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

Fang Liang[1,A,B,D,E], Na Ren[2,A,D,E], Hongxia Zhang[1,C], Jian Zhang[1,E], Qingguo Wu[1,D],
Rui Song[1,B], Zhenfeng Shi[1,E], Zhanxiu Zhang[3,B], Kuixiang Wang[3,B,E,F]

[1] Departments of Endocrinology, Xingtai People's Hospital, Hebei Medical University, China

[2] Departments of Endocrinology, 3rd Xiangya Hospital of Central South University, Changsha, China

[3] Departments of Joint Orthopedics, Xingtai People's Hospital, Hebei Medical University, China

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

Address for correspondence
Kuixiang Wang
E-mail: kxe721@163.com

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 *ApaI* 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 *ApaI* polymorphism and PCOS risk in all 4 genetic models. Subgroup analysis by ethnicity showed that *ApaI* 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, *ApaI* 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 *ApaI* 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, *ApaI*

256

F. Liang, et al. Meta-analysis of a polycystic ovary syndrome

Introduction

Polycystic ovary syndrome (PCOS) is a common multifaceted metabolic disease with a strong genetic component in women of fertile age.[1] The PCOS incidence increased and ranges from 5% to 10%, with the age of affected females ranging from 12 years to 45 years.[2–4] Being a complex multigenic and heteroplasmy disease, PCOS results in several disorders, such as infertility,[5] myocardial infarction,[6] dysfunctional uterine bleeding,[7] cardiovascular risk,[8] endometrial carcinoma,[9] insulin resistance (IR),[10] diabetes mellitus,[10] hyperandrogenism (hirsutism, acne, male pattern hair loss),[11] oligoanovulation and polycystic ovaries, dyslipidemia, amenorrhea, and hypertension, as well is associated with obesity and high levels of cholesterol.[12]

As a secosteroid hormone, vitamin D (VD) is acquired and synthesized from the diet and ultraviolet radiation.[13] Besides its calciotropic function, VD has potent non-classical properties, including immunomodulatory, anti-inflammatory, antioxidant, antiangiogenic, and antiproliferative properties.[14] 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.[10] Insulin resistance, which is commonly present in women with PCOS, may play an important role in the long-term complications of PCOS.[15] Accumulating evidence suggests that VD deficiency might be a causal factor in the pathogenesis of IR and the metabolic syndrome in PCOS.[16] 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.[17] Moreover, previous studies have revealed significant associations between *VDR* gene polymorphisms and PCOS.[18–20]

Considering the past establishment of the important functions of *VDR* gene *ApaI* polymorphism, many studies have explored the association between *VDR* gene *ApaI* polymorphism and PCOS risk.[20–26] 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 *ApaI* (rs7975232) 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 ApaI* OR VD receptor *ApaI*] 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 abovementioned 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 *ApaI* 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 χ^2 test in the control group for HWE. The strength of the association between *VDR* gene *ApaI* polymorphism and PCOS susceptibility was assessed by calculating the pooled ORs and 95% CI of the Z-test. *ApaI* 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. I^2 statistic were used to test

Table 1. Characteristics of experimental methods in the 7 studies included in the meta-analysis

Author (publication year)	Case selection	Control selection	Genotyping method	NOS score
Mahmoudi et al. (2009)[24]	NIH criteria	normal healthy women	PCR-RFLP	6
El-Shal et al. (2013)[23]	Rotterdam criteria	normal healthy women	PCR-RFLP	7
Dasgupta et al. (2015)[22]	Rotterdam criteria	normal healthy women	PCR-RFLP	5
Wehr et al. (2011)[19]	Rotterdam criteria	normal healthy women	genotyping assay	8
Huabin et al. (2016)[26]	Rotterdam criteria	a contemporaneous hospitalized woman with benign ovarian tumors	PCR-RFLP	5
Mahmoudi et al. (2015)[21]	NIH criteria	normal healthy women	PCR-RFLP	5
Jędrzejuk et al. (2015)[20]	Rotterdam criteria	normal healthy women	PCR-RFLP	7

NIH – National Institutes of Health; NOS – Newcastle-Ottawa Scale; PCR-RFLP – polymerase chain reaction – restriction fragments length polymorphism.

