Association between retinol-binding protein 4 and polycystic ovary syndrome: A meta-analysis

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Abstract. Studies have examined the association between retinol-binding protein 4 (RBP4) and polycystic ovary syndrome (PCOS). However, the results have been inconsistent. To investigate the association between RBP4 and PCOS, we performed a meta-analysis. The Cochrane Library, MEDLINE, the ISI Web of Science, and EMBASE were searched to identify all of the studies that examined the relationship between circulating RBP4 levels and PCOS. Standard mean difference (SMD) values and 95% confidence interval (CI) were estimated and pooled using meta-analysis methodology. A total of seven studies were involved in the meta-analysis, which included a total of 636 subjects (260 controls and 376 patients with PCOS). The RBP4 level was higher in PCOS patients than in non-PCOS patients (random effects MD (95% CI)=0.69, [0.20, 1.18], \(P=0.006\)). However, the RBP4 level was not higher in nonobese PCOS patients than in nonobese controls (random effects MD (95% CI)=0.38, [-0.21, 0.98], \(P=0.20\)). The effect size revealed that the RBP4 level was higher in overweight or obese PCOS patients than weight-matched controls (fixed effects MD (95% CI)=7.95, [5.96, 9.93], \(P<0.05\)). In the subgroup analysis by region, the RBP4 level was higher in PCOS patients in Asia than controls (random effects MD (95% CI)=0.85, [0.54, 1.15], \(P<0.05\)), but not in European PCOS patients compared with controls (random effects MD (95% CI)=0.34, [-1.12, 1.80], \(P=0.65\)). This subgroup analysis also showed that nonobese PCOS patients have higher RBP4 levels than controls in Asia. Our meta-analysis results indicated that RBP4 might be a useful tool for identifying PCOS women.

Key words: Retinol-binding protein 4, Polycystic ovary syndrome, Meta-analysis, Adipokine

POLYCYSTIC OVARY SYNDROME (PCOS) is the most common endocrinopathy in women of reproductive age, with a prevalence of up to 10%. It is a heterogeneous syndrome with the characteristics of hirsutism, acne, anovulation, hyperandrogenemia, polycystic ovaries, and infertility [1]. It is associated with an adverse metabolic profile including insulin resistance. There is a clear association between obesity, the development of PCOS, and the severity of its phenotypic, biochemical and metabolic features [2]. About 50% of PCOS patients are obese [3]. It is currently assumed that adipose tissue is not only the main energy reservoir but also a pivotal endocrine organ secreting a variety of bioactive cytokines named adipokines, such as leptin, resistin, adiponectin (APN), visfatin, omentin, chemerin, and so on. In patients with PCOS, dysfunction of adipose tissue has been observed with the over-production of pro-inflammatory adipokines such as tumor necrosis factor α (TNFa), and the reduced expression of some ‘beneficial adipokines’ such as APN [4].

Retinol-binding protein 4 (RBP4) is a protein synthesized mainly by hepatocytes, followed by adipocytes. It has been regarded as a novel adipokine since a study on adipose-specific glucose transporter type 4 (Glut4) knockout mice in 2005 [5]. Although it is a transport protein for vitamin A (retinol), RBP4 has been recognized as an adipokine that is implicated in the development of insulin resistance [2]. The effects of RBP4 are thought to be mediated by alterations in insulin signaling through insulin receptor substrate-1 and phosphoinositide 3-kinase activation [5]. Although the majority of studies support a role for RBP4 in mediating insulin resistance, several studies do not support such an effect.
In overweight women with PCOS compared with controls, enhanced expression of RBP4 in adipose tissue and elevated serum RBP4 have been described [7]. In a study involving 50 women with PCOS and 28 control women, the result showed indistinguishable levels of RBP4 between the two groups [8]. In a subsequent study on 27 lean women with PCOS and 19 age- and body mass index-matched controls, RBP4 levels were also shown to be similar between the two groups [9]. RBP4 therefore appears to show similar levels between PCOS cases and control women [2].

As the association between RBP4 and PCOS are far from clear, we addressed the potential role of RBP4 in women with PCOS and tested whether RBP4 might be a useful tool for identifying PCOS women. To our knowledge, no systematic review has been conducted to evaluate RBP4 levels in women with PCOS. Hence, the purpose of the present study was to review all of the available data, to provide a quantitative assessment of the association, and to summarize the results by performing a meta-analysis.

