A meta-analysis on the association between PPAR- γ Pro12Ala polymorphism and polycystic ovary syndrome

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Abstract

Purpose To investigate the influence of the peroxisome proliferator activated receptor gamma (PPAR- γ) Pro12Ala polymorphism on the susceptibility of polycystic ovary syndrome (PCOS) and body mass index (BMI), fast insulin levels, homeostasis model assessment of insulin resistance (HOMA-IR) in PCOS patients.

Methods PubMed, EMBASE, MEDLINE and CENTRAL databases were searched to identify eligible studies. We then

Capsule This meta-analysis indicated that Ala variant would decrease the risk of PCOS; X/Ala genotype lowered BMI and fast insulin levels, but had no impact on HOMA-IR in PCOS patients.

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conducted a meta-analysis to examine the association between Pro12Ala polymorphism and PCOS.

Results Seventeen eligible studies, including 2,149 patients and 2,124 controls were enrolled in this meta-analysis. Pro12Ala polymorphism was significantly associated with the susceptibility of PCOS (odds ratio [OR] 0.74, 95 % confidence interval [CI] [0.61, 0.90] for allele; OR 0.70, 95 % CI [0.57, 0.86] for genotype). In the European subgroup of PCOS, the X/Ala genotype was associated with lower BMI (mean difference [MD] −1.08, 95 % CI [−2.08, −0.09]) and fast insulin levels (MD −19.82, 95 % CI [−34.07, −5.58]). However, this polymorphism did not display an impact on HOMA-IR in PCOS patients.

Conclusions Ala variant would decrease the risk of PCOS and result in lower BMI and fast insulin levels in a European population, but had no impact on HOMA-IR in PCOS patients. Further studies are required to elucidate these associations more clear.

Keywords Polycystic ovary syndrome · PPAR- γ · Meta-analysis · Body mass index · Fast insulin · Insulin sensitivity

Introduction

The polycystic ovary syndrome (PCOS) is a common endocrinopathy affecting an estimated 5–10 % of adult women and diagnosed frequently during adolescence [1–3]. PCOS is primarily characterized by anovulation, hyperandrogenism, insulin resistance and polycystic ovaries by ultrasonography in most patients [4, 5]. Although the pathogenesis of PCOS remains elusive, both the genetic and environmental factors are involved in the development of this condition [6]. Evidence from different familial and monozygotic twins' studies indicates that the genetic factors play an important

role [7–9]. To date, a large number of candidate genes have been studied concerning their potential roles in the etiology of PCOS, such as *CYP11a*, *CYP17*, *CYP19*, *LHR*, *FSHR*, *IRS-1/2*, *INSR*, and *PPAR-γ*. Among them, the peroxisome proliferator activated receptor gamma (*PPAR-γ*) gene was presumed to be involved in this disease.

PPAR-γ is a ligand-activated transcription factor that belongs to the nuclear hormone receptor superfamily. It is expressed in adipose tissue, macrophages, intestines, ovaries and plays a crucial role in the regulation of energy storage, insulin sensitivity, adipocyte differentiation and lipid metabolism [10]. *PPAR-γ* was demonstrated to be up-regulated in the ovaries of PCOS patients [11], which was consistent with other animal studies [12–14]. Recently, many researchers focused on the association between Pro12Ala polymorphism (rs1801282) in *PPAR-γ* and PCOS, which may facilitate to elucidate the pathogenesis of this condition. However, the results were inconsistent.

To deal with these contradictory issues, we conducted a meta-analysis to examine the influence of the Pro12Ala polymorphism on the susceptibility of PCOS and body mass index (BMI), fast insulin levels, homeostasis model assessment of insulin resistance (HOMA-IR) in PCOS patients.

