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

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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, rs2228570, and rs11168266.

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; χ2, Chi-square

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The vitamin D receptor (VDR) (3) 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 the physiological effect of Vit D is dependent on 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, 16–20). Wehr and coworkers (21) reported that ApaI (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-

**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
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                                        | GTGGCCTATAGGGTGGT TGA         | 79  | 54                     |
|       | R                                        | CATACCTGGGCCCTGTAA GA        | 79  |                        |
| 2     | F                                        | TGTGCTGATTGTCCCAT T G         | 77  | 60                     |
|       | R                                        | CAGTGGTTACAGGCTAGCTG G        | 80  |                        |
| 3     | F                                        | CT TCCCACCTGCTCCTG TAC       | 80  | 60                     |
|       | R                                        | ATCTGGGTG T GTATCCCT TTC      | 79  |                        |
| 4     | F                                        | CTGAGCTC C C T G T G T G      | 79  | 60                     |
|       | R                                        | GGA GCTGAGAGGGAGG G AAA       | 80  |                        |
| 5     | F                                        | GCCCTCATGTCTTCTGT G T G       | 79  | 60                     |
|       | R                                        | GCTGGCAGCTACAGAGG G A G       | 80  |                        |
| 6     | F                                        | CGAGTGTAAAGGGCCCTC C T       | 81  |  58                    |
|       | R                                        | CTGCCCTCTGTCCC T ACT CC       | 79  |                        |
| 7     | F                                        | TGAACAGAAACTG GGG TAG G G     | 79  | 54                     |
|       | R                                        | T TGGGTAAAG T CAC C C C T T C | 78  |                        |
| 8     | F                                        | GAAGGGTGACCTGTG GAG T C       | 79  | 54                     |
|       | R                                        | AG G TGCAGTG AG CCG G A T     | 78  |                        |
| 9     | F                                        | AGGA AACACCTT GTGCCC T T C C | 79  | 54                     |
|       | R                                        | T C C C TGT GTGG C T AC T A C T | 79  |                        |
| 10    | F                                        | T C C T T T C AG C T C C A G A T T C | 77  | 60                     |
|       | R                                        | GCT CTCGCAAACCAGGA A AG T     | 78  |                        |
| 11    | F                                        | TGGTATCACCCGGTGAC GAG T       | 80  | 58                     |
|       | R                                        | GTGAGGAGG G GCTGTGGA GTA      | 79  |                        |
| UTR   | F                                        | CGAAGTGTGTTTG GCAATG G A      | 77  | 54                     |
|       | R                                        | GAGAG G G AACC C C A T AG G    | 81  |                        |
| UTR   | F                                        | GGA CAGAGACACC CTGC A C C T   | 80  | 56                     |
|       | R                                        | AGG GCT TC CACCTCA ACC        | 79  |                        |
| UTR   | F                                        | GT C C C TGT CAC A A G C T C   | 80  | 56                     |
|       | R                                        | C TCTTAGCCCTGTGGG T GA A       | 79  |                        |
| UTR   | F                                        | GGGTCTGGAAGAAGC AGT G A G     | 77  | 60                     |
|       | R                                        | CAT T C C CAAA ACT C A AG C A | 80  |                        |
| UTR   | F                                        | AG AAGG CGGT T T C C G AG T  | 78  | 60                     |
|       | R                                        | TCAAACAAGGGTGCTC TCCCT CA C  | 78  |                        |
| UTR   | F                                        | GGGG A GAAACT TAC ATTT G T G A A | 77  | 60                     |
|       | R                                        | C G A TCT CAGCTCACTGCA AC     | 79  |                        |
| UTR   | F                                        | A G GCTGAGCAGA AAG A AT T G   | 78  | 60                     |
|       | R                                        | TGAGGCAA C AGC TAT C C A      | 77  |                        |
| UTR   | F                                        | CCA ACC CAT CAGA AG G G A A   | 78  | 60                     |
|       | R                                        | TTCCAGTTACCGACGC C AG        | 79  |                        |
| UTR   | F                                        | GGGAGGGTGGC C C A A A AT G    | 78  | 60                     |
