J Assist Reprod Genet (2011) 28:225–232
DOI 10.1007/s10815-010-9506-4

GENETICS

Influence of gene variants related to calcium homeostasis on biochemical parameters of women with polycystic ovary syndrome

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Received: 8 September 2010 / Accepted: 29 October 2010 / Published online: 17 November 2010

Abstract
Purpose The purpose of this study was to evaluate the associations between polymorphisms in vitamin D receptor (VDR), parathyroid hormone (PTH), calcium sensing receptor (CASR), insulin receptor (INSR), and adiponectin (ADIPOQ) genes and biochemical characteristics of women with polycystic ovary syndrome (PCOS).
Methods Serum levels of LH, FSH, estradiol, testosterone, prolactin, SHBG, glucose, IGF-1, IGFBP-1, calcium, phosphorus, PTH, 25(OH)D, and $1,25(OH)_2$ D were measured in 56 women with PCOS. Furthermore, genotyping five, one, one, two, and two polymorphisms of the VDR, PTH, CASR, INSR, and ADIPOQ genes, respectively, were performed.
Results The VDR TaqI "CC" genotype was associated with elevated serum levels of LH (p=0.011). There were significant associations between decreased levels of SHBG and both VDR BsmI "GG" (p=0.009) and ADIPOQ BsmI "CC" (p=0.016) genotypes. Furthermore, patients with CaSR Hin1I "TG" genotype showed higher HoMA-IR (p=0.008). All these associations remained significant after Bonferroni correction. In addition, phosphorus correlated negatively with estradiol (r=−0.298, P=0.026) and positively with glucose (r=0.287, P=0.032).
Conclusions These data indicated for the first time that it is possible that the VDR and CASR gene variants through their effects on LH and SHBG levels, and insulin resistance are involved in pathogenesis of PCOS.

Capsule It is possible that VDR and CASR gene variants, at least in part, through their effects on LH and SHBG levels, and insulin resistance are involved in pathogenesis of PCOS.

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Keywords Calcium sensing receptor gene · Phosphorus · Polycystic ovary syndrome · Vitamin D receptor gene

Introduction

Polycystic ovary syndrome (PCOS) is a common multifaceted metabolic disease in women of fertile age which has a strong genetic component [1]. PCOS is associated with insulin resistance, hyperinsulinemia and central obesity [2]. On the other hand, insulin secretion is a calcium-dependent process [3] and positive correlations between serum levels of calcium and both insulin levels and insulin resistance has been reported [4]. Furthermore, vitamin D receptor (VDR), parathyroid hormone (PTH), and calcium sensing receptor (CaSR) gene variants appear to be involved in maintaining calcium homeostasis [5–7]. In addition, recent studies have demonstrated increased serum levels of PTH [8, 9], 25-hydroxyvitamin D [25(OH)D] and phosphorous [9] in women with PCOS and a different distribution of VDR

ApaI gene polymorphism between women with PCOS and controls was found [10]. Accordingly, these data support the hypothesis that the genes related to calcium homeostasis including VDR, PTH, and CaSR might have a role in pathogenesis of PCOS.

In recent years genes involved in insulin signaling pathway have been suggested as candidate genes for PCOS and significant associations between the syndrome and genetic variants in insulin receptor (INSR) [11] and adiponectin (ADIPOQ) [12] have been found. Also, previous epidemiologic studies have also shown significant associations of ADIPOQ gene variants with insulin resistance [13] and obesity [14]. Furthermore, a significant association between INSR gene variants and insulin resistance has been observed [15].

The present study was designed to investigate for the possible associations between the FokI, BsmI, ApaI, Tru9I, and TaqI polymorphisms of the VDR gene, the DraII polymorphism of the PTH gene, the Hin1I polymorphism of the CaSR gene, the NsiI and PmlI polymorphisms from the INSR gene, and the SmaI and BsmI polymorphisms of the ADIPOQ gene and metabolic and biochemical characteristics of women with PCOS. Selection criteria for these SNPs were based on (a) their use in previous genetic epidemiology studies (b) degree of heterozygosity (c) and position in the gene. Furthermore, the possible correlations of serum levels of calcium, phosphorus, PTH, 25(OH) D, and 1, 25(OH)2 D with the hormonal and metabolic characteristics of the syndrome were also evaluated.

