62.50)	2.286	0.522–10.011	1.22	0.269
	CC	2 (11.76)	1 (6.25)	0.800	0.057–11.298	0.03	0.868
	G	23(67.6)	20(62.5)	0.797	0.289–2.198	0.19	0.660
	C	11(32.3)	12(37.5)	1.255	0.455–3.459	0.19	0.660
rs1173979145 (prime UTR A/G)	TT	16(94.12)	16(100)	1.000	0.019–53.457	NAN	1.000
	TC	1(5.88)	–	0.333	0.013–8.793	0.97	0.324
	CC	–	–	1.000	0.019–53.457	NAN	1.000
	T	33(97.0)	32(100)	2.910	0.114– 74.079	0.96	1.359
	C	1(2.9)		0.344	0.013–8.745	0.96	1.359
rs11168276a (intron C/T)	GG	16(94.12)	16(100)	3.000	0.114–79.135	0.97	0.324
	GA	–	–	1.000	0.019–53.457	NAN	1.000
	AA	1(5.88)	–	0.333	0.013–8.793	0.97	0.324
	G	32(94.1)	32(100)	5.00	0.231–108.254	1.94	0.600
	A	2(5.8)		0.200	0.009–4.330	1.94	0.600
rs2228570b (start lostmissense variant A/C/G/T)	TT	1(5.88)	1 (6.25)	1.33	0.073–24.315	0.04	0.845
	TC	4(23.53)	6(37.50)	1.500	0.071–31.575	0.07	0.793
	CC	12(70.59)	9(56.25)	0.750	0.41–13.677	0.04	0.845
	T	6	8	1.556	0.473–5.117	0.53	0.465
	C	28	24	0.643	0.195–2.115	0.53	0.465
(rs121909790c missense variant C/G/T)	GG	17(100)	15(93.75)	0.886	0.017–47.358	Nan	1.000
	GA	–	1 (6.25)	3.387	0.128–89.369	1.08	0.844
	AA	–	–	1.129	0.021–60.369	1.10	0.295
	G	34(100)	31(96.8)	0.304	0.012–7.747	1.08	0.844
	A		1(3.1)	3.286	0.129–83.633	1.08	0.844

(rs10783218 splice region variant intron variant 3 prime UTR variant G/A)	CC	12(70.59)	15(93.75)	1.240	0.023–67.036	Nan	1.000
	CT	5(29.41)	1 (6.25)	0.160	0.016–1.560	2.97	0.084
	TT	–	–	0.806	0.015–43.598	Nan	1.000
	C	29(85.2)	31(96.8)	5.345	0.589–48.524	2.68	0.200
	T	5(14.7)	1(3.1)	0.187	0.021–1.699	2.68	0.200
(rs11168267 intron variant G/A/C)	CC	14(82.35)	11(68.75)	0.786	0.044–14.026	0.03	0.869
	CT	2(11.76)	4(25)	2.545	0.391–16.550	1.00	0.318
	TT	1(5.88)	1 (6.25)	1.273	0.071–22.720	0.03	0.869
	C	30	26	0.578	0.147–2.273	0.63	0.505
	T	4	6	1.731	0.440–6.810	0.63	0.505
(rs11168266d intron variant C/T)	GG	–	1 (6.25)	2.739	0.100–74.872	0.87	0.350
	GA	7(41.18)	4(25)	0.200	0.007–6.037	1.53	0.216
	AA	10	11(68.75)	0.365	0.013–9.979	0.87	0.350
	G	7(2.05)	6(18.75)	0.890	0.264–3.003	0.04	0.851
	A	27(79)	26(81.2)	1.123	0.333–3.791	0.04	0.851
(rs11168265e intron variant downstream gene variant C/T)	GG	5(29.41)	5(31.25)	1.667	0.251–11.071	0.28	0.595
	GA	7(41.18)	8(50)	1.143	0.230–5.670	0.03	0.03
	AA	5(29.41)	3(18.75)	0.600	0.090–3.986	0.28	0.595
	G	17	18	1.286	0.488–3.390	0.26	0.611
	A	17	14	0.778	0.295–2.051	0.26	0.611
(rs7975232 C/A)	GG	2(11.76)	1(6.25)	0.438	0.032–5.926	0.40	0.610
	GT	8(47.06)	7(43.75)	1.750	0.129–23.703	0.18	0.671
	TT	7(41.18)	8(50)	2.286	0.169–30.959	0.40	0.527
	G	12	9	0.717	0.253–2.036	0.39	0.532
	T	22	23	1.394	0.491–3.957	0.39	0.532
(rs731236f synonymous variant A/G)	TT	6(35.29)	4(25)	0.667	0.102–4.354	0.18	0.671
	TC	7(41.18)	8(50)	1.714	0.339–8.676	0.43	0.513
	CC	4(23.53)	4(25)	1.500	0.230–9.796	0.18	0.671
	T	19	16	0.789	0.300–2.080	0.23	0.632
	C	15	16	1.267	0.481–3.337	0.23	0.632
(rs3858733g prime UTR variant T/G)	AA	13 (76.47)	13 (81.25)	1.000	0.018–54.155	Nan	1.000
	CA	4 (23.53)	3 (18.75)	0.750	0.139–4.035	0.11	0.737
	CC	–	–	1.000	0.018–54.100	Nan	1.000
	A	30(88.2)	29(90.6)	1.289	0.265–6.267	0.10	1.000
	C	4(11.7)	3(9.37)	0.776	0.160–3.773	0.10	1.000
(rs97293 prime UTR variant G/C/T)	AA	8 (47.06)	8 (50.00)	2.000	0.150–26.734	0.28	0.595
	CA	7 (41.18)	7 (43.75)	1.000	0.238–4.198	0.00	1.000
	CC	2 (11.76)	1 (6.25)	0.0500	0.037–6.683	0.287	0.595
	C	11	9	0.818	0.285–2.347	0.14	0.708
	A	23	23	1.222	0.426–3.505	0.14	0.708
(rs28535633 prime UTR variant C/T)	GG	12 (70.59)	13 (81.25)	1.080	0.020–58.6555	Nan	1.000
	GA	5(29.41)	3 (18.75)	0.554	0.108–2.833	0.51	0.475
	AA	–	–	0.926	0.017–50.287	Nan	1.000
	G	29(85.2)	29(90.6)	1.667	0.364–7.629	0.44	0.709
	A	5(14.7)	3(9.37)	0.600	0.131–2.746	0.44	0.709