Materials and methods

Literature search and data extraction

A literature search of the PubMed, EMBASE, MEDLINE and Cochrane Central Register of Controlled Trials (CENTRAL) databases was conducted to identify all the articles about the association between *PPAR-γ* Pro12Ala polymorphism and PCOS before November 2011, using the keywords “(polycystic ovary syndrome or PCOS) and (peroxisome proliferator-activated receptor or PPAR or PPARgamma or PPARγ) and (gene or polymorphism or variant or mutation)”. Besides, all references cited in these studies and previously published review articles were reviewed to identify additional eligible researches. The search was not restricted by language. Eligible studies must meet the following criteria: (1) the study had at least one of following data: (I) genotype or allele frequency, (II) BMI, (III) fast insulin, (IV) HOMA-IR; (2) for the comparison of allele and genotype, eligible study should use an unrelated case-control design, and genotype distributions in the control group were consistent with Hardy–Weinberg equilibrium ($P > 0.01$). Two authors (J.H. and J.L.) independently screened the titles and abstracts to exclude obviously irrelevant reports. All full text copies of studies meeting

the inclusion criteria were retrieved and examined for the eligibility. Disagreements resolved by discussion between the authors.

Data extraction

Another two authors (L.W. and F.L.) independently extracted data using the checklist for data collection adapted from the Cochrane Handbook [15]. All disagreements about eligibility were resolved during a consensus with a third reviewer (X.L.). The data extracted included: author, year of publication, ethnicity of the population. For binary outcomes, the information on the numbers of cases and controls, allele and genotype distributions of Pro12Ala polymorphism was extracted. For continuous outcomes (BMI, fast insulin and HOMA-IR), information on the numbers of patients with different genotypes, mean values and standard differences (SD) was also extracted. The outcomes of fast insulin were converted from $\mu\text{IU/ml}$ to pmol/l (where needed, $\text{pmol/l} = \mu\text{IU/ml}$ multiply 6.965). The ethnic descents were categorized as European, Asian, African and others.

Statistical analysis

The effect measure of allele and genotype was odds ratio (OR) and its corresponding 95 % confidence interval (CI). For BMI, fast insulin and HOMA-IR, mean difference (MD) and 95 % CI were adopted. Since the Ala/Ala homozygote is extremely rare, Pro/Ala and Ala/Ala were referred to X/Ala for the pooled analysis. The association between the risk allele and PCOS was evaluated by comparing Ala allele to Pro allele. Besides, the impact of Pro12Ala polymorphism on BMI, fast insulin and HOMA-IR was also examined (patients with X/A genotype versus patients with Pro/Pro genotype). Forest plots were generated by using RevMan5.1 software (The Nordic Cochrane Centre, The Cochrane Collaboration, 2011).

Between-study heterogeneity was tested by Q-statistic test and I^2 metric [16, 17]. The I^2 metric is independent of the number of studies in the meta-analysis, and ranges between 0 % and 100 % ($I^2 < 25$ %: low heterogeneity; $25 \% < I^2 < 50$ %: moderate heterogeneity; $50 \% < I^2 < 75$ %: high heterogeneity; $I^2 > 75$ %: extreme heterogeneity). If there was heterogeneity between studies ($I^2 > 50$ %), random-effect model was adopted. In the absence of heterogeneity, both the random-effect and fixed-effect models were appropriate.

Egger's test and Begg's test were undertaken to detect publication bias using Stata software 11.0 (Stata Corporation, College Station, Texas). Log (OR)/the standard error of log (OR) and MD/the standard error of MD were adopted to yield funnel plots. Hardy–Weinberg equilibrium was

examined by the chi-square test with a web program (<http://ihg.gsf.de/cgi-bin/hw/hwa1.pl>).

Results

Quality control

A total of 82 studies were identified. Only 22 articles were concerned the influence of Pro12Ala polymorphism on the susceptibility, BMI, fast insulin and insulin resistance of PCOS [18–39]. Among them, five articles were excluded for: (1) participants derived from the same cohort [20, 21, 30]; (2) the study did not use an unrelated case–control design [26]; (3) genotype distribution of controls was not consistent with Hardy–Weinberg equilibrium [32]. Ultimately, 17 studies (including 2,149 patients and 2,124 controls) were enrolled in this meta-analysis. The data extracted were listed in Tables 1 and 2.

The power of our sample size to detect the correlation between this SNP and PCOS achieved 100 % ($\alpha=0.05$, effect size index=0.1). For the continuous data, the power of our sample size to detect the association between this

SNP and BMI, fast insulin and HOMA-IR in PCOS patients was 82.0 %, 70.5 % and 76.8 %, respectively ($\alpha=0.05$, effect size index=0.2).