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

Table 2. Characteristics of experimental groups in the 7 studies included in the meta-analysis

Author (publication year)	Region	Latitude	Ethnicity	Age (case/control)
Mahmoudi et al. (2009)[24]	Tehran, Iran	36°N	Asian	19–42/18–54
El-Shal et al. (2013)[23]	Zagazig, Egypt	30°N	Caucasian	29.8 ±5.6/29.3 ±6.2
Dasgupta et al. (2015)[22]	Hyderabad, India	18°N	Asian	no mention
Wehr et al. (2011)[19]	Graz, Austria	47°N	Caucasian	23–31/26–36
Huabin et al. (2016)[26]	Jiangxi, China	28°N	Asian	22.56 ±4.56/23.14 ±3.21
Mahmoudi et al. (2015)[21]	Tehran, Iran	36°N	Asian	19–42/19–44
Jędrzejuk et al. (2015)[20]	Lower Silesia, Poland	50°N	Caucasian	20–35

Table 3. Genotype frequencies of *VDR ApaI* polymorphisms in 7 studies included

Author (publication year)	Genotype frequency (AA/Aa/aa/total)		Allele-wise frequency (A/a)		HWE (p-value)
	case	control	case	control	
Mahmoudi et al. (2009)[24]	58/68/36/16	49/90/23/162	184/140	188/136	NS
El-Shal et al. (2013)[23]	63/65/22/150	68/64/18/150	191/109	200/100	0.800
Dasgupta et al. (2015)[22]	12/120/118/250	12/117/121/250	144/356	141/359	0.014
Wehr et al. (2011)[19]	142/274/127/543	48/60/37/145	558/528	156/134	0.155
Huabin et al. (2016)[26]	22/58/40/120	39/55/26/120	102/138	133/107	0.021
Mahmoudi et al. (2015)[21]	8/21/6/35	15/11/9/35	37/33	41/29	0.054
Jędrzejuk et al. (2015)[20]	19/52/19/90	32/49/17/98	90/90	113/83	0.204

NS – not significant; HWE – Hardy-Weinberg equilibrium.

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 = 1.08–1.84) 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. 2A–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,[21,22,24,26] we found statistically significant increased risk of PCOS in additive or homozygous 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 (a 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,[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 genetic 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/homozygous 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.[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
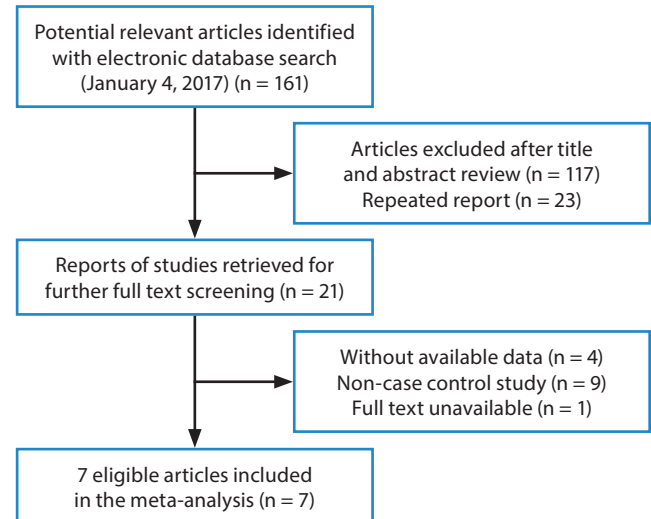
Fig. 1. Results of the literature search strategy

of the association of the *VDR* gene *ApaI* polymorphism and PCOS risk from 7 case-control studies,[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 score 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.[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