Note: The superscripts (<sup>a,b,c,d,e,f,g</sup>) on the SNPs number, points to the SNPs that show significant differences in biochemical or hormonal parameters in different genotypes, as presented in Table VI.

**Table VI** Effect of the SNP genotype in VDR on the studied variables in the study group.

SNP	Variable	Wild-type	Heterozygous	Vatiant	P-value
rs11168276a	SNP genotyping	GG	GA	AA	
	Vitamin D (ng/mL)	42.6±6.4	–	25.0±0.0	GG vs AA P=0.018
	FSH (IU/L)	6.0±1.3	–	3.2 0.0	GG vs AA p=0.049
rs2228570b	SNP genotyping	TT	TC	CC	
	Weight (kg)	85.0±0.0	61.5±12.8	55.7±9.6	TT vs CC P=0.046
rs11168266d	SNP genotyping	GG	GA	AA	
	Weight (kg)	–	51.7±7.3	63.7±12.6	AA vs AG P=0.039
rs3858733g	SNP genotyping	AA	AC	CC	
	Weight (kg)	55.6±9.2	69.0±16.1	–	AC vs AA p=0.049
	BMI (kg/m <sup>2</sup> )	32.5±7.7	42.9±9.9	–	AC vs AA p=0.042
rs121909790c	SNP genotyping	GG	GA	AA	
	LH (IU/L)	15.6±1.9	47.0±0.0	–	GG vs GA P=0.012
rs11168265e	SNP genotyping	GG	GA	AA	
	Weight (kg)	77.7±10.3	73.2±10.3	58.2±3.7	GG vs AA p=0.043
	Vitamin D (ng/mL)	45.0±2.4	44.8±6.6	57.3±2.3	AA vs AG P=0.008
rs731236f	SNP genotyping	TT	CT	CC	
	VitaminD (ng/mL)	46.0±1.2	44.0±6.7	54.8±5.5	CC vs CT P=0.024

Note: The superscripts (a,b,c,d,e,f,g,h) on the SNPs link these SNPs to the SNPs in the Table V.

