A meta-analysis of the relationship between vitamin D receptor gene Apal polymorphisms and polycystic ovary syndrome

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Abstract

Background. Emerging evidence from pre-clinical and clinical studies has shown that vitamin D (VD) plays an important role in the pathogenesis of polycystic ovary syndrome (PCOS). Potentially functional Apal polymorphism of vitamin D receptor (VDR) gene has been implicated in PCOS risk, but individually published studies have yielded inconclusive results.

Objectives. Studies on the associations of VDR gene polymorphisms with PCOS susceptibility reported conflicting results. The objective of this study was to perform a systematic meta-analysis to clarify this issue.

Material and methods. We searched for all publications regarding the associations mentioned above in PubMed, Web of Science, Embase, and China National Knowledge Infrastructure (CNKI) databases updated up to April 2017. A meta-analysis of the overall odds ratios (ORs) with 95% confidence interval (CI) was calculated with the fixed or random effect model.

Results. A total of 7 studies fulfilling the inclusion criteria were included in this meta-analysis (1,350 cases and 960 controls). Pooled ORs showed a significant association between Apal polymorphism and PCOS risk in all 4 genetic models. Subgroup analysis by ethnicity showed that Apal polymorphism was associated with the risk of PCOS in Asians (aa vs AA: OR = 1.54, 95% CI = 1.04–2.28, p = 0.03). However, Apal polymorphism (a vs A: OR = 1.34, 95% CI = 1.00–1.79, p = 0.02; aa+Aa vs AA: OR = 1.36, 95% CI = 1.04–1.79, p = 0.03) was associated with the risk of PCOS in Caucasians.

Conclusions. Our meta-analysis demonstrated that PCOS risk was significantly associated with VDR gene Apal polymorphism. However, due to the relatively small sample size in this meta-analysis, further studies with a larger sample size should be conducted to confirm the findings.

Key words: meta-analysis, polycystic ovary syndrome, vitamin D receptor, genetic polymorphisms, Apal polymorphism

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Introduction

Polycystic ovary syndrome (PCOS) is a common multifaceted metabolic disease with a strong genetic component in women of fertile age. The PCOS incidence increased and ranges from 5% to 10%, with the age of affected females ranging from 12 years to 45 years. Being a complex multigenic and heteroplasmy disease, PCOS results in several disorders, such as infertility, myocardial infarction, dysfunctional uterine bleeding, cardiovascular risk, endometrial carcinoma, insulin resistance (IR), diabetes mellitus, hyperandrogenism (hirsutism, acne, male pattern hair loss), oligoovulation and polycystic ovaries, dyslipidemia, amenorrhea, and hypertension, as well as associated with obesity and high levels of cholesterol.

As a secosteroid hormone, vitamin D (VD) is acquired and synthesized from the diet and ultraviolet radiation. Besides its calcitropic function, VD has potent non-classical properties, including immunomodulatory, anti-inflammatory, antioxidant, angiogenic, and anti-proliferative properties. It is well-known that the interaction of VD with target tissues is mediated by the VD receptor, a member of the steroid/thyroid hormone receptor family with the function of a transcriptional activator of many genes. There is accumulating evidence suggesting that the VD endocrine system is involved in a wide variety of biological processes, including IR and type 2 diabetes mellitus. Insulin resistance, which is commonly present in women with PCOS, may play an important role in the long-term complications of PCOS. Accumulating evidence suggests that VD deficiency might be a causal factor in the pathogenesis of IR and the metabolic syndrome in PCOS. The VDR gene is located on chromosome 12q13.1, consists of 11 exons and has an extensive promoter region capable of generating multiple tissue-specific transcripts. There are 4 single-nucleotide polymorphisms (SNPs) in the VDR gene, FokI (rs10735810), BsmI (rs1544410), ApaI (rs7975232), and TaqI (rs731236), which have been studied most frequently. Moreover, previous studies have revealed significant associations between VDR gene polymorphisms and PCOS.

Considering the past establishment of the important functions of VDR gene Apal polymorphism, many studies have explored the association between VDR gene Apal polymorphism and PCOS risk. However, individual studies yielded inconsistent and even conflicting results. This may be attributed to limited sample sizes and inadequate statistical power, which might affect their reliability. A meta-analysis is a statistical procedure of pooling the data from individual studies, increasing effective sample size, enhancing statistical power of the analysis, and producing a single estimate of an effect. Therefore, we performed a comprehensive meta-analysis to further evaluate the association of VDR gene Apal polymorphism and PCOS.

