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The -675 4G/5G polymorphism in the PAI-1 gene may not contribute to the risk of PCOS

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Abstract. – OBJECTIVE: The association between the -675 4G/5G polymorphism in PAI-1 gene and PCOS has been studied with inconclusive results. We sought to investigate this inconsistency by performing a comprehensive meta-analysis on the polymorphism.

MATERIALS AND METHODS: Searches were performed in the PubMed, Embase, CNKI and Wanfang databases, covering all papers. Statistical analysis was performed using Revman5.2 and STATA11.0 software. A total of 11 case-control studies were extracted on the polymorphism involving 1861 PCOS cases and 1187 controls.

RESULTS: The results showed that, no significant increased/decreased risk were found for the polymorphism for PCOS: OR = 1.03, 95% CI = 0.77-1.66, *p* = 0.52 for 4G4G + 4G5G vs. 5G5G; OR = 0.99, 95% CI = 0.66-1.49, *p* = 0.96 for 4G4G vs. 5G5G + 4G5G; OR = 1.08, 95% CI = 0.66-1.79, *p* = 0.76 for 4G4G vs. 5G5G; OR = 1.11, 95% CI = 0.78-1.58, *p* = 0.56 for 4G5G vs. 5G5G; OR = 1.00, 95% CI = 0.71-1.41, *p* = 0.99 for 4G vs. 5G. In the further subgroup analysis by ethnicity, we did not find a significant association between the polymorphism for PCOS risk in either Asians or Europeans.

CONCLUSIONS: Our findings demonstrated that -675 4G/5G polymorphism in the *PAI-1* gene might not be a risk factor for the development of PCOS.

Key Words:
PCOS, Polymorphism, Meta-analysis, PAI-1.

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disease in women of reproductive age with an estimation of effecting 5% to 10% of the population[1,2]. Epidemiology studies suggested that PCOS arises as a result of complex interactions of environmental and genetic factors[3-5]. In recent years, many individual studies have set out to determine whether there was an association between genetic polymorphisms and PCOS susceptibility, such as *PAI-1* polymorphisms.

The human *PAI-1* gene is located on the chromosome 7q21.3-22 region and many polymorphisms in the gene have been identified[6,7]. Among these polymorphisms, the -675 4G/5G polymorphism is one of the widely studied polymorphisms for PCOS. Several studies have performed to study the association between the polymorphism with the risk of PCOS; however, the results were inconclusive. Recently, Liu et al[8] reported a meta-analysis of the association between the -675 4G/5G polymorphism in the *PAI-1* gene and PCOS susceptibility[8]. They included 10 case-control studies with a total of 2079 cases and 1556 controls. The results showed that the -675 4G/5G polymorphism may increase the risk of PCOS, especially among Asian populations but not among Caucasians. However, there were several mistakes in that study, and the conclusion should be validated by other studies. In order to get more precision results, we carried out a meta-analysis including all eligible studies published to date to systematically and comprehensively estimate the association of the polymorphism and susceptibility to PCOS.

Materials and Methods

Literature Search Strategy

The databases of PubMed, Embase, CNKI and Wanfang were searched (the last search was updated in April 1, 2014) to identify all relevant publications on the association between *PAI-1* -675 4G/5G polymorphism and PCOS risk. The following search terms and their synonyms were used: PCOS and polymorphism and *PAI-1* and Chinese. We also manually searched the reference lists of all eligible studies and review articles to obtain additional usable data that can be included in the current meta-analysis.

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Inclusion Criteria and Exclusion Criteria

We selected eligible studies according to the following criteria: (1) They were original papers containing independent data with the association between the -675 4G/5G polymorphism in the *PAI-1* gene and PCOS susceptibility, (2) they provided sufficient data to calculate the odds ratio (OR) or P-value, (3) they used a case-control design, (4) the control population being consistent with Hardy-Weinberg equilibrium (HWE). The major reasons for exclusion of studies were (1) they were family studies, (2) they contained overlapping data, (3) they were review papers. If a study was subsequently updated, we selected the study with the largest sample size. Two investigators independently reviewed all studies to examine whether they fulfilled the inclusion criteria.

Data Extraction

Two independent investigators extracted the original data according to the inclusion criteria and exclusion criteria to ensure the accuracy of the retrieved information. The data extracted from each eligible study included the first author's name, year of publication, cancer type, ethnicity, source of controls, method adopted for genotyping, number of cases and controls and genotype frequencies. Disputes were settled by consulting the third person.