## Discussion

Our interest developed in this study on patients with PCOS, since it is shown in many investigations that Vit D and VDR play several essential roles in the female reproductive organs. The VDR spreads over most of the female reproductive tissues and are involved in uterus development during preparation for the process of embryo implantation (28, 29). In addition, the VDR are also present on the ovaries and are involved in the formation of vesicles process (folliculogenesis) and the synthesis of sex hormones (steroidogenesis of sex hormones) (6). However, our results in Saudis compared with or contrasted several of the findings reporting Vit D deficiency in PCOS.

In our study, the females suffering from PCOS were 16 in number and matched in age and BMI with the control group (17 in number). Vit D levels were higher in the PCOS, compared to the control group and the difference was significant statistically. The optimal level according to the »Reference Ranges« as

set by the Mayo Medical Laboratories (30) is 25–80 ng/mL. In our study all the females suffering from PCOS had optimal Vit D levels and amongst the normal controls only 1/17 (5.8%) had sub-optimal Vit D levels, indicating that in Saudis Vit D deficiency is not involved in the pathogenesis of PCOS. These results are in line with those reported by Wehr and colleagues on Austrian population (10).

When the hormone levels were compared, the level of LH was significantly higher in the PCOS compared to the controls, while the FSH levels did not differ significantly. However the LH/FSH ratio was significantly higher in the PCOS compared to the controls. These results are in agreement with the results of Polson et al. (31), Taylor et al. (32) and Daghestani et al. (33), but disagree with the results of Norman et al. (34) and Adams et al. (35). Other studies have also reported that PCOS is characterized by high levels of LH due to low levels of estrogen and progesterone resulting from lack of ovulation process (36). In addition, this may be due to the high sensitivity of the pitu-



itary gland to gonadotropin-releasing hormone (GnRH), as pointed out by Hayes et al. (37).

Interestingly, when Vit D level was correlated with all the studied parameters, a positive correlation was identified between the level of Vit D and FSH in the control group only. In the PCOS patients Vit D correlated negatively with FSH, but the correlation was not statistically significant (38). In an earlier study Zolkova et al. (39) had shown that there was an improvement in FSH level with specific doses of Vit D. However, a recent report failed to show any correlation between Vit D and FSH and LH in young females (40).

The present study also failed to show any association between Vit. D and BMI, though earlier investigations showed that Vit D associates with BMI (10, 11, 13). Recently, Duhil de Benaze (40) also failed to show any association between Vit D and BMI.

In the present study, the unique feature was that we conducted a comprehensive survey of the *VDR* gene by sequencing all exons and part of the introns on both sides of the exon. Our genotyping results showed that there were no significant associations of any of SNPs in *VDR* gene with PCOs in Saudi women. These results are in line with the results reported by Wehr et al. (21) who did not find any association between 8 polymorphisms in *VDR* gene and PCOS. In our study the frequency of genotypes and alleles of several of the SNPs were very different when the PCOS patients were compared with the controls, but the statistical significance was not achieved. This is probably due to the smaller number of sample size used in this study.

Interestingly, literature review revealed that there are remarkable population variations in the association between Vit D deficiency and genetic variants of *VDR*. Mahmoudi et al. (12) showed significant association of rs7975232 (GT) in *VDR* with severity of the disease in Iranian population, while no such association was found in Egyptians, despite significantly lower Vit D levels in PCOS (41). In Silesian women endogenous Vit D deficiency and *VDR* polymorphisms were not associated with «classic» PCOS phenotype (42), though, in the South Indian females the *VDR* gene polymorphism was shown as a remarkable inheritable risk factor for PCOS (43).

Many of the SNPs identified in our study have not been reported in any of the previous studies. Hence, studies on larger study groups are required to confirm association if any between the SNP and PCOS development.

Furthermore, in our study we also found that some of the genotypes affected the levels of the anthropometric and hormonal parameters. The variant rs731236 (CC) associated with the level of Vit D in the PCOs group, but not with LH level. This result is contrary to studies conducted by Ranjzad et al. (25)

and Bagheri et al. (23) who showed association between Vit D and the level of LH. We also identified several relationships between variables of the study and SNP genotypes which were not mentioned in previous studies on *VDR* and PCOS. These results show that the inter-individual variations may be a result of variations in the genotype of *VDR*. Further detailed studies are warranted to provide a clearer picture of the associations.

The major limitation of this study was the small sample size. However, as a pilot study it provided several interesting facts, which need confirmation by further more detailed studies. Secondly, during this study, the level of insulin was not measured. So we could not provide evidence to validate a number of studies that associate Vit D with insulin resistance. Also, the level of Vit D and its association with insulin resistance in type II diabetes could not be confirmed (13, 21). Further studies on a larger sample size are required to confirm these results.

