Plasminogen Activator Inhibitor-1 -675 4G/5G Polymorphism and Polycystic Ovary Syndrome Risk: A Meta Analysis

Ying Liu · Mei-Guo Sun · Rong Jiang · Rui Ding ·
Zhen Che · Yan-Yan Chen · Ci-Jiang Yao ·
Xiao-Xia Zhu · Ji-Yu Cao

Received: 11 October 2013 / Accepted: 29 December 2013 / Published online: 17 January 2014
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Abstract

Purpose Several studies have reported that excessive amounts of plasminogen activator inhibitor-1 (PAI-1) might increase the incidence of polycystic ovary syndrome (PCOS), but so far the published results were inconsistent. The aim of this study was to further investigate the association between PAI-1 gene polymorphism and the susceptibility to PCOS by performing a meta-analysis.

Methods A comprehensive literature search for relevant studies was conducted on google scholar, PubMed, the Chinese National Knowledge Infrastructure (CNKI) and the Chinese Biomedical Literature Database (CBM). This meta-analysis was performed using the STATA 11.0 software and the pooled odds ratio (OR) with 95 % confidence interval (CI) was calculated.

Results Ten case-control studies were included in this meta-analysis with a total of 2,079 cases and 1,556 controls. The results showed that PAI-1 -675 4G/5G polymorphism may increase the risk of PCOS, especially among Asian populations. However, there was no statistically significant association between the polymorphism and PCOS risk in Caucasians.

Conclusion Our meta-analysis suggests that PAI-1 -675 4G/5G polymorphism may contribute to increasing susceptibility

to PCOS in Asians. Detection of the PAI-1 gene polymorphism might be a promising biomarker for the susceptibility of PCOS.

Keywords Meta-analysis · PAI-1 · Polymorphism · PCOS

Introduction

Polycystic ovary syndrome (PCOS) is considered one of the most common reproductive endocrine disorders, affecting approximately 4 %–18 % of women of reproductive age [1–4]. PCOS is a complex condition characterized by hyperandrogenism, insulin resistance, chronic anovulation, infertility, and irregular menses [5]. The exact cellular and molecular mechanisms of PCOS remain elusive and it is believed to be multifactorial in etiology, where environmental risk factors operate in addition to susceptible genes, leading to a multitude of reproductive and metabolic defects [6]. Studies have shown that infertility is the most common symptom related to PCOS [7, 8]. Women with PCOS are more likely to develop complications of the metabolic syndrome such as obesity, hypertension, dyslipidemia, and diabetes, which are in turn severe risk factors for cardiovascular disease [7]. PCOS patients have elevated activation of the blood coagulation system [9]. Thrombus formation results from the disturbance between pro-thrombotic and anti-thrombotic factors that controls clotting balance: this disturbance may occur due to an ongoing stimulus to thrombogenesis, a defect of the natural anti-coagulant or fibrinolytic system [10].

Plasminogen activator inhibitor 1 (PAI-1), an important member of the serine protease inhibitor (SERPIN) family, is a major regulator of the endogenous fibrinolytic system. It suppresses fibrinolytic activity of the tissue-type plasminogen activator (tPA), which generates active plasmin from plasminogen, that then eliminates the fibrin [11]. However, evidences show that a -675 4G/5G polymorphism in the promoter of the

Capsule The PAI-1 4G allele is associated with an increased risk of developing PCOS in Asians and may be a marker for clinical evaluation.

Y. Liu · R. Ding · Z. Che · Y.-Y. Chen · C.-J. Yao · X.-X. Zhu ·
J.-Y. Cao (✉)

Department of Occupational and Environmental, School of Public
Health, Anhui Medical University, Meishan Road,
Hefei, Anhui, China
e-mail: qshq@163.com

M.-G. Sun

Department of Gynecology and Obstetrics, the First Affiliated
Hospital of Anhui Medical University, Meishan Road,
Hefei, Anhui, China

R. Jiang

Anhui Medical College, Furong Road, Hefei, Anhui, China

PAI-1 gene was associated with the elevated levels of plasma PAI-1 and the 4G allele leads to a higher activity than the 5G allele, because besides the binding site for the transcriptional activator, 5G allele also contains a binding site for a transcriptional repressor. Without the bound repressor, PAI-1 transcription is increased.