Statistical Analysis

Hardy-Weinberg equilibrium (HWE) of the control groups was tested by the χ^2 test for goodness of fitness. Crude ORs with 95% CIs were calculated to evaluate the strength of the association between the polymorphism and POCS risk. The pooled ORs were performed for the following genetic models: allele contrast (4G vs. 5G), homozygote (4G4G vs. 5G5G), heterozygote (G5G vs. 5G5G), dominant (4G4G+4G5G vs. 5G5G) and recessive (4G4G vs. 5G5G+4G5G) model. Heterogeneity assumption was evaluated by the chi-square based Q-test and I^2 statistics, $p > 0.05$ for the Q test or $I^2 < 50\%$ suggested a lack of heterogeneity. In this situation, the OR of each study was calculated by the fixed-effects model (the Mantel-Haenszel method). If $p < 0.05$ or $I^2 > 50\%$, the random-effects model (the DerSimonian and Laird method) was used. The evaluation of potential publication bias was performed using the Begg's funnel plots and Egger's test[9,10]. Subgroup analyses were performed by ethnicity. All statistical analyses were performed by Revman5.2.0 and STATA11.0. A level of $p < 0.05$ was accepted as statistically significant.

Results

Characteristics of Published Studies

Finally, 11 case-control studies concerning 1861 PCOS cases and 1187 controls were included for data analysis[1-7,11-14]. The characteristics of the included studies are shown in Table I. Genotype distributions of all control groups were in accord with HWE.

Table I. The characteristics of the included case-control studies.

Author	Year	Country	Case			Control			HWE
			5G5G	4G5G	4G4G	5G5G	4G5G	4G4G	
Glueck[1]	2006	USA	203	460	258	39	58	29	Yes
Idali[6]	2012	Iran	6	20	12	1	27	72	Yes
Karadeniz[3]	2007	Turkey	21	48	22	21	41	38	Yes
Kilicci[7]	2011	Turkey	39	29	32	28	42	30	Yes
Sales[4]	2013	Brazil	24	30	25	34	29	16	Yes
San Milian[2]	2004	Spain	17	37	18	17	17	8	Yes
Sun[5]	2010	China	20	62	60	36	51	26	Yes
Unsal[11]	2009	Turkey	13	24	7	32	26	12	Yes
Walch[12]	2005	Austria	29	50	27	24	46	32	Yes
Zhang[13]	2008	China	72	65	30	110	123	80	Yes
Zhao[14]	2005	China	18	25	58	6	20	16	Yes

Quantitative analysis

The 11 case-control studies included in the quantitative analysis yielded a total of 1861 PCOS cases and 1187 controls. Significant between-study heterogeneity was detected across studies for the 4G4G+4G5G vs. 5G5G model and thus we selected the random-effects model to summarize the ORs. Overall, we found no significant association between *PCOS* -675 4G/5G polymorphism and PCOS in total analysis (OR = 1.13, 95%CI = 0.77-1.66, p = 0.52) (Figure 1). Begg's funnel plots and Egger's test were performed to evaluate publication bias in the literature. Funnel plots of the genetic model seemed symmetrical (Figure 2). This was confirmed by the statistical data derived using Egger's test (t = -0.38, p = 0.714). Subgroup analyses suggested that the polymorphism did not contribute to the risk of PCOS in either Asians (OR = 0.77, 95%CI = 0.27-2.18, p = 0.62) or Europeans (OR = 1.17, 95%CI = 0.78-1.77, p = 0.45) (Figure 3).

Other genetic comparatives were also used to analyze the association (Figures not shown). The results for 4G4G vs. 5G5G+4G5G were as follows: OR = 0.99, 95%CI = 0.66-1.49, p = 0.96 for total analysis; OR = 0.90, 95%CI = 0.31-2.61, p = 0.85 for Asians, and OR = 0.95, 95%CI = 0.69-1.30, p = 0.74 for Europeans. The results for 4G4G vs. 5G5G were as follows: OR = 1.08, 95%CI = 0.66-1.79, p = 0.76 for total analysis; OR = 0.71, 95%CI = 0.17-2.95, p = 0.64 for Asians, and OR = 1.06, 95%CI = 0.68-1.65, p = 0.80 for Europeans. The results for 4G5G vs. 5G5G were as follows: OR = 1.11, 95%CI = 0.78-1.58, p = 0.56 for total analysis; OR = 0.77, 95%CI = 0.33-1.81, p = 0.55 for Asians, and OR = 1.22, 95%CI = 0.79-1.89, p = 0.37 for Europeans. The results for 4G vs. 5G were as follows: OR = 1.00, 95%CI = 0.71-1.41, p = 0.99 for total analysis; OR = 0.86, 95%CI = 0.38-1.94, p = 0.72 for Asians, and OR = 0.97, 95%CI = 0.73-1.28, p = 0.81 for Europeans.