Publication bias and heterogeneity

No publication bias was detected in all the five comparisons (Fig. 1). The heterogeneity examined is listed as follows: $P=0.79$, $I^2=0$ % for allele; $P=0.36$, $I^2=9$ % for genotype; $P=0.13$, $I^2=33$ % for BMI; $P<0.01$, $I^2=65$ % for fast insulin; $P<0.01$, $I^2=73$ % for HOMA-IR. According to the I^2 metric, the fixed-effect model was used for the allele, genotype and BMI comparisons. The random-effect model was adopted for the other two analyses.

Meta-analysis

Significant association was found between Pro12Ala and the susceptibility of PCOS (OR 0.74, 95 % CI [0.61, 0.90], $P=.002$ for allele; OR 0.70, 95 % CI [0.57, 0.86], $P=.0006$ for genotype). This association also existed in the European subgroup (OR 0.72, 95 % CI [0.58, 0.89], $P=.002$ for allele; OR 0.68, 95 % CI [0.54, 0.84], $P=.0005$ for genotype) (Fig. 2).

Table 1 Characteristics and Allele, genotype distributions of the studies enrolled in this meta-analysis

Author(year)	Country	Criteria for PCOS	Case/ Control(n)	Allele				Genotype			
				Cases		Controls		Cases		Controls	
				Ala	Pro	Ala	Pro	X/Ala	Pro/Pro	X/Ala	Pro/Pro
Hara (2002) [18]	USA	NICHD criteria	124/N/A	21	227	N/A	N/A	21	103	N/A	N/A
Korhonen (2003) [19]	Finland	Anovulation and polycystic ovaries	135/115	34	236	44	186	31	104	39	76
Orio (2004) [22]	Italy	NICHD criteria	120/120	7	233	5	235	7	113	5	115
Tok (2005) [23]	Turkey	Hyperandrogenism, oligoamenorrhoea and polycystic ovaries	60/60	6	114	13	107	6	54	13	47
Hahn (2005) [24]	Germany	NICHD criteria	102/104	N/A	N/A	N/A	N/A	23	79	24	80
Haap (2005) [25]	Germany	Rotterdam criteria	53/546	11	95	145	947	10	43	139	407
Wang (2006) [27]	China	Rotterdam criteria	201/147	18	384	12	282	18	183	11	136
Yilmaz (2006) [28]	Turkey	Rotterdam criteria	100/100	15	185	22	178	15	85	22	78
Antoine (2007) [29]	USA	NICHD criteria	267/168	56	478	39	297	54	213	34	134
Dragojevič (2008) [39]	Slovenia	NICHD criteria	69/N/A	19	119	N/A	N/A	19	50	N/A	N/A
Koika (2009) [31]	Greek	NICHD criteria	156/56	21	291	10	102	20	136	8	48
Xita (2009) [33]	Greek	NICHD criteria	180/140	30	330	N/A	N/A	30	150	N/A	N/A
San-Millán (2010) [34]	Spain	NICHD criteria	161/113	N/A	N/A	N/A	N/A	20	141	21	92
Chae (2010) [35]	Korea	Rotterdam criteria	184/256	15	353	29	483	13	171	26	230
Christopoulos (2010) [36]	Greek	Rotterdam criteria	183/148	20	346	19	277	17	166	17	131
Bidzińska-Speichert (2011) [37, 38]	Poland	Rotterdam criteria	54/51	25	83	27	75	19	35	21	30

N/A not available; NICHD National Institute of Child Health and Human Development

For the comparisons of BMI and fast insulin, the results of total group analysis indicated that no association existed (MD -0.45 , 95 % CI $[-1.24, 0.33]$, $P=.26$; MD -13.03 , 95 % CI $[-29.85, 3.78]$, $P=.13$, respectively). However, these parameters were significantly lower in patients carried X/Ala genotype in the European subgroup (MD -1.08 , 95 % CI $[-2.08, -0.09]$, $P=.03$ for BMI; MD -19.82 , 95 % CI $[-34.07, -5.58]$, $P=.006$ for fast insulin). Pro12Ala polymorphism did not display an impact on HOMA-IR in PCOS patients neither in the total group (MD -0.23 , 95 % CI $[-0.67, 0.22]$, $P=.31$) nor in the European subgroup (MD -0.32 , 95 % CI $[-0.77, 0.14]$, $P=.17$) (Fig. 3).