Materials and Methods

Participants

In the present study, fifty-six women with PCOS 19 to 41 years of age were diagnosed on the basis of the NICHD criteria [16]. PCOS was defined by the presence of menstrual dysfunction i.e. oligomenorrhea (fewer than six menstrual periods in the preceding year) or amenorrhea (absence of periods for more than 6 months), and clinical hyperandrogenism (i.e. hirsutism: Ferriman-Gallwey score >6) and/or hyperandrogenemia. Furthermore, patients with any other cause of oligomenorrhea or hyperandrogenism, such as nonclassic congenital adrenal hyperplasia, androgen secreting tumours, Cushing's syndrome, or hyperprolactinaemia were excluded. In addition, all subjects had polycystic ovaries by ultrasonography, but this was not a required inclusion criterion. All 56 women with PCOS were Iranian and genetically unrelated. Subjects were excluded from additional study if they were taking drugs known to affect calcium metabolism (e.g. corcicosteroids, anticonvulsants). All subjects were informed about the aims of the study and gave written consents. The present study was performed in accordance with the principles of the Declaration of Helsinki and the Institutional Review Boards of the Institute and the Ethical Committee of the Institute have reviewed and approved this study. Body mass index (BMI) was calculated as body weight divided by height squared (kg/m2) and obesity was defined as BMI ≥30 kg/m^2. Seventeen (30.4%) patients had a BMI <25 kg/m2; these women were considered normal weight. The 21 (37.5%) patients with a 25≤ BMI <30 were considered overweight. Eighteen (32.1%) patients had a BMI ≥30 kg/m2; these women were considered obese. The free androgen index (FAI) was calculated by dividing total testosterone (converted to nmol/L) by the SHBG level.

Biochemical measurements

Specimens of venous blood were obtained during a spontaneous bleeding episode or progestin-induced menstrual cycle after an overnight fast and were immediately centrifuged and sera were aliquoted and stored at −20C until analysis. In all 56 women with PCOS serum concentrations of Luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol, testosterone, prolactin, sex hormone binding globulin (SHBG), glucose, insulin, insulin-like growth factor 1 (IGF-1), insulin-like growth factor binding protein (IGFBP-1), total calcium, phosphorus, intact parathyroid hormone (iPTH), 25(OH) D, and 1, 25-dihydroxyvitamin D [1, 25$(OH)_2$ D] were measured. Insulin resistance was assessed by calculating the homeostasis model assessment of insulin resistance index (HoMA-IR) according to the formula: [fasting insulin (μIU/ml)×fasting glucose (mg/dl)]/405 [17].

LH, FSH, and prolactin were measured with immunoradiometric assay (IRMA) methods (Izotop, Budapest, Hungary), and estradiol and testosterone were measured with radioimmunoassay (RIA) methods (Orion, Diagnostica, Espoo, Finland). The SHBG was assayed by enzyme linked immunosorbent assay (ELISA) method (IBL GmbH, Hamburg, Germany). Serum insulin levels were measured by an IRMA (BioSource Europ S.A., Nivelles, Belgium). The assay methods for IGF-1 and IGFBP-1 were ELISA (BioSource Europ S.A., Nivelles, Belgium) and immunoenzymometric assay (IEMA) (Medix Biochemica, Kaunianen, Finland), respectively. Serum concentrations of the two vitamin D metabolites, 25(OH)D, and 1,25$(OH)_2$D were determined by RIA method, and PTH were determined by an IRMA (DRG instruments GmbH, Marburg, Germany). Serum glucose, calcium, and phosphorus measurements were performed using standard methods (CinnaGen Inc., Tehran, Iran). The intra- and inter assay coefficients of variation were 1.4 and 3.1% for LH respectively, 2.5 and 2.7% for FSH, 1.5 and 1.9% for prolactin, 7.3 and 5.1% for

estradiol, 7.5 and 7.0% for testosterone, 2.2 and 6.5% for insulin, 7.8 and 13.3% for IGF1, 4.4 and 4.4% for IGFBP1, 5.7 and 4.5% for PTH, 6.4 and 7.1% for 25(OH)D, and 12.4 and 13.8% for $1,25(OH)_2D$.