Materials and Methods

Search strategy
The Cochrane Library, MEDLINE, the ISI Web of Science, and EMBASE were searched with English to identify literature published through February 01, 2014 that addressed both the exposure (retinol-binding protein 4) and the outcome (polycystic ovary syndrome) of interest. Our overall search terms were retinol-binding protein 4 OR RBP4 AND polycystic ovary syndrome OR PCOS. We also reviewed the reference lists of identified articles for more studies. This systematic review was conducted in accordance with the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines.

Inclusion and exclusion criteria
To be considered for the meta-analysis, the studies had to be case control or cohort studies, which reported RBP4 levels in women with PCOS compared with healthy controls. Papers defining PCOS according to revised 2003 Rotterdam criteria were included. National Institutes of Health (NIH) criteria, or other compatible criteria (Supplementary data) were excluded in our study. Conference abstracts were also included if they included sufficient information to extract effect estimates. Literature reviews, articles of research on the drug, articles in which the mean and standard deviation (SD) could not be calculated, and studies with no healthy control group were excluded.

Data extraction
Two investigators independently reviewed the titles and the abstracts of all identified citations. To settle disagreements, a third investigator was consulted. The following information was extracted from each study: general characteristics of the study (the first author’s last name, journal, the year of publication, the study design, the country where the population was studied, study size, and number of cases), characteristics of the PCOS and control groups (criteria, selection, age, BMI), methodology (PCOS definition, RBP4 measurement method) and results (RBP4 means and SD) were recorded.

Statistical analysis
The primary variables, RBP4 levels in patients with PCOS, were reported as standard mean differences (SMD) and the corresponding 95% confidence intervals (CIs). The SMD in circulating RBP4 was calculated for all of the studies that were eligible for the meta-analysis, and the results were combined using fixed- or random-effects modeling, as appropriate. Publication bias was assessed using both Begg’s funnel plot and Egger’s test. Begg’s test is based on the adjusted rank correlation, which determines whether there is significant correlation between the effect estimates and their variances [10, 11]. Heterogeneity in the results of the different studies was examined using $\chi^2$ tests for significance (a $P$-value <0.1 was considered statistically significant) and the I$^2$ test ($I^2 > 50\%$: significant heterogeneity; $I^2 < 25\%$: insignificant heterogeneity), which can be interpreted as the percentage of total variation across several studies owing to heterogeneity [12]. A sensitivity analysis was conducted to identify whether the summary results had been significantly influenced by one study that investigated the association between circulating RBP4 levels and PCOS. Subgroup analyses were conducted by region, nonobese or not. All statistical analyses were performed with Review Manager 5.2 and Stata version 11.0. $P< 0.05$ was considered statistically significant.

Results

Description of the studies
The literature search identified a total of 105 potentially relevant articles. Of these, 55 were duplicates, 28
were excluded after reading the title or abstract because of obvious irrelevance, 22 potentially relevant articles remained for further full-text evaluation. Of these, three articles were excluded because they were research on the drugs. Two articles were excluded because of no healthy controls. Another two articles were excluded because data was represented by the median. One article was excluded since it had no detailed data. Another article was excluded because it was a retrospective study. Five studies were excluded for defining PCOS according to NIH criteria, or other compatible criteria. Another one was excluded because it was a duplicate publication. At last, a total of seven studies (376 cases and 260 controls) were included in our meta-analysis [7, 13-18]. A flow chart describing the process of study inclusion/exclusion is displayed as Fig. 1. Among the seven studies, two were conducted in Europe and five in Asia. The main characteristics of the included studies are presented in Table 1.

**Main analysis**

A meta-analysis of the seven studies, which included a total of 636 subjects (260 controls and 376 patients with PCOS), was performed [7, 13-18]. Data were all extracted as means±SD, the \( P \) value of heterogeneity between studies was with significance (\( P<0.05 \), Fig. 2), so we used the random effect model (random effects model).