Recently, several studies have indicated that PAI-1 4G/5G polymorphism might significantly relate to the increased risk of PCOS. However, the results are inconsistent and controversial. Therefore, we attempt to perform this meta-analysis of all eligible studies to provide a more comprehensive and reliable conclusion by reevaluating the association between PAI-1 4G/5G polymorphism and PCOS risk.

Materials and methods

Search strategy

Studies which evaluated the link between PAI-1 polymorphism and PCOS risk were all searched in the databases of google scholar, PubMed, the Chinese National Knowledge Infrastructure (CNKI) and the Chinese Biomedical Literature Database (CBM) with the following terms: plasminogen activator inhibitor 1, PAI-1, polymorphism, polymorphisms, mutation, variant, variants, polycystic ovary syndrome, and PCOS. There is no limit on languages. Besides, all references cited were also reviewed to identify additional studies.

Inclusion criteria

Regarding PCOS susceptibility and the polymorphism, studies which met the following criteria were identified: (1) Clinical case–control or cohort studies concentrated on the role of PAI-1 4G/5G polymorphism in the pathogenesis of PCOS. (2) All patients should meet the PCOS definition specified in the 1990 National Institute of Health–National Institute of Child Health and Human Development conference or in 2003 Rotterdam conference by European society for human reproduction and embryology (ESHRE) and American society for reproductive medicine (ASRM). (3) The clear numbers or genotype frequency in case and control groups must be showed in the articles. (4) The genotype frequencies distribution of the controls should consist with Hardy–Weinberg equilibrium (HWE), which was performed for evaluating the qualities of the studies. Studies were excluded if they did not confirm to all the inclusion criteria.

Data extraction

Two authors independently extracted data from each included study independently according to the selection criteria. We compared the results and made decision by consensus of all members of our group. For each included study, data on first author, year of

publication, study design, ethnicity of the study, number of cases and controls, mean age of participants, diagnostic standards of cases in each study, and the number of cases and controls for –675 4G/5G genotype were extracted.

Statistical analysis

Crude ORs with their 95 % CIs were used to estimate the strength of association between the PAI-1 4G/5G polymorphism and PCOS susceptibility. The pooled ORs were calculated for the allele contrasts, recessive genetic model, dominant genetic model, and additive comparison. Subgroup analyses were also performed by ethnicity.

A random or fixed effect model was employed based on the heterogeneity assumption [12, 13]. Heterogeneity assumption was examined by the chi-square based Q test [14]. The random effect model was used as the pooling method in the presence of substantial heterogeneity ($I^2 > 50\%$), otherwise, the fixed effect was performed to assess the pooled OR. The genotypic frequency distribution in the control was checked for consistence with the Hardy–Weinberg equilibrium (HWE) in all the included studies. The potential publication bias was assessed using Begg's test or Egger's linear regression test by visual examination of the funnel plot, and $p < 0.05$ was regarded as representative of statistically significant publication bias. To estimate the stability of the results of the meta-analysis, case definition influence on the pooled evaluation, one-way sensitivity analyses were performed. All statistical tests were used with STATA version 11.0 (Stata Corporation, College station, TX, USA). All p -values tested were two-tailed.

Results

Figure 1 illustrates the study selection process. There were altogether 754 relevant papers under the search words (PubMed 20, google scholar 523, CBM 25, and CNKI 176). However, 20 articles searched in PubMed can be found in google scholar and 25 articles searched in CBM can also be identified in CNKI, of which 673 were excluded and a total of 26 articles were identified through literature search and screening of title and/or abstract. Of these, 3 articles were not concentrated on PAI-1 gene, 6 articles were not related to PAI-1 gene polymorphism, 2 meta-analysis and 2 studies without clear data. During the extraction of data, 1 article was excluded owing to overlapping data [25], and 2 articles were excluded as the distribution of genotypes deviated from HWE [26, 27]. 10 studies met all inclusion criteria and were included in the meta-analysis [15–24].

Characteristics of included studies

In total, 10 included studies provided data for 2,079 cases and 1,556 controls. Selected details of the individual studies are

Fig. 1 Flow diagram of the study selection process

listed in Table 1. Of all the studies, there were five for Caucasians, four for Asians, and one for mixed ethnicities.

The results of HWE test for the distribution of the genotype in control population are shown in Table 2.