Genotype analysis

Genomic DNA was extracted from peripheral blood leukocytes with a commercial isolation kit (BioNEER, Daejeon, Korea) according to manufacturer's instructions. In the present investigation, genotyping was done by PCR-RFLP method. Details of the studied polymorphisms, PCR conditions, and RFLP conditions are presented in Table 1. All SNPs are named after their respective restriction enzymes. The PCR products were digested overnight with the appropriate restriction enzymes (Fermentas, Leon-Rot, Germany) and were separated by 2 to 3% agarose gels. Bands in gels stained with ethidium bromide for visualization under ultraviolet light. To check for genotyping error rate, approximately 10% of the samples were randomly selected and genotyped in duplicate and 5% of the samples were confirmed by DNA sequencing in individuals of each different genotype and all the results were concordant.

Statistical analysis

The deviation of the genotype frequencies from Hardy-Weinberg equilibrium for each SNP was examined using the χ^2 test. The continuous variables, which failed the normality test (the Kolmogorov-Smirnov goodness-of-fit test), were logarithmically transformed before analysis and their geometric mean (geometric standard error of the mean) were presented. The relations between continuous variables were assessed using Pearson correlation coefficients. Partial correlation coefficients were used for evaluation of independent relations. The nonparametric Kruskal Wallis H test was used to determine the association between the three genotype groups of all SNPs and clinical and biochemical parameters. When the Kruskal Wallis one-way analysis showed a significant effect of genotypes, the nonparametric Mann-Whitney U two-tailed test with Bonferroni correction was applied as post-hoc analysis. For statistical analyses, we used SPSS software, version 15.0 (SPSS Inc. Chicago, IL, USA) and a $P<0.05$ was considered statistically significant.

Results

Clinical, biochemical, and genetic characteristics of the women studied are presented on Table 2. None of the genotype frequency distributions deviated significantly from the Hardy-Weinberg equilibrium ($P>0.05$).

As shown in Table 3, we observed significant associations between VDR, PTH, CaSR, and ADIPOQ gene polymorphisms and biochemical parameters of women with PCOS. In addition to the observed significant associations in the Table 3, the difference in BMI for VDR FokI genotypes was statistically significant (Kruskal Wallis H test, $P=0.043$). Women carrying the FokI "CC" genotype had higher BMI as compared with individuals in the "CT" genotype (Mann-Whitney U test, $P=0.025$), but the difference did not remain significant after Bonferroni correction ($P=0.075$).

Calculation of the pearson coefficients showed that in women with PCOS, the concentrations of phosphorus were negatively correlated with estradiol ($r=-0.298$, $P=0.026$) and calcium ($r=-0.301$, $P=0.024$). Also, partial correlation analysis indicated that the correlation between phosphorus and estradiol was age, BMI, calcium, PTH, 25(OH) D, 1, 25 $(OH)_2$ D, and insulin-independent. Furthermore, in these women, glucose correlated positively with phosphorous ($r=0.287$, $P=0.032$) and FSH ($r=0.327$, $P=0.014$), and negatively with calcium ($r=-0.273$, $P=0.029$). These correlations remained significant after adjusting for age and BMI. In addition, in the obese women with PCOS, positive correlations between phosphorus and both IGF-1 ($r=0.535$, $P=0.022$) and IGFBP-1($r=0.499$; $p=0.035$), and negative correlations between IGFBP-1 and both testosterone ($r=-0.587$; $p=0.010$) and FAI ($r=-0.524$; $p=0.025$) were found. These correlations were also age and BMI-independent. However, the correlations between phosphorus and both IGF-1 and IGFBP-1 did not remain significant after additional adjustment for 1, $25(OH)_2$ D.