### Table 1  Characteristics of the studies included in meta-analysis

<table>
<thead>
<tr>
<th>References</th>
<th>Country</th>
<th>Region</th>
<th>Study design</th>
<th>Participants’ age, years (case-control)</th>
<th>N case/control</th>
<th>BMI (kg/m(^2)) (case-control)</th>
<th>RBP4 assay</th>
<th>PCOS diagnosis criteria</th>
<th>DM was excluded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chan TF [13]</td>
<td>Taiwan, China</td>
<td>Asia</td>
<td>case-control</td>
<td>27.9±5.2 – 29.3±4.8</td>
<td>37/37</td>
<td>22.2±4.1 – 20.8±2.9</td>
<td>ELISA</td>
<td>revised 2003 Rotterdam criteria</td>
<td>yes</td>
</tr>
<tr>
<td>Jeon YE [14]</td>
<td>Korea</td>
<td>Asia</td>
<td>case-control</td>
<td>23.72±5.26 – 24.92±2.94</td>
<td>54/36</td>
<td>23.09±4.54 – 19.77±1.51</td>
<td>Bradford method</td>
<td>revised 2003 Rotterdam criteria</td>
<td>yes</td>
</tr>
<tr>
<td>Mellati AA [16]</td>
<td>Iran</td>
<td>Asia</td>
<td>case-control</td>
<td>22.45±5.48 – 23.10±6.46</td>
<td>75/53</td>
<td>27.45±4.82 – 26.35±5.22</td>
<td>ELISA</td>
<td>revised 2003 Rotterdam criteria</td>
<td>yes</td>
</tr>
<tr>
<td>Olszanecka-Glinianowicz M [17]</td>
<td>Poland</td>
<td>Europe</td>
<td>case-control</td>
<td>24.8±4.8 – 25.7±4.9</td>
<td>83/67</td>
<td>29.0±8.7 – 28.3±7.0</td>
<td>ELISA</td>
<td>Rotterdam/ESHRE/ASRM criteria from 2003</td>
<td>yes</td>
</tr>
<tr>
<td>Tan BK [7]</td>
<td>United Kingdom</td>
<td>Europe</td>
<td>case-control</td>
<td>31.4±5.4 – 32.0±4.1</td>
<td>10/10</td>
<td>30.5±2.8 – 29.3±2.5</td>
<td>EIA</td>
<td>Rotterdam/ESHRE/ASRM criteria from 2003</td>
<td>yes</td>
</tr>
<tr>
<td>Yildizhan R [18]</td>
<td>Turkey</td>
<td>Asia</td>
<td>case-control</td>
<td>26.4±3.01 – 25.4±2.62</td>
<td>57/27</td>
<td>26.9±4.2 – 23.8±3.83</td>
<td>ELISA</td>
<td>revised 2003 Rotterdam criteria</td>
<td>yes</td>
</tr>
</tbody>
</table>

ELISA, enzyme-linked immunosorbent assay; EIA, enzyme immunoassay; DM, diabetes mellitus; ESHRE, European Society of Human Reproduction and Embryology; ASRM, American Society for Reproductive Medicine.

Fig. 1  Flow chart of article selection for meta-analysis
Jia et al. effect model (fixed effects MD (95% CI)=7.95, [5.96, 9.93]), and test for overall effect was with significance (P<0.05). The effect size revealed that the RBP4 level was higher in overweight or obese PCOS patients than weight-matched controls.

In the subgroup analysis by region, the RBP4 level was higher in PCOS patients in Asia than controls (I²=58%, random effects MD (95% CI)=0.85, [0.54, 1.15]), and test for overall effect was with significance (P<0.05) (Fig. 4A). By contrast, the RBP4 level in European PCOS patients was not higher than controls (I²=88%, random effects MD (95% CI)=0.34, [-1.12, 1.80]), and the test for overall effect was with no significance (P=0.65) (Fig. 4B).

Furthermore, we conducted a sensitivity analysis by omitting one study at a time and calculating the pooled
Association between RBP4 and PCOS

SMD for the remainder of the studies. When the study by Olszanecka-Glinianowicz et al. (2012) was excluded, there was no material change in the direction of the effect while heterogeneity among studies was reduced ($P=0.08$, $I^2=49\%$). While in nonobese cases, when the study by Olszanecka-Glinianowicz et al. (2012) was excluded, the effect size revealed that the RBP4 level was higher in nonobese PCOS patients than in nonobese controls ($I^2=25\%$, fixed effects MD (95% CI)=0.65, [0.39, 0.91]).