Discussion

Via a comprehensive meta-analysis, we evaluated the association of one common polymorphism in the *PAI-1* gene with the risk of PCOS. Although potential sources of heterogeneity could not be easily eliminated, the present study, to our knowledge, is the most comprehensive meta-analysis to date dealing with the association of the -675 4G/5G polymorphism with PCOS.

In this meta-analysis, we included a total of 11 case-control studies. The pooled results indicated that there were no obvious associations between *PAI-1* -675 4G/5G polymorphism and PCOS risk under all models. Thus, the *PAI-1* -675 4G/5G polymorphism could not be suggested as a PCOS risk factor.

Recently, Liu performed a meta-analysis to assess the association between the *PAI-1* -675 4G/5G polymorphism and the risk of PCOS. Although Liu made great contribution to the topic in their study, unfortunately, there are quite a few mistakes in that meta-analysis, especially in including literatures and genotype data. Firstly, they included two overlapped studies in the meta-analysis[15,16] When performed the meta-analysis,

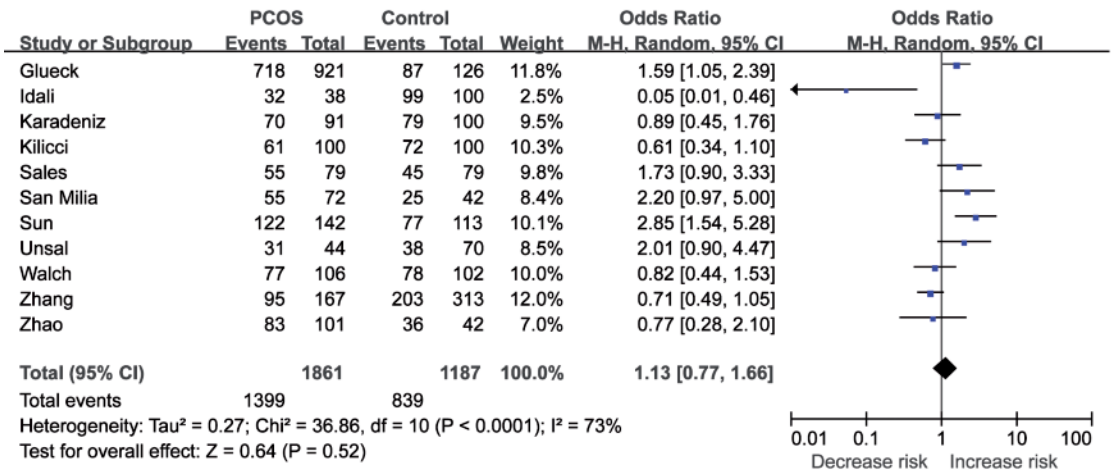

Figure 1. The association between the *PAI-1*-675 4G/5G polymorphism and PCOS risk (4 G4G+4G5G vs. 5G5G): total analysis.