Table 1 Characteristics of studies included in the meta-analysis

First author	Year	Ethnicity	Country	Mean age Case/Control	Diagnostic standard	Case/Control	Genotyping method
Glueck	1999	Caucasian	America	NR/NR	Clinical	144/234	PCR
San Millan				24.6/31.1	Clinical	72/42	PCR-RFLP
Walch	2004	Caucasian	Spain	27.9/28.8	Clinical	106/102	PCR
Zhao	2005	Caucasian	Australia	29/30	ESHRE/ASRM	101/42	PCR
Glueck	2005	Asian	China	NR/NR	ESHRE/ASRM	921/126	PCR
Karadeniz	2006	Caucasian	America	24.3/26.4	ESHRE/ASRM	91/100	PCR
Zhang	2007	Caucasian	Turkey	29.0/30.2	ESHRE/ASRM	167/218	PCR
	2008	Asian	China				
Lin	2009	Asian	China	27.7/27.4	ESHRE/ASRM	126/97	PCR
Sun	2009	Asian	China	26.2/26.1	ESHRE/ASRM	142/107	PCR
Sales	2013	Mixture	Brazil	30.1/27.4	ESHRE/ASRM	209/488	PCR

NR not report

Clinical, meet the mandatory criteria, and the absence of exclusion criteria

NIH, the 1990 National Institute of Health Conference on PCOS and the 1995 Sero Symposium on PCOS

ESHRE/ASRM, the European Society of Human Reproduction/Embryology and the American Society for Reproductive Medicine

Table 2 Characteristics of case–control studies included in a meta-analysis of the link between the PAI-1 4G/5G polymorphism and PCOS

First author	Year	Ethnicity	Case			Control			<i>P</i> for HWE
			4G4G	4G5G	5G5G	4G4G	4G5G	5G5G	
Glueck	1999	Caucasian	38	78	28	47	102	85	0.11
San Millan	2004	Caucasian	18	37	17	8	17	17	0.33
Walch	2005	Caucasian	27	50	29	32	46	24	0.35
Zhao	2005	Asian	58	25	18	16	20	6	0.95
Glueck	2006	Caucasian	258	460	203	29	58	39	0.41
Karadeniz	2007	Caucasian	22	48	21	38	41	21	0.12
Zhang	2008	Asian	30	65	72	15	80	123	0.69
Lin	2009	Asian	55	56	15	23	47	27	0.77
Sun	2009	Asian	60	62	20	26	51	30	0.64
Sales	2013	Mixture	139	67	3	323	145	20	0.48

PAI-1 4G/5G polymorphism and the risk of PCOS

Table 3 lists the main results of this meta-analysis. Since heterogeneity was observed, so random-effects model was used. The results revealed that PAI-1 4G/5G polymorphism may increase the risk of PCOS (allele contrasts, OR=1.366, 95%CI 1.108–1.684, $p=0.003$; recessive genetic model, OR=1.418, 95%CI 1.025–1.961, $p=0.035$; dominant genetic model, OR=1.658, 95%CI 1.258–2.185, $p<0.001$; additive comparison, OR=1.918, 95%CI 1.255–2.934, $p=0.003$) (Fig. 2). In order to explore potential sources of heterogeneity, a stratified analysis was conducted to assess effect estimated in subgroups defined by ethnicity. The results suggested that the polymorphism is associated with increased risk of PCOS in Asian (allele

contrasts, OR=1.836, 95%CI 1.523–2.214, $p<0.001$; recessive genetic model, OR=2.463, 95%CI 1.806–3.361, $p<0.001$; dominant genetic model, OR=1.867, 95%CI 1.397–2.495, $p<0.001$; additive comparison, OR=3.150, 95%CI 2.139–4.639, $p<0.001$) (Fig. 3), but no evidence for any association in Caucasians.

Sensitivity analysis

Sensitivity analysis was performed to estimate the influence of each individual study on the pooled OR by omitting each individual studies. The analysis results showed that no individual study significantly affected the pooled ORs under any genetic models of PAI-1 gene polymorphism (data were not shown).