Discussion

In this report, significant associations of VDR gene polymorphisms with serum levels of LH and SHBG in women with PCOS were observed, which to our knowledge has not been reported previously. These data also indicated for the first time that in the syndrome, CaSR, PTH, and ADIPOQ gene variants had been associated with HoMA-IR and serum levels of the phosphorus and SHBG, respectively. Furthermore, our study is the first to show a negative correlation between serum levels of phosphorus and estradiol, a positive correlation between phosphorus and glucose, and a negative correlation between calcium and glucose in women with PCOS, as well as the positive correlations between phosphorus and both IGF-1 and IGFBP-1, and negative correlations between IGFBP-1 and both testosterone and FAI in obese women with PCOS.

In the present study women carrying the VDR BsmI "GG" genotype had lower serum SHBG as compared with individuals in the "AA" genotype, and therefore it seems

Table 1 Information for the studied markers in the VDR, PTH, CaSR, INSR, and ADIPOQ genes

Gene/SNP (SNP ID)	Location (Base change)	Forward Primer Reverse Primer	PCR program (35 cycles)	PCR fragment size (bp)	Restriction enzyme, Incubation temperature	Alleles: RFLP fragments size (bp)
VDR/FokI (rs10735810)	Exon 2 (C/T)	5′-AGCTGGCCCTGG CACTGACTCTGCTCT-3′ 5′-ATGGAAACACCTTGC TTCTTCTCCCTC-3′	93°C 45 s,66°C 30 s, 72°C 45 s	265	FoKI, 55°C	Allele C: 265 Allele T: 169 +96
VDR/BsmI (rs1544410)	Intron 8 (G/A)	5′-GGCAACCTGAAGGGA GACGTA-3′ 5′-CTCTTTGGACCTCAT CACCGAC-3′	93°C 45 s,66°C 30 s, 72°C 45 s	461	BsmI, 37°C	Allele A: 461 Allele G: 258 +203
VDR/ApaI (rs7975232)	Intron 8 (C/A)	5′-CAGAGCATGGACAGG GAGCAAG-3′ 5′-GCAACTCCTCATGGCT GAGGTCTCA-3′	93°C 45 s,66°C 30 s, 72°C 45 s	740	ApaI, 37°C	Allele A: 740 Allele C: 530 +210
VDR/Tru9I (rs757343)	Intron 8 (G/A)	5′-AATACTCAGGCTCTGC TCTT-3′ 5′-CATCTCCATTCCTTGA GCCT-3′	93°C 45 s,56°C 30 s, 72°C 45 s	331	Tru9I, 65°C	Allele G: 331 Allele A: 178 +153
VDR/TaqI (rs731236)	Exon 9 (T/C)	5′-CAGAGCATGGACAG GGAGCAAG-3′ 5′- GCAACTCCTCATGGC TGAGGTCTCA-3′	93°C 45 s,66°C 30 s, 72°C 45 s	740	TaqI, 65°C	Allele T: 495 +245 Allele C: 290+245+205
PTH/DraII (rs6256)	Exon 3 (C/A)	5′-CATTCTGTGTACTATA GTTTG-3′ 5′-GAGCTTTGAATTAGCA GCATG-3′	93°C 45 s,56°C 30 s, 72°C 45 s	600	DraII, 37°C	Allele A: 600 Allele C: 420 +180
CaSR/Hin1I (rs1801725)	Exon 7 (G/T)	5′-CTGAGCTTTGATGAGC CTCAGAAGGAC-3′ 5′-CACTGATGACAAGC TCTGTGAACTGGA-3′	93°C 45 s,63°C 30 s, 72°C 45 s	269	Hin1I, 37°C	Allele T: 269 Allele G: 241 +28
INSR/NsiI (rs2059806)	Exon 8 (A/G)	5′-CGGTCTTGTAAGGG TAACTG-3′ 5′-GAATTCACATTCCCA AGACA-3′	93°C 45 s,56°C 30 s, 72°C 45 s	324	NsiI, 37°C	Allele G: 324 Allele A: 239 +85
INSR/PmlI (rs1799817)	Exon 17 (T/C)	5′-CCAAGGATGCTGTGT AGATAAG-3′ 5′-TCAGGAAAGCCAGCC CATGTC-3′	93°C 45 s,56°C 30 s, 72°C 45 s	317	PmlI, 37°C	Allele T: 317 Allele C: 274 +43
ADIPOQ/SmaI (rs2241766)	Exon 2 (T/G)	5′-GAAGTAGACTCTGCT GAGATG G-3′ 5′-TATCAGTGTAGGAGGT CTGTGATG-3′	93°C 45 s,56°C 30 s, 72°C 45 s	372	SmaI, 30°C	Allele T: 372 Allele G: 216 +156
ADIPOQ/BsmI (rs1501299)	Intron 2 (C/A)	5′-GGCCTCTTTCATCAC AGACC-3′ 5′-AGATGCAGCAAAGC CAAAGT-3′	93°C 45 s,58°C 30 s, 72°C 45 s	196	BsmI, 37°C	Allele A: 196 Allele C: 146 +50