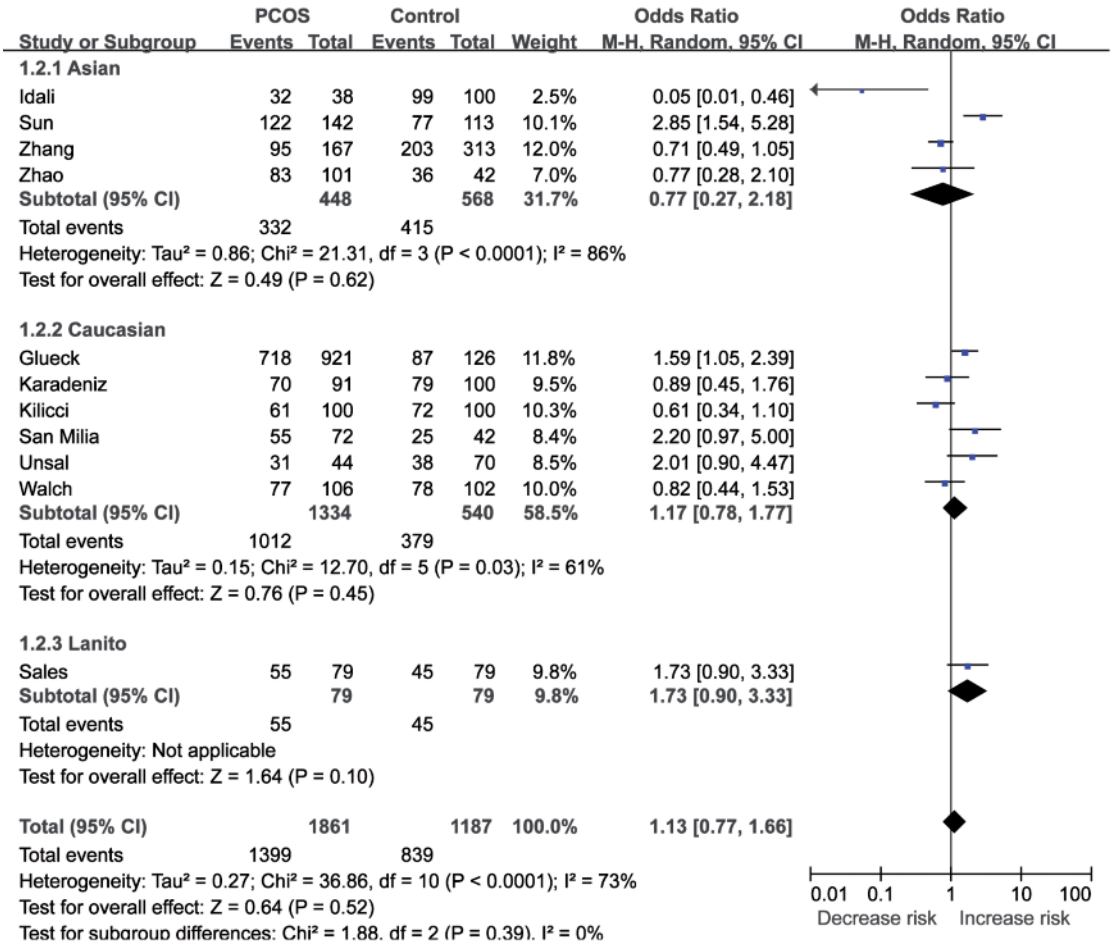

Figure 2. The association between the *PAI-1* -675 4G/5G polymorphism and PCOS risk (4 G4G+4G5G vs. 5G5G): subgroup analysis by ethnicity.

one important thing was to exclude the overlapped data and literatures. The reference of 22 and 15 should be excluded when performed the meta-analysis[15,16]. Actually, the reference 22 and 23 were from the same authors[16,5], and the reference 22 should be excluded[16]. In addition, the reference

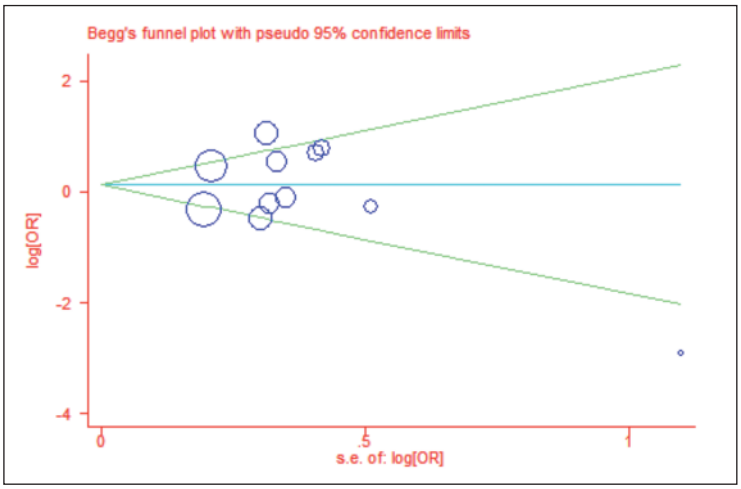

Figure 3. Publication bias for the association between the *PAI-1* -675 4G/5G polymorphism and PCOS risk (4 G4G+4G5G vs. 5G5G).

15 and 19 were from the same authors[1,15], and the reference 15 should also be excluded[15]. In spite of included the wrong study, they also missed Idali's study[6]. Secondly, when comparing the genotype data used for meta-analysis by Liu et al[8] with those presented in the primary literatures, we found the data of Sales et al[4] was different with the primary data. Compared with Liu's study, we performed the current meta-analysis by developing a detailed study protocol, meticulous search for published studies, and using explicit methods for study selection, data extraction and data analysis. Thus, our results were more conclusive.

Several limitations should be acknowledged when interpreting the results of this meta-analysis. First, there was a potential language bias, because the PubMed, EMbase, CNKI and Wanfang search engines were used to identify articles and to exclude articles written in languages other than English and Chinese. This might not have prevented the researchers from accessing all relevant studies. Second, the overall outcomes were based on individual unadjusted ORs; a more precise estimation should be adjusted by age, environmental and other confounding factors. Finally, this meta-analysis could not address the gene-gene and gene-environmental interactions in the association between polymorphism and PCOS risk. Future studies are needed to assess the possible gene-gene and gene-environment interactions in the association between polymorphism and PCOS risk.

Conclusions

The -675 4G/5G polymorphism in the *PAI-1* gene might not contribute to the risk of PCOS. Further studies should perform to validate these results.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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