|       | R                                        | AATGATTT CAT C C CATA AG G T C | 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 signs 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) (kg/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 °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 °C for 40 s; annealing at 54 °C 40s, and extension step at 72 °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 ± 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 (χ^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 ± 7.5 ng/mL), while in the PCOS they were 30.0–60.0 ng/mL (47.2 ± 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-
trol 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 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/m²)    | 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) n (%) | PCOS (N=16) n (%) |
|----------------------|----------------------|-------------------|
| Suboptimal Vit D     | 1(5.8)               | – (–)             |
| provision (20–30 ng/mL) |                     |                   |
| Optimal Vit D        | 14(82.3)             | 12(75)            |
| level (30–50 ng/mL)  |                      |                   |
| Upper normal         | 2(11.7)              | 4(25)             |
| (50–70 ng/mL)        |                      |                   |
| 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/m²)                     | 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
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       | X²       | 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  | 0.015–8.745 | 0.96     | 1.559   |
| 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) | 5.00 | 0.19–125.785 | 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 | 0.073–5.435 | 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) | 1.806 | 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 | 0.89–17.655 | 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 | 0.89–17.655 | 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  | 0.015–8.793 | 0.97     | 0.324   |
|                       | CC        | –        | –    | 1.000  | 0.19–53.4577 | 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  | 0.015–8.745 | 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  | 0.19–53.457 | Nan      | 1.000   |
|                       | AA        | 1(5.88)  | –    | 0.333  | 0.015–8.793 | 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 lost) (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(66.25) | 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 G/A | 12(70.59) | 15(95.75) | 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  | 0.015–9.979    | 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 gene | GG                 | 5(29.41) | 5(31.25)  | 1.667  | 0.251–11.071   | 0.28| 0.595  |
|            | downstream gene variant C/T| 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    | 0.03| 0.03   |
| 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  | 0.169–50.959   | 0.40| 0.527  |
| rs731236f  | synonymous variant  | GG                 | 5(29.41) | 3(18.75)  | 0.600  | 0.090–3.986    | 0.28| 0.595  |
|            | A/G                 | 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  |
| rs3858733g | prime UTR variant T/G| GG                 | 13       | 13(81.25) | 1.000  | 0.018–54.155   | Nan| 1.000  |
|            | T/G                 | 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  |
|            | G/C/T               | 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       | 13(81.25)| 1.080  | 0.020–58.6555  | Nan| 1.000  |
|            | C/T                 | 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 (a,b,c,d,e,f,g) 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 | Variant | P-value |
|-----------|---------------------------|-----------|--------------|---------|---------|
| rs11168276a | SNP genotyping            | GG        | GA           | AA      |         |
| rs2228570b | Vitamin D (ng/mL)         | 42.6±6.4  | –            | 25.0±0.0| GG vs AA| 0.018   |
|           | FSH (IU/L)                | 6.0±1.3   | –            | 3.2±0.0 | GG vs AA| 0.049   |
| rs11168266d | SNP genotyping            | GG        | GA           | AA      |         |
| rs3858733g | Weight (kg)               | 85.0±0.0  | 61.5±12.8    | 55.7±9.6| TT vs CC| 0.046   |
| rs121909790c | SNP genotyping            | AA        | AC           | CC      |         |
| rs11168265e | Weight (kg)               | 55.6±9.2  | 69.0±16.1    | –       | AC vs AA| 0.049   |
|           | BMI (kg/m²)               | 32.5±7.7  | 42.9±9.9     | –       | AC vs AA| 0.042   |
| rs751236f  | SNP genotyping            | GG        | GA           | AA      |         |
| rs11168256e | LH (IU/L)                 | 15.6±1.9  | 47.0±0.0     | –       | GG vs GA| 0.012   |
|           | Weight (kg)               | 77.7±10.3 | 73.2±10.3    | 58.2±3.7| GG vs AA| 0.043   |
|           | Vitamin D (ng/mL)         | 45.0±2.4  | 44.8±6.6     | 57.3±2.3| AA vs AG| 0.008   |
| rs3858736f | SNP genotyping            | TT        | CT           | CC      |         |
| rs11168265f | Vitamin D (ng/mL)         | 46.0±1.2  | 44.0±6.7     | 54.8±5.5| CC vs CT| 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 (58). 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 rs7975252 (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 rs751236 (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.

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**Conflict of interest statement**

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