## Conclusion

This study shows that in Saudis Vit D deficiency does not contribute to PCOS development and comparing the results with those in the literature highlight the wide variations that exist in the prevalence of Vit D deficiency in PCOS in different populations. During this study, extensive polymorphisms are revealed in the *VDR* gene on sequencing. Though no significant differences are observed in the frequency of these variants in the Saudi PCOS and normal population, but several variants influence the demographic and hormonal parameter levels in the studied population. Since extensive population differences exist and studies on *VDR* variants are few, it is suggested that further detailed studies are conducted to obtain a clearer picture of the association between *VDR* gene polymorphisms and PCOS etiology.

*Acknowledgements.* The authors extend their appreciation to the National Plan for Science, Technology and Innovation (MAARIFAH), King Abdul-Aziz City for Science and Technology, Kingdom of Saudi Arabia, grant Number No 08-MED 604-2. We thank all the subjects for their cooperation and participation in the study. We would also like to thank all the participants (researchers, technicians and nurses) for their notable contribution.

*Funding.* This Work was funded by the National Plan for Science, Technology and Innovation (MAARIFAH), King Abdul-Aziz City for Science and Technology, Kingdom of Saudi Arabia, grant Number No 08-MED 604-2.

## Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

## References

- Uitterlinden, AG, Fang, Y, van Meurs JB, van Leeuwen H, Pols HA. Vitamin D receptor gene polymorphisms in relation to Vitamin D related disease states. *The Journal of Steroid Biochemistry and Molecular Biology* 2004; 89: 187–93.
- Mahmoudi T. Genetic variation in the vitamin D receptor and polycystic ovary syndrome risk. *Fertility and Sterility* 2009; 92: 1381–3.
- Darwish H, and DeLuca HF. Vitamin D-regulated gene expression. *CRC Crit Rev Eukaryotic Gene Expression* 1993; 3: 89–116.
- Agic A, Xu H, Altgassen C, Noack F, Wolfler MM, Diedrich K, Friedrich M, Taylor RN, Hornung D. Relative expression of 1,25-dihydroxyvitamin D<sub>3</sub> receptor, vitamin D 1 $\alpha$ -hydroxylase, vitamin D 24-hydroxylase, and vitamin D 25-hydroxylase in endometriosis and gynecologic cancers. *Reproductive Sciences* 2007; 14: 486–97.
- Lerchbaum E, Obermayer-Pietsch B. Mechanisms in endocrinology: Vitamin D and fertility: a systematic review. *European Journal of Endocrinology* 2012; 166(5): 765–78.
- Parikh G, Varadinova M, Suwandhi P, Araki T, Rosenwaks Z, Poretsky L, Seto-Young D. Vitamin D regulates steroidogenesis and insulin-like growth factor binding protein-1 (IGFBP-1) production in human ovarian cells. *Hormone and Metabolic Research* 2010; 42: 754–7.
- Collinson P. Laboratory medicine is faced with the evolution of medical practice. *J Med Biochem* 2017; 36: 211–5.
- Hahn S, van Halteren WB, Roesler S, Schmidt M, Kimmig R, Tan S, Mann K, Janssen OE. The combination of increased ovarian volume and follicle number is associated with more severe hyperandrogenism in German women with polycystic ovary syndrome. *Experiments in Clinical Endocrinology and Diabetes* 2006; 114: 175–81.