Table 3 Summary ORs and 95%CI for contrasts in PAI-1 4G/5G polymorphism

SNPs	Contrast	Odds ratio		Model	Heterogeneity	
		OR(95%CI)	P_{OR}		$I^2(\%)$	P_H
PAI-1	4G vs. 5G	1.366(1.108–1.684)	0.003	R	70.4	<0.001
	4G4G vs. 4G5G+5G5G	1.418(1.025–1.961)	0.035	R	70.1	<0.001
	4G4G +4G5G vs. 5G5G	1.658(1.258–2.185)	<0.001	R	48.0	0.044
	4G4G vs. 5G5G	1.918(1.255–2.934)	0.003	R	66.5	0.001
Subgroup						
Caucasian						
	4G vs. 5G	1.151(0.844–1.569)	0.373	R	72.8	0.005
	4G4G vs. 4G5G+5G5G	1.050(0.820–1.346)	0.698	F	54.8	0.065
	4G4G +4G5G vs. 5G5G	1.449(0.959–2.190)	0.078	R	60.3	0.039
	4G4G vs. 5G5G	1.317(0.747–2.323)	0.342	R	68.4	0.013
Asian						
	4G vs. 5G	1.836(1.523–2.214)	<0.001	F	0	0.693
	4G4G vs. 4G5G+5G5G	2.463(1.806–3.361)	<0.001	F	0	0.924
	4G4G +4G5G vs. 5G5G	1.867(1.397–2.495)	<0.001	F	41.9	0.160
	4G4G vs. 5G5G	3.150(2.139–4.639)	<0.001	F	20.0	0.290

OR odds ratio, 95%CI confidence interval, R random-effects model, F fixed-effects model

Fig. 2 The association of PAI-1 polymorphism and PCOS. Meta-analysis for the association between PAI-1 4G/5G polymorphism and PCOS under an additive comparison (4G4G vs. 5G5G) in the total populations using a random-effects model. The *squares* and *horizontal lines* correspond to the study specific OR and 95%CI. The area of the *squares* reflects the weight (inverse of the variance). The *diamond* represents the summary OR and 95%CI

Publication bias

Begg's funnel plot and Egger's linear regression test were used to assess the publication bias. The shape of the funnel plots did not reveal any evidence of obvious asymmetry under all the genetic model of PAI-1 -675 4G/5G polymorphism (Fig. 4). Egger's test also did not display any statistical evidence of publication bias as well.

Discussion

As an important type of statistical method, meta-analysis has been widely used to analyze the results of original studies,

which had similar objectives and research procedures while gaining conflicting results [28]. When compared with a single study, meta-analysis contains a relatively larger sample size which greatly reduces the potential bias, enhances statistical power, and gets more reliable results [29]. Meta-analysis cannot correct the biases of individual studies, but lead to a statistical conclusion with a greater precision and power, and addresses the heterogeneity of the results. Therefore, a significant association between an allele and a specific disease could be resulted from population stratification, or due to errors in identifying and analyzing the relevant genotype information of a population. Furthermore, a true association between an allele and a disease may also owing to the physically allele locus which is currently unclear [30]. Meta-analysis cannot

Fig. 3 The association of PAI-1 polymorphism and PCOS. Meta-analysis for the association between PAI-1 polymorphism and PCOS under regressive genetic model (4G4G vs. 4G5G+5G5G) in Asians using a fixed-effects model. The *squares* and *horizontal lines* correspond to the study specific OR and 95%CI. The area of the *squares* reflects the weight (inverse of the variance). The *diamond* represents the summary OR and 95%CI

Fig. 4 Begg's funnel plot analysis was used to detect publication bias for the additive comparison (4G/4G vs. 5G/5G) of the PAI-1 4G/5G polymorphism. No asymmetry was found as indicated by the *p*-value of the Egger's test

solve all the above problems, but it does help to minimize the chances of errors resulting from randomness if it is conducted properly.

Recent advances in genetics demonstrate that the unusual expression of certain genes might contribute to the development of PCOS [31, 32]. Polymorphism in the PAI-1 gene and its association with PCOS has been studied in different ethnicities over the years. Many previous studies have described significant associations between the PAI-1 gene polymorphism and the risk of PCOS. Evidences showed that polymorphism in the PAI-1 gene can cause an alteration in expression and activity of PAI-1, which have an impact on women's susceptibility to PCOS. However, the precise mechanism between the blood level of PAI-1 and the development of PCOS is unclear. The overproduction of PAI-1, which could result from the polymorphism, may lead to disorders in the ovarian plasminogen-plasmin pathway and anovulation in women with PCOS [33]. But till now the controversial results failed to reveal any significant effects of PAI-1 4G/5G polymorphism on the risk of developing PCOS.