that "GG" genotype is a risk factor for PCOS, because it increases the bioavailability of androgens in women with PCOS. Interestingly, our study is also the first to show an association between VDR TaqI "CC" genotype and serum levels of LH. The molecular mechanism through which this polymorphism influences LH levels is not known at present; however, previous studies have shown significant associations between VDR gene variants and insulin resistance on one side [18], and insulin resistance and LH on the other [19]. Furthermore, it has been suggested a modulating role of 1, $25(OH)_2$ D in the control of FSH secretion [20]. Therefore, these findings are consistent with a recent report [10] that showed the VDR gene variants might have a role in pathogenesis of PCOS.

In this study, we also observed a significant association between PTH DraII gene polymorphism and serum levels

Table 2 Clinical, biochemical, and genetical variables of the study subjects

Variable	PCOS (n=56)
Age (years)	28.5(0.6)
BMI (kg/m^2)	28.4(0.7)
HoMA-IR[a]	3.5(1.1)
LH (IU/l)	7.8(0.8)
FSH (mIU/ml)	5.1(0.3)
LH/FSH[a]	1.3(1.1)
Estradiol[a] (pg/ml)	33.8(1.1)
Testosterone[a] (ng/dl)	57.6(1.1)
FAI[a]	3.4(1.1)
Prolactin (μg/l)	11.9(0.8)
SHBG (nmol/l)	62.3(2.9)
Glucose (mg/dl)	90.4(1.8)
Insulin[a] (μIU/ml)	15.9(1.1)
IGF-1[a] (ng/ml)	88.8(1.1)
IGFBP-1[a] (ng/ml)	3.4(1.1)
Calcium (mg/dl)	9.5(0.1)
Phosphorous (mg/dl)	3.9(0.1)
iPTH[a] (pg/ml)	21.4(1.1)
25(OH)D[a] (ng/ml)	32.7(1.1)
1,25(OH)$_2$ D[a] (pg/ml)	34.2(1.0)
Gene, SNP, Genotypes, Alleles	
VDR, FokI, CC: CT: TT, C: T	27(48.2): 23(41.1): 6(10.7), 77(68.8): 35 (31.2)
VDR, BsmI, AA: AG: GG, A: G	5(8.9): 32(57.2): 19(33.9), 42(37.5): 70 (62.5)
VDR, ApaI, AA: AC: CC, A: C	12(21.4): 28(50.0): 16(28.6), 52(46.4): 60 (53.6)
VDR, Tru9I, GG: GA: AA, G: A	46(82.1): 10(17.9): 0(0.0), 102(91.1): 10(8.9)
VDR, TaqI, TT: TC: CC, T: C	24(42.9): 26(46.4): 6(10.7), 74(66.1): 38(33.9)
PTH, DraII, AA: AC: CC, A: C	3(5.4): 13(23.2): 40(71.4), 19(17.0): 93 (83.0)
CaSR, Hin1I, TT: TG: GG, T: G	4(7.1): 16(28.6): 36(64.3), 24(21.4): 88 (78.6)
INSR, NsiI, GG: GA: AA, G: A	30(53.6): 22(39.3): 4(7.1), 82(73.2): 30 (56.8)
INSR, PmlI, TT: TC: CC, T: C	5(8.9): 21(37.5): 30(53.6), 31(27.7): 81 (72.3)
ADIPOQ, SmaI, TT: TG: GG, T: G	38(67.9): 16(28.6): 2(3.6), 92(82.1): 20 (17.9)
ADIPOQ, BsmI, AA: AC: CC, A: C	5(8.9): 19(33.9): 32(57.2), 29(25.9): 83 (74.1)