Genetic model	Subgroups	Number of studies	Heterogeneity		Effect model	Meta-analysis		
			I² [%]	p-value		OR (95% CI)	Z-test	p-value
a/A	total	7	14	0.24	fixed	1.20 (1.04~1.37)	2.57	0.01
	Asians	4	51	0.08	random	1.18 (0.91~1.54)	1.23	0.21
	Caucasians	3	0	0.74	fixed	1.25 (1.04~1.50)	2.36	0.02
aa/AA	total	7	0	0.53	fixed	1.41 (1.08~1.84)	2.51	0.01
	Asians	4	23	0.28	fixed	1.54 (1.04~2.28)	2.13	0.03
	Caucasians	3	0	0.64	fixed	1.31 (0.91~1.89)	1.44	0.15
aa/AA+Aa	total	7	14	0.32	fixed	1.11 (0.91~1.36)	1.05	0.29
	Asians	4	46	0.14	fixed	1.17 (0.90~1.50)	1.17	0.24
	Caucasians	3	0	0.57	fixed	1.04 (0.75~1.43)	0.22	0.83
aa+Aa/AA	total	7	44	0.10	fixed	1.34 (1.00~1.79)	1.95	0.05
	Asians	4	67	0.03	random	1.35 (0.74~2.47)	0.98	0.33
	Caucasians	3	0	0.52	fixed	1.36 (1.04~1.79)	2.22	0.03

A

Study or Subgroup	Experimental		Control		Weight	Odds Ratio M-H, Random, 95% CI
	Events	Total	Events	Total		
1.1.1 Asians						
Dasgupta et al	356	500	359	500	20.2%	0.97 [0.74, 1.28]
Mahmoudi et al	140	324	136	324	16.4%	1.05 [0.77, 1.44]
Mahmoudi T et al	33	70	29	70	4.1%	1.26 [0.65, 2.46]
Huabin Cao et al	138	240	107	240	12.7%	1.68 [1.17, 2.41]
Subtotal (95% CI)		**1134**		**1134**	**53.4%**	**1.18 [0.91, 1.54]**
Total events	667		631			

Heterogeneity: Tau² = 0.03; Chi² = 6.11, df = 3 (P = 0.11); I² = 51%
Test for overall effect: Z = 1.25 (P = 0.21)

Study or Subgroup	Experimental		Control		Weight	Odds Ratio M-H, Random, 95% CI
1.1.2 Caucasian						
El-Shal AS et al	109	300	100	300	14.4%	1.14 [0.82, 1.60]
Wehr et al	528	1086	124	290	21.9%	1.27 [0.98, 1.65]
Jedrzejuk D,et al	90	180	83	196	10.3%	1.36 [0.91, 2.05]
Subtotal (95% CI)		**1566**		**786**	**46.6%**	**1.25 [1.04, 1.50]**
Total events	727		307			

Heterogeneity: Tau² = 0.00; Chi² = 0.46, df = 2 (P = 0.79); I² = 0%
Test for overall effect: Z = 2.34 (P = 0.02)

Total (95% CI)		**2700**		**1920**	**100.0%**	**1.20 [1.04, 1.37]**
Total events	1394		938			

Heterogeneity: Tau² = 0.00; Chi² = 6.96, df = 6 (P = 0.32); I² = 14%
Test for overall effect: Z = 2.57 (P = 0.01)
Test for subgroup differences: Chi² = 0.10. df = 1 (P = 0.75). I² = 0%

0.01 0.1 1 10 100
Favours [experimental] Favours [control]

B

Study or Subgroup	Experimental		Control		Weight	Odds Ratio M-H, Fixed, 95% CI
	Events	Total	Events	Total		
4.1.1 Asians						
Dasgupta et al	118	130	121	133	12.2%	0.98 [0.42, 2.26]
Huabin Cao et al	40	62	26	65	10.0%	2.73 [1.33, 5.60]
Mahmoudi et al	36	94	23	72	17.8%	1.32 [0.69, 2.52]
Mahmoudi T et al	6	14	9	24	4.2%	1.25 [0.33, 4.79]
Subtotal (95% CI)		**300**		**294**	**44.2%**	**1.54 [1.04, 2.28]**
Total events	200		179			

Heterogeneity: Chi² = 3.87, df = 3 (P = 0.28); I² = 23%
Test for overall effect: Z = 2.13 (P = 0.03)

Study or Subgroup	Experimental		Control		Weight	Odds Ratio M-H, Fixed, 95% CI
4.1.2 Caucasian						
El-Shal AS et al	22	85	18	86	14.7%	1.32 [0.65, 2.69]
Jedrzejuk D,et al	19	38	17	49	8.2%	1.88 [0.79, 4.48]
Wehr et al	127	269	37	85	32.9%	1.16 [0.71, 1.90]
Subtotal (95% CI)		**392**		**220**	**55.8%**	**1.31 [0.91, 1.89]**
Total events	168		72			