Material and methods

Literature search

Eligible studies were systematically searched in PubMed, Web of Science, Embase, and China National Knowledge Infrastructure (CNKI) databases up to April 2017, with keywords including: [PCOS OR Polycystic Ovary Syndrome] and [VDR Apal OR VD receptor Apal] and [polymorphism OR mutation OR variation OR SNP]. All studies that showed potential relevance of genetic association were assessed by examining their titles and abstracts. All published studies matching the aforementioned eligibility criteria were obtained and tested for their eligibility for incorporation in the present meta-analysis (Fig. 1).

Inclusion and exclusion criteria

Studies were chosen if they met the following criteria: 1) published studies; 2) evaluated association between VDR gene Apal polymorphism and PCOS risk; 3) a case-control or cohort study based on unrelated individuals; 4) sufficient data for examining odds ratios (ORs) with 95% confidence interval (CI); and 5) genotype distributions of polymorphism of the control population consistent with Hardy-Weinberg equilibrium (HWE). The most recent article was used to extract data if the authors published more than 1 article with the same study data. Case reports, editorials, reviews, abstracts from conferences, republished or duplicate studies, and studies with insufficient information on data extraction were excluded.

Data extraction and quality assessment

The following information was extracted independently by 2 authors from each study: 1) name of the 1st author; 2) year of publication; 3) country of origin; 4) ethnicity of the study population; 5) genotype distribution or allele frequencies; and 6) sample sizes of cases and controls, and the SNPs included (Table 1). The 2 authors independently assessed the articles for compliance with the inclusion/exclusion criteria, resolved disagreements and reached a consistent decision.

Statistical analysis

Review Manager v. 5.3 software (Cochrane Collaboration, Oxford, UK) was used for all statistical analyses. Genotype frequency was assessed by the χ² test in the control group for HWE. The strength of the association between VDR gene Apal polymorphism and PCOS susceptibility was assessed by calculating the pooled ORs and 95% CI of the Z-test. Apal genetic models were used for analyses: allelic model, common model, risk model, and additive model; the p-values were corrected for multiple testing using the false discovery rate. I² statistic were used to test
the heterogeneity among studies, and studies with $I^2 < 50\%$ were considered to be of low heterogeneity. Publication bias was assessed by funnel plot. A p-value $<0.05$ was considered significant for all tests.

**Results**

**Characteristics of the studies**

A comprehensive flowchart of the selection process of the studies is shown in Fig. 1. Our initial search of the literature yielded 161 publications. After reading the titles and abstracts, 21 potential studies were included for full-text view. After reading full texts, 4 studies were excluded for not reporting usable data. Finally, a total of 7 case-control studies in 7 articles were identified and met our inclusion criteria, encompassing 1,350 PCOS patients and 960 controls in total. The main characteristics of these selected studies were summarized in Table 1, Table 2 and Table 3, including 1st author, publication year, country of origin, ethnicity of the study group, genotype distribution, and HWE. Generally, most of the studies (>80%) scored 5 stars or more in the Newcastle-Ottawa scale (NOS), and indicated modest to decent quality (Table 1).

**Meta-analysis of VDR ApaI polymorphism and PCOS susceptibility**

The heterogeneity of the 7 selected studies were employed to assess the overall association between the VDR gene ApaI polymorphism and the risk of PCOS. When $I^2 > 50\%$, we selected random-effects, and for $I^2 < 50\%$ we selected the fixed model. Variant allele genetic model (a compared with A: $p = 0.01$; OR = 1.2, 95% CI = 1.04–1.37), additive/homozygous genetic model (aa compared with AA: $p = 0.01$; OR = 1.41, 95% CI = 1.08–1.74) and risk genetic model (aa+Aa compared with AA: $p = 0.01$; OR = 1.29, 95% CI = 1.05–1.59) showed the risk of the occurrence of PCOS in response to the VDR gene ApaI polymorphism, whereas the common model (aa compared with AA+Aa: $p = 0.29$; OR = 1.11, 95% CI = 0.91–1.36) did not show any risk of PCOS associated with VDR gene ApaI polymorphism (Fig. 2 A–D, Table 4).