Summary of main results

In this meta-analysis, 10 case-control studies were included with a total of 2,079 PCOS cases and 1,556 healthy controls. When all the eligible studies were pooled into this meta-analysis, the results showed that PAI-1 4G/5G polymorphism was associated with increased risk of PCOS, suggesting the polymorphism may be a risk factor for PCOS. In order to explore potential source of heterogeneity, we also performed subgroup analysis based on ethnicity. Our results demonstrated that PAI-1 4G/5G polymorphism was significantly associated with increased risk of PCOS among Asian populations. But we found no statistically significant associations between

the polymorphism and PCOS risk in Caucasians. Although ethnic diversity in susceptibility to PCOS is well understood, the molecular basis is not fully investigated. One likely hypothesis for this difference was that large difference in common single nuclear polymorphisms that could affect the risk of PCOS are mostly due to natural selection and genetic drift [34]. No significant publication bias was detected in this meta-analysis, indicating our statistically robust results. Our results are partially consistent with several previous studies, suggesting that polymorphism of PAI-1 gene may be linked with an increased risk of PCOS, and may be a useful biomarker for predicting an individual's susceptibility to PCOS.

Implication of the findings

In Asians, the PAI-1 4G/4G genotype may account for increased cases of PCOS when compared with heterozygous genotype of the PAI-1. However, this does not indicate this homozygous genotype will increase the risk of developing PCOS in an individual. Most pregnant women with the PAI-1 4G/4G genotype will not develop PCOS and most women suffering from PCOS do not carry the PAI-1 4G/4G genotype. The results so far did not reveal close relationship between PAI-1 gene polymorphism and the risk of PCOS. The uncertain link between the two factors doesn't guarantee the PAI-1 gene polymorphism to be used as a target gene for the prediction and diagnosis of PCOS. Even taken other possible genetic risk factors, including those related to fibrinolysis or thrombogenesis, into consideration, use PAI-1 gene polymorphism to predict the risk of PCOS remains weak and imprecise compared with already established clinical or demographic data, such as family history of PCOS. Furthermore, the consequences of interactions between genetic thrombophilia tendencies and characteristics such as obesity

and smoking that are associated both with PCOS and increased PAI-1 generation remain to be defined.

Limitations of the study

Similar to other meta-analyses, this analysis also bears some shortages and limitations. First, the sample size of this meta-analysis is relatively small, which may not have abundant statistical power in assessing the role of PAI-1 polymorphism in the development of PCOS. Second, a language bias may have existed because this meta-analysis contained only English and Chinese articles due to database limitations. Some studies in coincidence with the inclusion criteria in other languages published in specific journals might not be identified and included in this meta-analysis. Besides, all included articles were published studies, and unpublished studies that had null results were missed, which also might bias the results. Third, although no publication biases were found by observing symmetry of funnel plot, Begg's and Egger's test, pooled analyses of original data could not be prevented from possible publication bias. Fourth, significant heterogeneity was observed, which may affect the statistical power of this meta-analysis. Some factors that are highly likely to be sources of heterogeneity are taken into consideration. The first candidate is various different definitions used in different studies, because previous studies used different clinical standard. Another candidate is the inappropriate criteria of controls entered into the case-control studies. There is also the confounding of interaction with other outcomes associated with the metabolic syndrome. Since PCOS is being considered as another phenotypic expression related to the metabolic syndrome such as dislipidemia, obesity, hypertension, and diabetes, including or excluding controls suffering from above-mentioned disease would produce heterogeneity. Interestingly, PAI-1 polymorphism has been also studied for predisposition to obesity, diabetes, and other syndromes related to PCOS [35–37].

Conclusions

The present meta-analysis indicates that PAI-1 polymorphism may be a potential risk factor contributing to PCOS, especially among Asian populations. Thus we may use this gene polymorphism as a useful biomarker for predicting an individual's susceptibility to PCOS. However, due to the limitations mentioned above, more and more detailed studies are required to confirm our findings further.

Acknowledgments This work received no specific grant from any funding agency in the commercial, or public sectors. We really appreciated the patience and suggestions from the reviewers and editors.

Conflict of interest We declare that we have no conflict of interest.

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