We conducted analysis with Begg’s test and Egger’s test. Begg’s funnel plot had the expected funnel shape (Fig. 5A, 5B). Begg’s test ($P=0.548$) and Egger’s test for publication bias ($P=0.219$) indicated that there was no publication bias in our analysis.

**Discussion**

The prevalence of PCOS varies depending on which criteria are used to make the diagnosis, but is as high as 15%–20% when the European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM) criteria are used [19]. Clinical manifestations include oligomenorrhea or amenorrhea, hirsutism, and frequent infertility. About 50% of PCOS patients are obese. Although obesity is an increasingly prevalent health problem worldwide, women with PCOS have a greater risk of

**Fig. 4A** Forest plot of RBP4 in PCOS patients in Asia

**Fig. 4B** Forest plot of RBP4 in PCOS patients in Europe

**Fig. 5A** Begg’s funnel plot had the expected funnel shape, Begg’s test ($P=0.548$)

**Fig. 5B** Egger’s publication bias plot, Egger’s test ($P=0.219$)
overweight, obesity, and central obesity [20]. Women with PCOS are more likely to have central distribution of body fat, which is associated with insulin resistance (IR) and hyperandrogenemia. The presence of obesity can also magnify IR. Obesity is thus one of the crucial parameters and an independent risk factor of PCOS [21]. However, the molecular mechanisms involved in this complex interplay between obesity, IR, hyperandrogenemia, and diabetic and cardiovascular risk factors are only incompletely understood.

RBP4 has recently been identified as an adipocyte-secreted protein that has been shown to cause IR by enhancing hepatic gluconeogenesis and impairing insulin signaling in skeletal muscle [5]. Most studies have shown that serum RBP4 levels are also elevated in IR humans with obesity, impaired glucose tolerance, and type 2 diabetes and correlate inversely with insulin sensitivity and directly with the components of metabolic syndrome [22, 23]. Reduction in body weight after dietary interventions, especially with a carbohydrate-restricted diet, results in decreased serum RBP4 levels [24]. RBP4 has therefore widely been regarded as one of the key mediators linking adiposity with systemic IR and potentially with adiposity-related disorders. Up until now, there have been only a few studies investigating the involvement of RBP4 in the pathophysiology of PCOS. And these have yielded conflicting results [7, 25, 26].

In the present meta-analysis of seven observational studies, the results showed that the RBP4 level was higher in PCOS patients than in non-PCOS patients. This finding suggested that elevated serum RBP4 was related to the pathophysiology of PCOS. However, the precise cause of this increase in PCOS is unknown. One possible postulated mechanism is up-regulation of mRNA RBP4 by 17β-estradiol, a hormone derived from testosterone that is mainly responsible for PCOS-associated clinical hyperandrogenism [7]. However, in contrast to these results, a few previous studies [8, 26, 27] have stated that there are no differences in RBP4 levels between PCOS cases and controls and have suggested a primary role of RBP4 in the development of PCOS. It is possible that this process involves other molecular mechanisms that remain to be elucidated.