Discussion

It has been demonstrated that both ovarian and metabolic dysfunctions play critical roles in the pathophysiology of PCOS [40], but the underlying mechanism is still elusive. In the last decade, tens of studies were focused on the association between PPAR- γ Pro12Ala polymorphism and PCOS. But the results were varied and no convincing evidence had

been provided. Though San-Millán et al. [34] had done an earlier meta-analysis, only 9 studies were enrolled and no analysis of BMI was conducted. In this meta-analysis, 17 researches were eligible and were stratified into two subgroups (European and Asian) to avoid the heterogeneity of ethnicity. We found Pro12Ala polymorphism was significantly associated with the susceptibility of PCOS either in the total group or in the European subgroup. The common Pro allele would increase the risk of PCOS, whereas the Ala variant would be protective against this disease, which was consistent with the meta-analysis of San-Millán. Besides, BMI and fast insulin levels were showed to be lower in patients carried X/Ala genotype in European subgroup. However, no association between this polymorphism and HOMA-IR was yielded.

Though PCOS is clinically heterogeneous, obese and insulin resistance are very common and play a central pathogenic role. Since PPAR- γ is mainly expressed in adipose tissue, the Pro12Ala polymorphism was originally studied to examine its potential impact on obesity. A meta-analysis comprising 32,000 non diabetic subjects revealed positive correlation between this polymorphism and BMI in the

Table 2 Parameters of BMI, HOMA-IR and fasting insulin in patients with PCOS

First author	BMI (kg/m ²)	HOMA-IR (mUmM/l)	Fast insulin (pmol/l)	n
Hara	33.9 $\pm$ 8.7	5.18 $\pm$ 6.09	132.0 $\pm$ 123.7	21
	36.1 $\pm$ 8.1	6.54 $\pm$ 5.48	165.0 $\pm$ 121.7	103
Orio	30.2 $\pm$ 5.4	4.6 $\pm$ 0.4	149.7 $\pm$ 47.4	7
	30.3 $\pm$ 5.5	4.4 $\pm$ 0.6	153.9 $\pm$ 45.3	113
Tok	27.33 $\pm$ 4.82	1.88 $\pm$ 1.2	64.7 $\pm$ 38.9	6
	24.56 $\pm$ 4.78	2.91 $\pm$ 1.18	97.8 $\pm$ 38.4	54
Hahn	30.0 $\pm$ 7.6	2.9 $\pm$ 1.9	70.0 $\pm$ 52.6	22
	30.0 $\pm$ 9.4	4.5 $\pm$ 4.1	110.0 $\pm$ 100.6	79
Wang	22.6 $\pm$ 2.9	N/A	N/A	18
	22.4 $\pm$ 3.8	N/A	N/A	183
Yilmaz	21.78 $\pm$ 6.65	2.56 $\pm$ 0.62	102.7 $\pm$ 42.8	15
	24.7 $\pm$ 6.78	3.55 $\pm$ 1.50	133.4 $\pm$ 50.1	85
Antoine	34.5 $\pm$ 9.9	2.74 $\pm$ 1.68	174.5 $\pm$ 127.9	54/40 ^a
	34.9 $\pm$ 9.1	2.31 $\pm$ 1.36	147.2 $\pm$ 110.6	213/148 ^a
Dragojevič	25.2 $\pm$ 5.2	2.18 $\pm$ 1.67	77.3 $\pm$ 57.8	19
	28.9 $\pm$ 7.2	2.49 $\pm$ 1.53	84.3 $\pm$ 48.8	50
Koika	24.4 $\pm$ 3.49	2.08 $\pm$ 1.19	63.5 $\pm$ 30.8	20
	25.76 $\pm$ 6.69	2.35 $\pm$ 2.56	99.4 $\pm$ 42.1	136
Xita	26.6 $\pm$ 6.9	3.3 $\pm$ 2.2	N/A	30
	28.2 $\pm$ 7.6	3.2 $\pm$ 2.4	N/A	150
Chae	24.1 $\pm$ 5.0	3.8 $\pm$ 2.0	120.5 $\pm$ 57.0	13
	22.0 $\pm$ 4.8	2.9 $\pm$ 2.2	88.5 $\pm$ 55.7	171
San-Millán	N/A	3.9 $\pm$ 3.6	114 $\pm$ 99	20
	N/A	3.2 $\pm$ 2.4	98 $\pm$ 68	141
Bidzińska-	28.50 $\pm$ 8.48	N/A	N/A	19
Speichert	26.02 $\pm$ 6.69	N/A	N/A	35