Heterogeneity: Chi² = 0.91, df = 2 (P = 0.64); I² = 0%
Test for overall effect: Z = 1.44 (P = 0.15)

Total (95% CI)		**692**		**514**	**100.0%**	**1.41 [1.08, 1.84]**
Total events	368		251			

Heterogeneity: Chi² = 5.11, df = 6 (P = 0.53); I² = 0%
Test for overall effect: Z = 2.51 (P = 0.01)
Test for subgroup differences: Chi² = 0.34. df = 1 (P = 0.56). I² = 0%
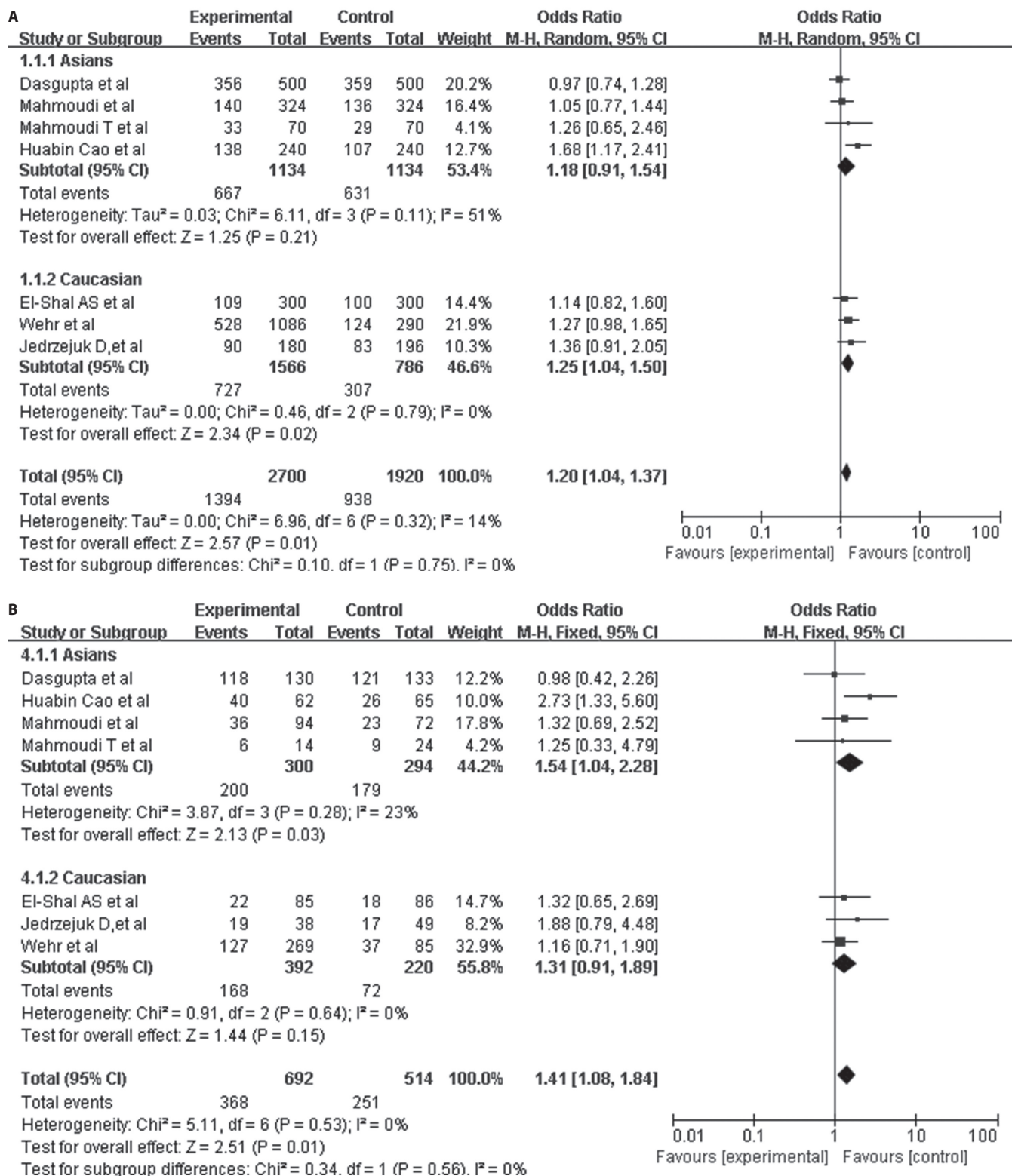
0.01 0.1 1 10 100
Favours [experimental] Favours [control]