**Subgroup analysis**

Subgroup analysis based on the ethnicity of the study group was performed to detect any relationship between VDR gene ApaI polymorphism and PCOS risk. Then, we conducted a subgroup analysis stratified by population...
(Caucasian vs Asian). In the Asian population,\textsuperscript{21,22,24,26} we found statistically significant increased risk of PCOS in additive or homozygotic genetic model (aa compared with AA: \(p = 0.03\); \(OR = 1.54\), 95% CI = 1.04–2.28) (Fig. 2B). However, other genetic models, i.e., allele model (aa compared with A: \(p = 0.21\); OR = 1.18, 95% CI = 0.91–1.54), common model (aa compared with AA+AA: \(p = 0.24\); OR = 1.17, 95% CI = 0.90–1.50) and risk model (aa+AA compared with AA: \(p = 0.33\); OR = 1.35, 95% CI = 0.74–2.47) did not show any risk of PCOS associated with VDR gene ApaI polymorphism (Fig. 2A, 2C, 2D). In the Caucasian population,\textsuperscript{20,23,25} we found statistically significant increased risk of PCOS in allele genetic model (a compared with A: \(p = 0.02\); OR = 1.34, 95% CI 1.00–1.79) and risk model (aa+AA compared with AA: \(p = 0.03\); OR = 1.36, 95% CI = 1.04–1.79) (Fig. 2A, 2D). However, in other genetic models, i.e., additive/homozygotic model (aa compared with AA: \(p = 0.15\); OR = 1.31, 95% CI = 0.91–1.89) and common model (aa compared with AA+AA: \(p = 0.83\); OR = 1.04, 95% CI = 0.75–1.43) did not show any risk of PCOS associated with VDR gene ApaI polymorphism (Fig. 2B, 2C).

**Publication bias**

Funnel plot was carried out to estimate the publication bias among the studies included in this meta-analysis (Fig. 3–6). The emergence of the shape of the funnel plots has not revealed any evidence of publication bias for all the comparison models (a compared with A, aa compared with AA, aa+AA compared with AA, and aa compared with AA+AA).

**Discussion**

Lately, genetic susceptibility to PCOS has led to increasing interest in the study of polymorphisms of genes. This has resulted in the investigation of a number of candidate genes as a way to analyze the possible connection between modulations of PCOS risk across various populations.\textsuperscript{20–27} To date, various reports have been published that have evaluated the possible association of VDR gene ApaI polymorphism and PCOS development, but the findings from different studies were inconsistent and contradictory. Hence, pooled analysis with sufficient power was needed to summarize individual studies. In the present meta-analysis, we aimed to obtain summary estimates for the strength of the association of the VDR gene ApaI polymorphism and PCOS risk from 7 case-control studies,\textsuperscript{20–26} as pooling of the data from individual studies has the advantage of reducing random errors. Also, most of the included studies scored 5 or more stars in NOS quality assessment criteria and suggested good to moderate quality by clearly stating the sample size, genotype, inclusion criteria of patients, and characteristics of healthy controls.

Our novel findings concerning gene models analysis are the following: the frequency of haplotype ApaI “a” was significantly increased in PCOS women compared to that in the controls, while the additive “aa” and risk “aa+Aa” genotype appeared to confer an increased risk for PCOS. The pathophysiological mechanism of these associations is still unclear. Previously, Mahmoudi et al. also reported a relationship between ApaI polymorphisms and PCOS risk.\textsuperscript{21} A previous report by Dasgupta et al. reported that VDR gene polymorphisms have not shown