- Ngo DT, Chan WP, Rajendran S, Heresztyn T, Amarasakera A, Sverdlov AL, O'Loughlin PD, Morris HA, Chirkov YY, Norman RJ, Horowitz JD. Determinants of insulin responsiveness in young women: impact of polycystic ovarian syndrome, nitric oxide, and vitamin D. *Nitric Oxide* 2011; 25: 326–30.
- Wehr E, Pilz S, Schweighofer N, Giuliani A, Kopera D, Pieber TR, Obermayer-Pietsch B. Association of hypovitaminosis D with metabolic disturbances in polycystic ovary syndrome. *European Journal of Endocrinology* 2009; 161(4): 575–82.
- Yildizhan R, Kurdoglu M, Adali E, Kolusari A, Yildizhan B, Sahin HG, Kamaci M. Serum 25-hydroxyvitamin D concentrations in obese and non-obese women with polycystic ovary syndrome. *Archives of Gynecology and Obstetrics* 2009; 280(4): 559–63.
- Mahmoudi T, Gourabi H, Ashrafi M, Yazdi RS, Ezabadi Z. Calcitropic hormones, insulin resistance, and the polycystic ovary syndrome. *Fertility and Sterility* 2010; 93: 1208–14.
- Li HW, Brereton RE, Anderson RA, Wallace AM, Ho CK. Vitamin D deficiency is common and associated with metabolic risk factors in patients with polycystic ovaries syndrome. *Metabolism* 2011; 60: 1475–81.
- Perovic Blagojevic I, Eror T, Pelivanovic J, Jelic S, Kotur-Stevuljevic J, Ignjatovic S. Women with polycystic ovary syndrome and risk of cardiovascular disease. *J Med Biochem* 2017; 36: 259–69.
- Bikle, D. D. Vitamin D metabolism and function in the skin. *Molecular and Cellular Endocrinology* 2011; 347(1): 80–9.
- Kinuta K, Tanaka H, Moriwake T, Aya K, Kato S, Seino Y.; Vitamin D is an important factor in estrogen biosynthesis of both female and male gonads. *Endocrinology* 2000; 141: 1317–24.
- Holick MF; Vitamin D deficiency. *New England Journal of Medicine* 2007; 357: 266–81.
- Koshiyama H, Sone T, Nakao K. Vitamin-D-receptor-gene polymorphism and bone loss. *Lancet* 1995; 345: 990–1.
- Dusso AS, Brown AJ, Slatopolsky E. Vitamin D. *Am J Physiol Renal Physiol* 2005; 289(1): F8-28.
- Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, Sambrook PN, Eisman JA. Prediction of bone density from vitamin D receptor alleles. *Nature* 1994; 367: 284–7.
- Wehr E, Trummer O, Giuliani A, Gruber HJ, Pieber TR, Obermayer-Pietsch B. Vitamin D-associated polymorphisms are related to insulin resistance and vitamin D deficiency in polycystic ovary syndrome. *European Journal of Endocrinology* 2011; 164: 741–9.
- Mahmoudi T, Majidzadeh-A K, Farahani H, Mirakhorli M, Dabiri R, Nobakht H, Asadi A. Association of vitamin D receptor gene variants with polycystic ovary syndrome: A case control study. *Int J Reprod Biomed (Yazd)* 2015; 13(12): 793–800.
- Mcgrath JJ, Saha S, Burne TH, Eyles, D W. A systematic review of the association between common single nucleotide polymorphisms and 25-hydroxyvitamin D concentrations. *The Journal of Steroid Biochemistry and Molecular Biology* 2010; 121(1): 471–7.
- Bagheri M, Rad IA, Jazani NH, Nanbakhsh F. 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 (Buchar)* 2012; 7(4): 303–8.
- Ranjzad F, Mahban A, Shemirani AI, Mahmoudi T, Vahedi M, Nikzamir A, Zali MR. Influence of gene variants related to calcium homeostasis on biochemical parameters of women with polycystic ovary syndrome. *Journal of Assisted Reproduction and Genetics* 2011; 28: 225–32.
- 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; 7(2):116-21. Epub 2013 Jul 31.
- The Rotterdam ESHRE/ASRM sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long term health risks related to polycystic ovary syndrome (PCOS). *Human Reproduction*,