Because there was obvious heterogeneity across the studies, we explored the possible variables that could explain why the results varied from study to study. We performed subgroup and sensitivity analyses to investigate the underlying causes. Obesity is one of the clinical characteristics of the PCOS along with oligomenorrhea, hirsutism, and infertility. However, not all obese females have PCOS and not all PCOS patients are obese. PCOS prevalence rates for underweight, normal-weight, overweight, mildly obese, moderately obese, and severely obese women were 8.2%, 9.8%, 9.9%, 5.2%, 12.4%, and 11.5%, respectively [19]. In the subgroup analysis by nonobese or not, the result suggested that the RBP4 level was not higher in nonobese PCOS patients than in nonobese controls, while the RBP4 level was higher in overweight or obese PCOS patients than weight-matched controls. RBP4 levels were associated with obesity, insulin resistance, and type 2 diabetes not only in mouse models of obesity and insulin resistance, but also in humans with these conditions. Hence, increased RBP4 levels might contribute to impaired insulin-stimulated glucose uptake in muscle and elevated hepatic glucose production, both characteristics of type 2 diabetes [26]. In Hahn et al.’s cohort study, RBP4 levels increased with BMI. In addition, RBP4 levels correlated with waist circumference and body fat but not with parameters of IR [26]. Their data were in accordance with study results from Janke et al., who did not observe a relationship between the homeostasis model assessment (HOMA) index and adipose RBP4 expression, or with circulating RBP4 levels [28]. Engeli et al. found that in obese women, a 5% weight loss improved the HOMA index by 20%, but this change was not associated with a significant change in RBP4 levels [29]. In addition, no differences in RBP4 levels between lean PCOS women and BMI-matched controls were found. Therefore, RBP4 levels do not appear to be influenced by PCOS per se. Aigner et al. suggested an intriguing relationship of RBP4 with several androgens [30], and Tan et al. demonstrated increased serum RBP4 levels in women with PCOS may be regulated by gonadal and sex steroids [7]. However, two other studies demonstrated that RBP4 level is not influenced by hyperandrogenism in PCOS patients [26, 31]. In nonobese cases, when the study by Olszanecka-Glinianowicz et al. (2012) which was conducted in Europe was excluded, the effect size revealed that the RBP4 level was higher in nonobese PCOS patients in Asia than in nonobese controls. At the same time, heterogeneity of the group significantly decreased. So heterogeneity mainly came from the study in European populations. Such discrepancy is perhaps due to different regions or the number of the studies in Europe involved in this analysis. So the result is more reliable in PCOS patients in Asia.

In the subgroup analysis by region, the RBP4 level
was higher in PCOS patients in Asia than controls, but not in European PCOS patients compared with controls. The discrepancy between the results may be possibly due to the following factors: (1) PCOS has a strong genetic basis and the underlying genetics may be quite different in subjects with different sub-phenotypes of PCOS. Under the influence of environment, lifestyles, and genetic factors, the relation between RBP4 and PCOS pathophysiology might be discrepant in different regions; (2) there are only two studies for European PCOS, and the results of these two studies are completely opposite. To show definitive results about RBP4 in European PCOS, further studies are needed.

The current meta-analysis has some limitations in spite of several advantages compared to individual studies. First, some potential confounding factors or modifiers were not controlled for. For instance, one of the included studies did not clarify whether diabetes was excluded [15]. Second, the small sample sizes in some subgroup analysis may have limited statistical power to estimate the RBP4 level in PCOS patients. Third, the relationship between RBP4 level and IR in PCOS patients was not addressed in the current meta-analysis since no related data were provided by the original publications. Fourth, the underlying genetics may be quite different in subjects with different sub-phenotypes of PCOS. The criteria of the Rotterdam Revised 2003 stated that subjects fulfilled the following 2 out of 3 items were diagnosed as PCOS: (1) oligomenorrhea or amenorrhea for at least 6 months; (2) clinical and/or biochemical signs of hyperandrogenism; (3) polycystic ovaries (the presence of 12 or more follicles in each ovary measuring 2–9 mm in diameter), and/or increased ovarian volume (10 mL) [32]. However, almost all the studies did not specify the number of subjects for each subset of phenotypes. Thus, the majority of included studies did not provide the data for sub-phenotypes of PCOS versus controls. Thus, we were unable to examine the association between RBP4 level and sub-phenotypes of PCOS.

In conclusion, our meta-analysis results indicated that the RBP4 level was higher in PCOS patients than in non-PCOS patients. In the subgroup analysis by nonobese or not, the result suggested that the RBP4 level was not higher in nonobese PCOS patients than in nonobese controls. By contrast, the RBP4 level was higher in overweight or obese PCOS patients than weight-matched controls. In the subgroup analysis by region, the RBP4 level was higher in PCOS patients in Asia than controls, but not in European PCOS patients compared with controls. This subgroup analysis also showed that nonobese PCOS patients have higher RBP4 levels than controls in Asia. Further investigation is necessary to clarify the association between the RBP4 level and PCOS patients.

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Disclosure

There are no conflicts of interest that interfere with the impartiality of the research, and there are no potential conflicts of interest that are not fully declared within the text of the article.

References