Fig. 2. A – allelic model, B – additive model

a significant association with PCOS, which is inconsistent with several previous independent studies.[22] 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 *ApaI* "a" and risk "aa+Aa" genotype appeared to confer an increased risk for PCOS in Caucasians. This finding may help to explain

C

Study or Subgroup	Experimental Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
3.2.1 Asians						
Dasgupta et al	118	250	121	250	35.1%	0.95 [0.67, 1.35]
Huabin Cao et al	40	120	26	120	9.5%	1.81 [1.02, 3.22]
Mahmoudi et al	36	162	26	162	11.1%	1.49 [0.85, 2.62]
Mahmoudi T et al	6	35	9	35	4.1%	0.60 [0.19, 1.91]
Subtotal (95% CI)		567		567	59.9%	**1.17 [0.90, 1.50]**
Total events	200		182			
Heterogeneity: Chi² = 5.52, df = 3 (P = 0.14); I² = 46%						
Test for overall effect: Z = 1.17 (P = 0.24)						
3.2.2 Caucasian						
El-Shal AS et al	22	150	18	150	8.4%	1.26 [0.65, 2.46]
Jedrzejuk D,et al	19	90	17	98	7.1%	1.28 [0.62, 2.64]
Wehr et al	127	543	37	145	24.6%	0.89 [0.58, 1.36]
Subtotal (95% CI)		783		393	40.1%	**1.04 [0.75, 1.43]**
Total events	168		72			
Heterogeneity: Chi² = 1.13, df = 2 (P = 0.57); I² = 0%						
Test for overall effect: Z = 0.22 (P = 0.83)						
Total (95% CI)		1350		960	100.0%	**1.11 [0.91, 1.36]**
Total events	368		254			
Heterogeneity: Chi² = 6.96, df = 6 (P = 0.32); I² = 14%						
Test for overall effect: Z = 1.05 (P = 0.29)						
Test for subgroup differences: Chi² = 0.31. df = 1 (P = 0.58). I² = 0%						

Odds Ratio M-H, Fixed, 95% CI

0.01 0.1 1 10 100
Favours [experimental] Favours [control]

D

Study or Subgroup	Experimental Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
2.1.1 Asians						
Dasgupta et al	238	250	238	250	9.2%	1.00 [0.44, 2.27]
Huabin Cao et al	98	120	81	120	14.0%	2.14 [1.18, 3.91]
Mahmoudi et al	104	162	113	162	18.3%	0.78 [0.49, 1.24]
Mahmoudi T et al	27	35	20	35	6.5%	2.53 [0.90, 7.12]
Subtotal (95% CI)		567		567	48.0%	**1.35 [0.74, 2.47]**
Total events	467		452			
Heterogeneity: Tau² = 0.24; Chi² = 9.15, df = 3 (P = 0.03); I² = 67%						
Test for overall effect: Z = 0.98 (P = 0.33)						
2.1.2 Caucasian						
El-Shal AS et al	87	150	82	150	18.6%	1.15 [0.73, 1.81]
Jedrzejuk D,et al	71	90	66	98	12.5%	1.81 [0.94, 3.50]
Wehr et al	401	543	97	145	21.0%	1.40 [0.94, 2.07]
Subtotal (95% CI)		783		393	52.0%	**1.36 [1.04, 1.79]**
Total events	559		245			
Heterogeneity: Tau² = 0.00; Chi² = 1.29, df = 2 (P = 0.52); I² = 0%						
Test for overall effect: Z = 2.22 (P = 0.03)						
Total (95% CI)		1350		960	100.0%	**1.34 [1.00, 1.79]**
Total events	1026		697			
Heterogeneity: Tau² = 0.07; Chi² = 10.77, df = 6 (P = 0.10); I² = 44%						
Test for overall effect: Z = 1.95 (P = 0.05)						
Test for subgroup differences: Chi² = 0.00. df = 1 (P = 0.99). I² = 0%						