Continuous values are mean (SE). Geometric mean (geometric standard error of the mean) is presented for HoMA-IR, LH/FSH, estradiol, testosterone, FAI (free androgen index), insulin, IGF-1, IGFBP-1, PTH, 25(OH) D, and 1,25(OH)$_2$D; qualitative variables presented as number (percent).

[a] Significance was tested on log-transformed values.

of phosphorus in women with PCOS. Previous studies have shown a significant association between the polymorphism and serum levels of PTH [6]. On the other hand, recently it has been reported that women with PCOS have higher serum levels of phosphorus [9] and PTH [8]. Accordingly, it is possible that the PTH DraII gene polymorphism by increasing serum levels of phosphorus and PTH is involved in pathogenesis of PCOS.

Previous studies have shown that the Hin1I polymorphism, located in codon 986, resulting in an amino acid exchange from alanine to serine in the intracellular C-terminal tail of the CaSR. This polymorphism (A986S) appears to be involved in maintaining calcium homeostasis and the "T" or "S" allele was associated with higher calcium and lower phosphorus concentrations [7, 21]. Our findings indicated that the insulin resistance was lower in women with PCOS who were homozygous for CaSR Hin1I "G" allele (GG) compared with those who were heterozygous (TG). It can be hypothesized that such an association might be related to the association between the "G" allele and lower levels of calcium [7, 21], as well as positive correlations between calcium and both insulin resistance and insulin levels [4]. Such mechanism is speculative at the present but biologically plausible. Furthermore, from the findings reported by Squires et al. [22], it appears that activation of CaSR inhibits insulin secretion. Therefore, our results provide evidence that CaSR Hin1I gene polymorphism might be a candidate for the genetic contribution to the development or the severity of insulin secretion in women with PCOS; however, the exact molecular mechanism underlying this association is largely undetermined.

Interestingly, in the present study we demonstrated a significant association between the ADIPOQ BsmI "CC" genotype and decreased levels of SHBG in the women with PCOS. It is possible that decreased adiponectin levels in subjects with ADIPOQ BsmI "CC" genotype [12, 23], because of positive correlation between adiponectin and SHBG [13, 24], decreases serum levels of SHBG in subjects with "CC" genotype, which observed in this study. Therefore, it seems that "CC" genotype is a risk factor for PCOS, because it increases the bioavailability of androgens in women with PCOS, as a negative correlation between adiponectin and FAI has been found [24]. Furthermore, in accordance with these findings, a higher frequency of "C" allele in women with PCOS than that in the controls was observed [12].

Another interesting finding, which has not been reported previously, was negative correlations between serum levels of phosphorus and estradiol, and between calcium and glucose in women with PCOS. Given that we previously found that phosphorus levels were directly related to PCOS risk [10], our present study suggest that the increased risk might be mediated through decreased levels of estradiol. Interestingly, the administration of estrogen resulted in reduction of serum phosphorus [25]. Previous studies have also shown that increase in serum levels of estrogen in the luteal phase can lead to a decrease in phosphorus levels

Table 3 Clinical and biochemical characteristics in relation to VDR, PTH, CaSR, and ADIPOQ gene polymorphisms in 56 women with PCOS

