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Short Communication

Genetic polymorphism of vitamin D receptor gene affects the phenotype of PCOS

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ABSTRACT

Aims: Polycystic ovary syndrome (PCOS), a common female endocrine disorder, represents a wide range of clinical manifestations and disease severity. Recent studies suggest an association between gene variants involved in vitamin D metabolism and common metabolic disturbances in PCOS. We aimed to examine the association of vitamin D receptor (VDR) gene variant with PCOS susceptibility and the severity of disease phenotype.

Methods: All participants, including 260 PCOS women (cases) and 221 normoovulatory women (controls), were recruited from a reproductive endocrinology clinic. Cases were divided into the severe and mild PCOS phenotype groups, based on their clinical and paraclinical features. An adenosine to guanine single nucleotide polymorphism of VDR gene (rs757343) was genotyped using the PCR–RFLP method.

Results: Distributions of genotypes and alleles did not differ between cases and controls, indicating that this SNP is not associated with increased risk for PCOS. However, this SNP was found to be associated with the severity of the PCOS phenotype. In particular, presence of the A allele is associated with a 74% increased risk of severe phenotype development (OR, 1.74; 95% CI, 1.07–2.82).

Conclusion: The genetic variant of the VDR was found to have an association with severity of clinical features of PCOS, but none with disease risk.

1. Introduction

Polycystic ovary syndrome (PCOS), the most common gynecological endocrinopathy, characterized by chronic anovulation and hyperandrogenism, is a multigenic disorder. There is a controversy regarding its diagnostic criteria resulting in considerable phenotypic heterogeneity in PCOS; it is not clear whether the various phenotypes available behave in a manner suggestive of their being part of the same disorder and their having similar inheritance patterns(Azziz et al., 2009; Broekmans et al., 2006; Zhang et al., 2009). Although the genetic components of this disorder have not been clearly illustrated, familial clustering suggests contribution of a genetic component to its pathogenesis. Over the past decade, a number of candidate genes involved in steroidogenesis (Gaasenbeek et al., 2004; Qin et al., 2006), insulin signaling pathway (Jin et al., 2006; Lee et al., 2008) and gonadotropin secretion (Li et al., 2011b) have been investigated to be associated with increased susceptibility to PCOS, but none is strong enough to correlate alone with susceptibility to the disease.

There is an increasing evidence that supports the contribution of vitamin D deficiency to metabolic disturbances in women with PCOS, including insulin resistance (IR) (Hahn et al., 2006; Li et al., 2011a; Wehr et al., 2011b), obesity (Hahn et al., 2006) (Yildizhan et al., 2009), hypertension (Wehr et al., 2009) and menstrual dysfunction (Wehr et al., 2011a), findings supported by the fact that vitamin D regulates about 3% of the human genome, including genes that are crucial for glucose and lipid metabolism, via its nucleoprotein receptor that binds to vitamin D response elements found in the promoter region of responsive genes(Darwish and DeLuca, 1993; Potera, 2009). Consistent evidence also suggests that polymorphisms in the vitamin D receptor (VDR) gene are associated with vitamin D deficiency in PCOS and its metabolic and endocrine disturbances (Mahmoudi, 2009; Wehr et al., 2011b). The VDR Cdx2 'AA' genotype is reported as an associated marker with lower fasting insulin and homeostatic model assessment-IR (Wehr et al., 2011b) and the ApaI 'CC' genotype was associated with an increased risk for PCOS(Mahmoudi, 2009).

Abbreviations: PCOS, polycystic ovary syndrome; VDR, vitamin D receptor; SNP, single nucleotide polymorphisms; PCR–RFLP, polymerase chain reaction–restriction-fragment length polymorphism; OR, odds ratio; CI, confidence interval; IR, insulin resistance; A, adenosine; C, cytidine; G, guanosine; T, thymidine; NIH, national institute of health; FSH, follicle-stimulating hormone; LH, luteinizing hormone; SHBG, sex hormone binding globin; tTes, total testosterone; A4, androstenedione; DHEA-S, Dehydroepiandrosterone sulfate; BMI, body mass index; HWE, Hardy–Weinberg equilibrium; df, degree of freedom.

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This study sought to investigate the relationship between the VDR gene single nucleotide polymorphism (SNP), PCOS susceptibility and the severity of disease phenotype. Because of the functional effect of the exon 8 in binding to vitamin D, the Tru9I polymorphism (rs757343) in this exon was selected.

2. Material and methods

2.1. Study population

The study population included 260 women with PCOS (cases) based on NIH criteria (Zawadzki and Dunaif, 1992) who referred to the reproductive endocrinology research center and a group of 221 eumenorrheic nonhirsute women (controls) who came for their annual gynecologic exam. Using the National Institute of Health criteria, we defined PCOS as the presence of ovulatory dysfunction and clinical hyperandrogenism and/or hyperandrogenemia, after exclusion of other known related disorders.

Although there is no consensus regarding the severity classification of PCOS, we subdivided our cases, based on their clinical manifestations into two subgroups as follows: 1 — Severe phenotype including women with both severe hirsutism (Ferriman_Gallwey Score ≥ 12) and severe menstrual dysfunction either amenorrhea or severe oligomenorrhea, defined as menstrual cycle > 90 days; 2 — Mild phenotype including the remaining PCOS participants.

Blood samples were obtained after overnight fasting for the hormonal assays from all PCOS women and 40 randomly selected controls in the early follicular phase (days 3–5) of a spontaneous menstrual cycle or a progestin induced menstrual cycle.