Odds Ratio M-H, Random, 95% CI

0.01 0.1 1 10 100
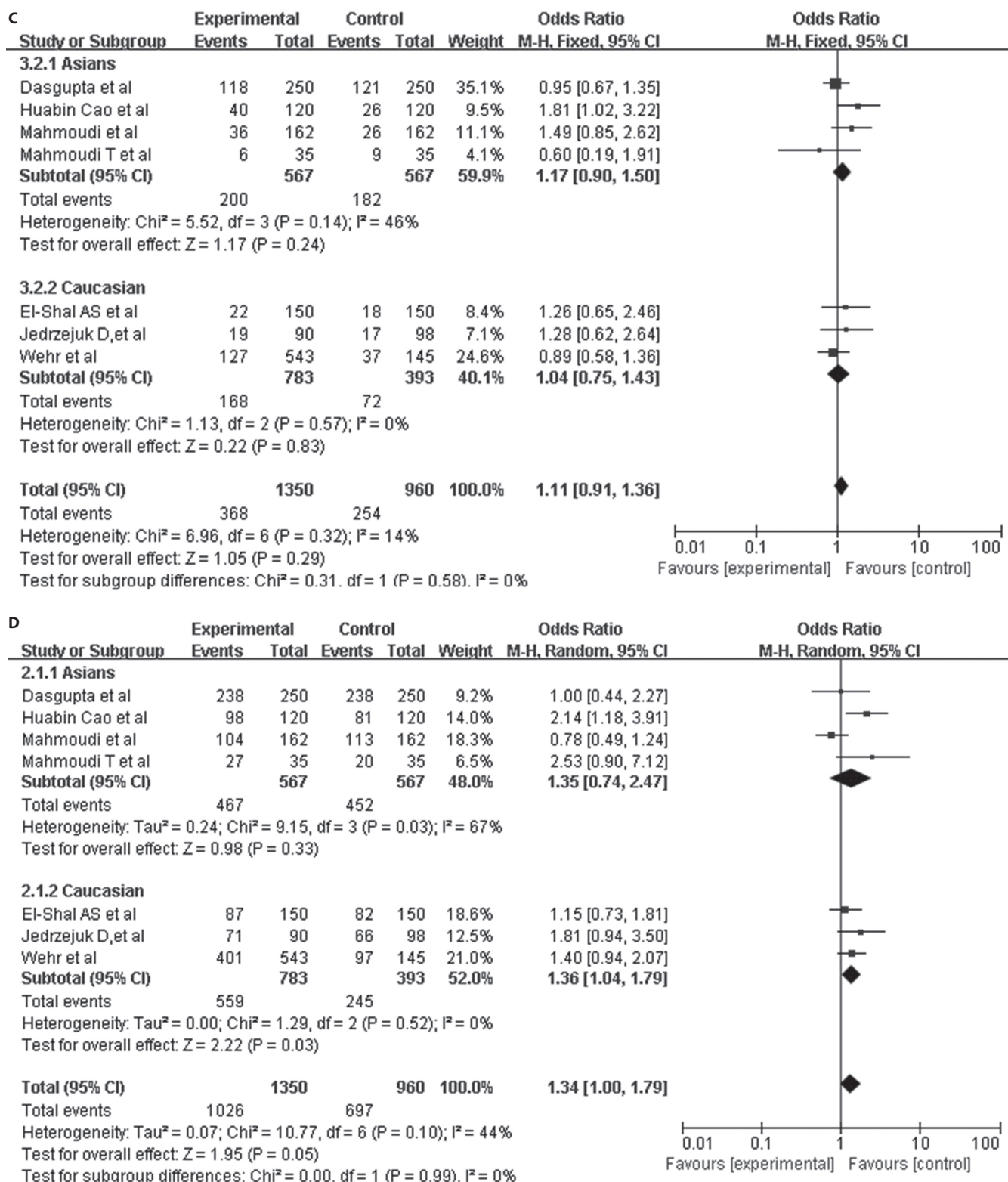Favours [experimental] Favours [control]

Fig. 2. A – allelic model. B – additive model (cont.)