- Volume 19, Issue 1, 1 January 2004, Pages 41–47, <https://doi.org/10.1093/humrep/deh098>
28. Bagot CN, Troy PJ, Taylor HS. Alteration of maternal *Hoxa10* expression by in vivo gene transfection affects implantation. *Gene Therapy* 2000; 7: 1378–84.
  29. Du H, Daftary GS, Lalwani SI, Taylor HS. Direct regulation of *HOXA10* by 1,25-(OH)<sub>2</sub>D<sub>3</sub> in human myelomonocytic cells and human endometrial stromal cells. *Molecular Endocrinology* 2005; 19: 2222–33.
  30. Kennel KA, Drake MT, Hurley DL. Vitamin D deficiency in adults: when to test and how to treat. *Mayo Clin Proc* 2010 Aug; 85(8): 752–7; quiz 757–8. doi:10.4065/mcp.2010.0138.
  31. Polson DW, Wadsworth J, Adams J, Franks S. Polycystic ovaries—a common finding in normal women. *The Lancet* 1988; 331(8590): 870–2.
  32. Taylor AE, McCourt B, Martin KA, Anderson EJ, Adams JM, Schoenfeld D, Hall JE. Determinants of abnormal gonadotropin secretion in clinically defined women with polycystic ovary syndrome. *Journal of Clinical Endocrinology & Metabolism* 1997; 82(7): 2248–56.
  33. Daghestani M, Daghestani M, El-Mazny A. The Correlation between Anthropometric Measurements, Insulin and Reproductive Hormones in Saudi Females with and without Polycystic Ovary Syndrome. *Medical Journal of Cairo University* 2008; 76(2): 385–92.
  34. Norman RJ, Shamin M, Masters S. Ethnic differences in insulin and glucose response to glucose between white and Indian women with polycystic ovary syndrome. *Fertility and Sterility* 1995; 63(1): 58–62.
  35. Adams JM, Taylor AE, Crowley WF, & Hall JE. Polycystic ovarian morphology with regular ovulatory cycles: insights into the pathophysiology of polycystic ovarian syndrome. *Journal of Clinical Endocrinology & Metabolism* 2004; 89(9): 4343–50.
  36. Marshall JC, Eagleson CA. Neuroendocrine aspects of polycystic ovary syndrome. *Endocrinology and Metabolism Clinics of North America*, 1999; 28: 295–324.
  37. Hayes FJ, Seminara SB, Crowley WF Jr. Hypogonadotropic hypogonadism. *Endocrinol Metab Clin North Am* 1998 Dec; 27(4): 739–63, vii. Review.
  38. Waldstreicher J, Santoro NF, Hall JE, Filicori M, Crowley WF Jr. Hyperfunction of the hypothalamic-pituitary axis in women with polycystic ovarian disease: indirect evidence for partial gonadotroph desensitization. *J Clin Endocrinol Metab* 1988; 66(1): 165–72.
  39. Žofková I, Scholz G, Starka L. Effect of calcitonin and 1, 25 (OH) 2-vitamin D<sub>3</sub> on the FSH, LH and testosterone secretion at rest and LHRH stimulated secretion. *Hormone and Metabolic Research* 1989; 21(12): 682–5.
  40. Duhil de Bénazé G, Brauner R, Souberbielle JC. There is no association between vitamin D status and characteristics of central precocious puberty in girls. *Eur J Pediatr* 2017 Dec; 176(12): 1677–80. doi: 10.1007/s00431-017-3022-9. Epub 2017 Sep 29.
  41. El-Shal AS, Shalaby SM, Aly NM, Rashad NM, Abdelaziz AM. Genetic variation in the vitamin D receptor gene and vitamin D serum levels in Egyptian women with polycystic ovary syndrome. *Mol Biol Rep* 2013 Nov; 40(11): 6063–73. PubMed PMID: 24078159.
  42. Jedrzejuk D, Łaczmanski Ł, Milewicz A, Kuliczowska-Plaksej J, Lenarcik-Kabza A, Hirnle L, Zaleska-Dorobisz U, Lwow F. Classic PCOS phenotype is not associated with deficiency of endogenous vitamin D and VDR gene polymorphisms rs731236(TaqI), rs7975232 (ApaI), rs1544410 (BsmI), rs10735810 (FokI): a case-control study of lower Silesian women. *Gynecol Endocrinol* 2015; 31(12): 976–9. doi:10.3109/09513590.2015.1062865. Epub 2015 Sep 30. PubMed PMID: 26422783.
  43. Siddamalla S, Reddy TV, Govatati S, Erram N, Deenadayal M, Shivaji S, Bhanoori M. Vitamin D receptor gene polymorphisms and risk of polycystic ovary syndrome in South Indian women. *Gynecol Endocrinol* 2017 Sep 3: 1–5. doi:10.1080/09513590.2017.1371128. [Epub ahead of print] PubMed PMID: 28868946.
  44. Han FF, Lv YL, Gong LL, Liu H, Wan ZR, Liu LH. VDR Gene variation and insulin resistance related diseases. *Lipids Health Dis* 2017 Aug 19; 16(1): 157–169 doi: 10.1186/s12944-017-0477-7. PubMed PMID: 28822353; PubMed Central PMCID: PMC5563043.

Received: January 16, 2018

Accepted: February 28, 2018