Circulating levels of Follicle-Stimulating Hormone (FSH), Luteinizing hormone (LH) and Sex Hormone Binding Protein (SHBG) were measured by Imuno-Enzymo Metric Assay method (IEMA kit, Diagnostic Biochem Canada Inc., Ontario, Canada); the assay sensitivities were 1 IU/L, 0.2 IU/L and 0.1 nmol/L respectively and the intra assay coefficients of variation were 5.9, 5.1and 7.9% respectively. Circulating levels of total Testosterone (tTes), Androstenedione (A4) and Dehydroepiandrosterone sulfate (DHEA-S) were determined by the Enzyme Immuno Assay method (EIA kit, Diagnostic Biochem Canada Inc., Ontario, Canada); the assay sensitivities were 0.022, 0.05and 0.11 ng/mL respectively and the intra assay coefficients of variation were 7.6, 6.7 and 5.9% respectively.

2.2. Genotype analysis

Genomic DNA was extracted from peripheral lymphocytes as previously described (Miller et al., 1988). A 331 bp DNA target fragment which contains the polymorphism was amplified by the polymerase chain reaction (PCR) using the following primers: forward: 5′ AAT ACT CAG GCT CTG CTC TT 3′; reverse: 5′ CAT CTC CAT TCC TTG AGC CT 3′ as described by Ye et al. (2000). Amplification was carried out in a DNA Thermal cycler (Corbett co. Australia) using the initial denaturation at 95 °C for 3 min; 40 cycles of denaturation at 94 °C for 45 s, annealing at 56 °C for 30 s, and extension at 72 °C for 45 s; and a final extension at 72 °C for 10 min. The PCR products were subjected to restriction digestion analysis. Digestion with Tru9I (Fermentase Co. Canada) resulted in 178 and 153 bp fragments for A allele and a 331 bp fragment for G allele. The fragments were separated by electrophoresis on 2% agarose gels and DNA fragments were visualized by gel documentation (Optigo Co. City, Holland).

2.3. Statistical analyses

Variables were checked for normal distributions with the one-sample Kolmogorov–Smirnov test. Genotype and allele frequencies were determined for the polymorphism, following which the Chi-square goodness of fit was used to test the deviation from Hardy–Weinberg equilibrium. A dominant model of inheritance was exclusively assumed due to the low frequency of individuals being homozygous for the A allele. Genotype frequency comparisons were conducted using logistic regression analysis, with P values < 0.05 being considered statistically significant. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to assess risk.

The statistical analyses were performed using SPSS statistical package, version 15.0 (SPSS Inc., Chicago, IL, USA).

The study protocol was approved by the Ethics Committee of Research Institute for Endocrine Disorders, and all participants gave written informed consent.

3. Results

Characteristics of women with and without PCOS and those with severe and mild PCOS phenotypes are shown in Tables 1 and 2 respectively. Results demonstrated that the body mass index (BMI) was significantly different between case and control groups as well as between women with severe and those with mild PCOS. It has been shown that HOMA-IR had higher values in women with severe PCOS compared to those with the mild phenotype.

3.1. Allele and genotype frequency

Allele and genotype frequencies of VDR polymorphism (rs757343) in studied groups are shown in Table 3. Genotype distributions in the control and patient groups were in Hardy–Weinberg equilibrium (HWE). The allele and genotype frequencies of rs757343 were similar between PCOS patients and controls (P > 0.05) indicating that this VDR variant does not confer an altered effect on PCOS risk. Yet, there was a significant difference in allelic frequency of the SNP between mild and severe cases (P = 0.043). Overall, significantly elevated risk for severe PCOS was found for A allele versus G allele (OR, 1.74; 95% CI, 1.07–2.82, P = 0.025). In analysis, under a dominant model GG genotype was predominantly present in those with mild phenotype and combined genotypes (GA + AA) were significantly (P = 0.037) associated with increased risk of severe phenotype compared with the GG genotype (OR: 1.8, 95% CI: 1.05–3.26), results which remained after further adjustment for BMI (OR: 1.88, 95% CI: 1.05–3.4).

4. Discussion

The present study aimed to examine the influence of genetic variant of the VDR gene (rs757343) on the severity of PCOS and disease susceptibility. Alleles and genotypes were equally distributed among cases and controls and no difference in the risk for PCOS in association with this variant was observed. Our results indicated that the combined genotype (GA + AA), in comparison with GG genotype of rs757343, was significantly associated to an increased risk of severity of PCOS phenotype.

Genetic association studies have reported a link between SNPs of the VDR gene and certain metabolic and endocrine parameters of

Table 1
Characteristics of the study subjects.

Characteristics	PCOS (n = 260)	Controls (n = 221)	P values
Age (years)	26.7 ± 5.3	30.8 ± 5.7	<0.001
BMI (Kg/m2)	26.8 ± 6.6	25.4 ± 4.3	0.014
Systolic blood pressure (mmHg)	106 ± 13.2	106 ± 12.5	NS
Diastolic blood pressure (mmHg)	68.8 ± 10.2	67.2 ± 9.8	NS
Waist (cm)	87.6 ± 14.4	86.1 ± 9.7	NS
Hip (cm)	103 ± 12.5	101 ± 9.9	0.045
Waist/hip	0.87 ± 0.6	0.87 ± 0.5	NS
Tru 91 alleles frequencies			
G	0.7308	0.7240	NS
A	0.2692	0.2760	NS