**Table 4. Meta-analysis of VDR gene ApaI polymorphism and polycystic ovary syndrome (PCOS) susceptibility**

<table>
<thead>
<tr>
<th>Genetic model</th>
<th>Subgroups</th>
<th>Number of studies</th>
<th>Heterogeneity</th>
<th>Effect model</th>
<th>Meta-analysis</th>
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<tr>
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<td></td>
<td></td>
<td>(I^2) (%)</td>
<td>p-value</td>
<td>OR (95% CI)</td>
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<td>a/A total</td>
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<td>0.24</td>
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<td>1.20 (1.04–1.37)</td>
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<td></td>
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<td>random</td>
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<tr>
<td></td>
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<td>0.74</td>
<td>fixed</td>
<td>1.25 (1.04–1.50)</td>
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<td>aa/AA total</td>
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<td>fixed</td>
<td>fixed</td>
<td>1.41 (1.08–1.84)</td>
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<tr>
<td></td>
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<tr>
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<td>0.32</td>
<td>fixed</td>
<td>fixed</td>
<td>1.11 (0.91–1.36)</td>
</tr>
<tr>
<td></td>
<td>Asians</td>
<td>4</td>
<td>0.14</td>
<td>fixed</td>
<td>1.17 (0.90–1.50)</td>
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<tr>
<td></td>
<td>Caucasians</td>
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<td>0.57</td>
<td>fixed</td>
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<tr>
<td>aa+Aa/AA total</td>
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<td>0.10</td>
<td>fixed</td>
<td>random</td>
<td>1.34 (1.00–1.79)</td>
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<tr>
<td></td>
<td>Asians</td>
<td>4</td>
<td>0.03</td>
<td>random</td>
<td>1.35 (0.74–2.47)</td>
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<tr>
<td></td>
<td>Caucasians</td>
<td>3</td>
<td>0.52</td>
<td>fixed</td>
<td>1.36 (1.04–1.79)</td>
</tr>
</tbody>
</table>

**Fig. 1. Results of the literature search strategy**
a significant association with PCOS, which is inconsistent with several previous independent studies. Also, they found some contradictory results of increased PCOS risk and suggested that this may have been a result of different experimental designs or methods, and that the issue warranted further investigation. In comparison with previously published reports, the present study has major improvements, as it included only specific PCOS cases of relevant published studies. When we studied the Asian and Caucasian population separately, we found strong evidence that variant additive "aa" confers susceptibility to PCOS in Asians, while haplotype Apal "a" and risk "aa+Aa" genotype appeared to confer an increased risk for PCOS in Caucasians. This finding may help to explain

**Fig. 2.** A. Allelic model. B. Additive model
the individual differences in the susceptibility to PCOS. A study by El-Shal et al. reported that ApaI was associated with a higher PCOS risk more than control genes in Egyptian women. However, more experimental studies with a larger sample size or alternative methods must be applied for further investigation to verify such findings, as only the mutant genes showed a significant outcome.

As it has been established that PCOS is a complex, multifactorial disease influenced by both environmental and genetic factors, a single genetic variant is normally insufficient to prevent susceptibility toward this disease. The important feature of this gene polymorphism is that its occurrence can vary sufficiently among different races or ethnic populations.
Prior to reaching a final conclusion, limitations of this meta-analysis should also be acknowledged. Firstly, we found significant heterogeneity in the overall analysis. Many factors might have contributed to this heterogeneity, e.g., variation in patients’ characteristics might have been an important source of heterogeneity. Some studies used matched controls (e.g., age- and sex-matched), while other studies did not perform matching. Secondly, only reports published in English were considered in the present study. The 3rd and the most important limitation is that the studies searched for in this pooled data analysis were indexed by the selected electronic web-databases (i.e., PubMed, Web of Science, Embase). There is, therefore, a possibility that some pertinent articles published in other languages and/or indexed in other databases (which are not known to us) may have been missed. The 4th limitation is that since the relevant complete data is not available for most of the time, we failed to adjust the confounding factors, such as age, sex and PCOS severity in this meta-analysis. The 5th constraint was that we were unsuccessful in computing the gene and environmental interactions because of lack of sufficient information in the primary studies.

Despite the abovementioned drawbacks, there are some strong points of our meta-analysis that support the reliability of the present results. Firstly, this meta-analysis involved a large set of harmonized individual level data from 7 independent studies, which provided enough statistical power to confirm our results. Secondly, funnel plot indicated no publication bias. Also, all the included studies were of good to modest quality, fulfilling the preset needful criteria as tested by NOS quality assessment scale. Thirdly, although plenty of meta-analyses considering various case-control studies have been performed in the past, we further analyzed the relationship from the point of ethnicity subgroup. In summary, this data suggests that the VDR gene Apal polymorphism is associated with PCOS. Therefore, VDR gene Apal polymorphism is considered to be one of the possible factors of PCOS predisposition. Furthermore, it is possible that the VDR gene, at least in part, through its effects on insulin resistance and serum levels of insulin, is involved in the pathology of PCOS. However, further studies are needed to confirm the findings and clarify the biological mechanisms by which the polymorphism influences the PCOS risk.
Conclusions

Our meta-analysis demonstrated that PCOS was significantly associated with VDR gene Apal polymorphism. However, due to the relatively small sample size in this meta-analysis, further studies with a larger sample size should be conducted to confirm the findings.

References