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.[23] 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,[28] 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.
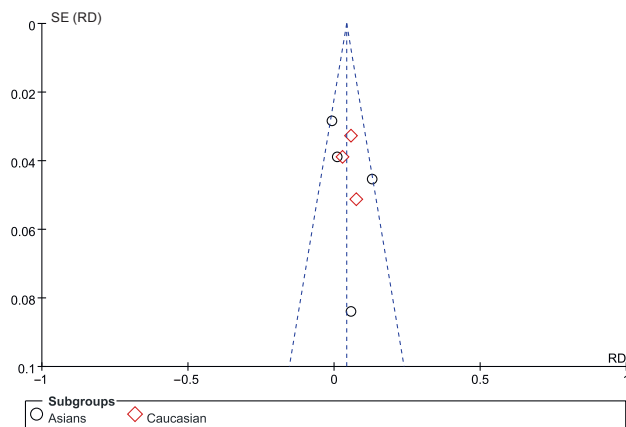
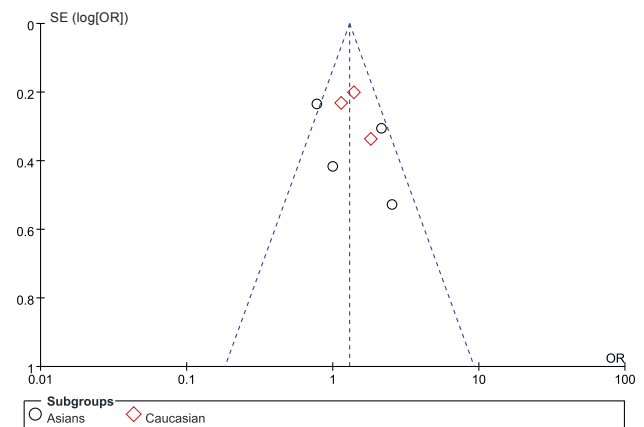**Fig. 3.** Publication bias of allelic model

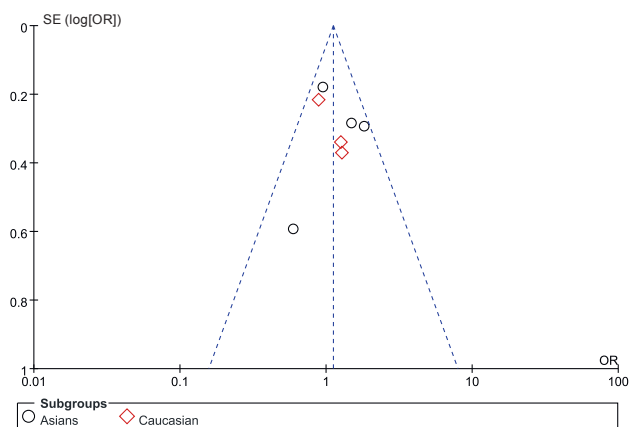**Fig. 5.** Publication bias of dominant model
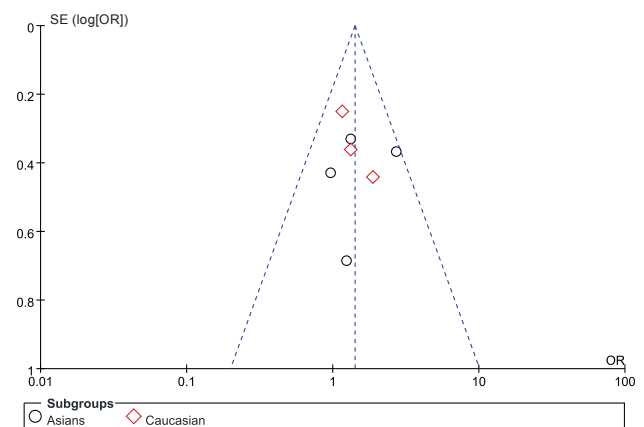

Fig. 4. Publication bias of recessive model

Fig. 6. Publication bias of addictive model

Prior to reaching a final conclusion, limitations of this meta-analysis should also be acknowledged.[29–31] 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 there 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.[32,33]

In summary, this data suggests that the *VDR* gene *ApaI* polymorphism is associated with PCOS. Therefore, *VDR* gene *ApaI* 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 *ApaI* 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.

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