Gene/SNP	SHBG (nmol/l)	LH (IU/l)	Phosphorous (mg/dl)	HoMA-IR[a]
VDR/BsmI				
AA (n=5)	88.4(7.7)			
AG (n=32)	60.5(3.6)			
GG (n=19)	58.3(4.7)			
P value[b]	0.025			
P value[c]	AA:GG(0.009)			
P value[d]	0.027			
VDR/TaqI				
TT (n=24)		7.1(0.9)		
TC (n=26)		7.6(1.4)		
CC (n=6)		11.8(1.5)		
P value[b]		0.025		
P value[c]		TT:CC(0.011)		
P value[d]		0.033		
PTH/DraII				
AA (n=3)			3.7(0.3)	
AC (n=13)			4.5(1.2)	
CC (n=40)			3.7(0.8)	
P value[b]			0.043	
P value[c]			AC:CC(0.014)	
P value[d]			0.042	
CaSR/Hin1I				
TT (n=4)				1.1(0.3)
TG (n=16)				1.8(0.2)
GG (n=36)				1.2(0.1)
P value[b]				0.027
P value[c]				TG:GG(0.008)
P value[d]				0.024
ADIPOQ/BsmI				
AA (n=5)	74.4(6.9)			
AC (n=19)	71.9(4.9)			
CC (n=32)	54.6(3.5)			
P value[b]	0.016			
P value[c]	AC:CC(0.016)			
P value[d]	0.048			

Continuous values are mean (SE). Geometric mean (geometric standard error of the mean) is presented for HoMA-IR. Normal ranges for SHBG, LH, and Phosphorous are 15–120 (nmol/l), 0.7–9.0 (IU/l), and 2.5–5.0 (mg/dl), respectively.

[a] Significance was tested on log-transformed values.

[b] Kruskal Wallis H test

[c] Mann-Whitney U test

[d] Mann-Whitney U test with Bonferroni correction

[26]. Furthermore, it has been also reported that estrogens directly or indirectly impact vitamin D signaling pathway [27], and vice versa [28]. Accordingly, our findings provide additional clues as to the cross-talk that exists between estradiol and vitamin D/PTH signaling pathway. On the other hand, the exact pathophysiological mechanism of the correlation between calcium and glucose, which is observed in this study, is still unclear. However, previous studies have shown positive correlations between total serum calcium and both insulin levels and insulin resistance [4] and insulin secretion is a calcium-dependent process [3]. These findings may increase our understanding of the complex pathogenesis of PCOS.

Finally, our findings are in concordance with previous studies where a positive correlation between serum levels of phosphorous and IGF1 [29] and a negative correlation between IGFBP-1 and testosterone [30] were found; to our knowledge, ours is the first study that demonstrates the association in obese women with PCOS. Our present study also offered a direct correlation between phosphorus and IGFBP-1 in these women. Accordingly, it is possible that the high levels of phosphorus in women with PCOS compared with controls [9] is part of the homeostatic response to compensate for the increasing testosterone, and is driven by the rising IGFBP-1 levels. Based on these observations it seems that in the obese women with PCOS,

IGF-1/IGFBP-1 system to have a greater impact on serum levels of phosphorus than does vitamin D and PTH status.

Although our small sample size, this study was well designed and to our knowledge this was the first that focuses on the role of calcium metabolism-related gene variants on metabolic and biochemical characteristics of women with PCOS and provided important points for further studies. In conclusion, our data demonstrated for the first time that it is possible that, at least in part, the VDR TaqI and BsmI gene variants through their effects on serum levels of LH and SHBG, the CasR Hin1I gene variant through its effect on insulin resistance, and the ADIPOQ BsmI gene variant through its effect on SHBG levels are involved in the pathogenesis of PCOS. Furthermore, there was crosstalk between phosphorus and both estradiol and insulin/IGF-1 system, through which phosphorus may affect the pathogenesis of the syndrome. However, further studies with increased numbers of PCOS patients are required to validate these findings.

Acknowledgments The authors thank all the subjects for participating. This work was supported by a grant from the Research Center for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences.

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