Values are mean $\pm$ SD

For each study, the parameters of patients with X/Ala and Pro/Pro genotype are expressed in the first and second lines, respectively

^aFigure before the slash represents the number of patients for “BMI”, that after the slash represents the number of patients for “HOMA-IR” and “fast insulin”

subgroup of European and Asian, though no correlation was found in global comparison [41]. In another study, Masud et al. [42] reported Ala carriers had higher BMI than none-carriers in the subgroup of $\text{BMI} \geq 27 \text{ kg/m}^2$; no correlation was found in total group and in the subgroup of $\text{BMI} < 27 \text{ kg/m}^2$. However, our meta-analysis indicated that PCOS patients with Ala variant had lower BMI only in European subgroup and was not related to the degrees of BMI.

Deeb et al. [43] found that Ala variant of PPAR- γ displayed lower transactivation capacity. This minor allele may lead to less efficient stimulation of PPAR- γ target genes and predispose to lower levels of adipose tissue mass accumulation, which may be responsible for improved insulin sensitivity. Tönjes et al. [41] reported that fasting insulin levels was significantly lower in nondiabetic individuals with Ala/Ala genotype compared with those subjects with Pro/Pro genotype, while the pooled X/Ala genotype was associated with significantly decreased fast glucose and HOMA-IR only in the obese subgroup ($\text{BMI} > 30 \text{ kg/m}^2$). It seemed that the Pro12Ala polymorphism had an apparent effect on HOMA-IR only in markedly obese individuals. However, since most the studies enrolled in our meta-analysis had no

information about the degrees of BMI, we failed to make this comparison in an obese subgroup.

In conclusion, this meta-analysis indicated that Ala allele would decrease the susceptibility of PCOS and result in lower BMI and fast insulin levels in European patients with PCOS. No correlation between this polymorphism and HOMA-IR was detected. This conclusion supported the idea that Pro12Ala polymorphism played a role in the etiology of PCOS and implied that populations without Ala allele may need to pay more attention to the early prevention and diagnosis of this disease. However, there are some limitations that should not be neglected in this study. Firstly, there are high heterogeneities in the meta-analysis of fast insulin and HOMA-IR, which may decrease the reliability of the results. PCOS is a heterogeneous disease, but we did not classify our patients due to the clinical phenotypes. Secondly, since most of the original studies derived from European population and the metabolic parameters seemed to be different in PCOS patients with different ethnic descents [44–47], our findings may not be extended to other descent. Thirdly, due to the limited numbers of original researches enrolled, our results are not conclusive. Further studies are needed to elucidate these associations more clear.

Fig. 1 Begg's funnel plots for the test of publication bias. **a–d** represent the funnel plots for comparisons of genotype, BMI, fast insulin and HOMA-IR in order

Fig. 2 Forest plots of the association between Pro12Ala polymorphism and PCOS. Weight, OR and its corresponding 95 % CI of each study are indicated. **a** Association of the alleles and PCOS. *Events* and

Total indicate the numbers of Ala and Pro allele, respectively. **b** Association of the genotypes and PCOS. *Events* and *Total* indicate the numbers of X/Ala and Pro/Pro genotypes, respectively

Fig. 3 Forest plots of the association between Pro12Ala polymorphism and BMI (a), fast insulin (b), HOMA-IR (c) in PCOS patients. Weight, MD and its corresponding 95 % CI of each study are indicated. *Total* indicates the numbers of patients in each study

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Conflict of interest The authors have no conflicts of interest to declare.

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