Association between the Pro12Ala polymorphism of PPAR-γ gene and the polycystic ovary syndrome: A meta-analysis of case–control studies

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A B S T R A C T

Several studies have been conducted to examine the association between PPAR-γ2 Pro12Ala polymorphism and polycystic ovary syndrome (PCOS), but the results remain inconsistent. To make a more precise estimation of the relationship, a meta-analysis was performed. In the current meta-analysis, a total of 17 case–control studies, including 2176 cases and 2373 controls, were selected. Odds ratios (ORs) and 95% confidence intervals (CIs) for Pro/Ala+Ala/Ala versus Pro/Pro genotype in all population and different nationality groups, and homeostasis model assessment-insulin resistance (HOMA-IR) of different genotype were evaluated. In the overall analysis, significant association between PPAR-γ2 Pro12Ala polymorphism and reduced risk of PCOS was observed (OR = 0.75; 95%CI, 0.62–0.91; p = 0.003). Stratified analysis showed that significantly strong association was presented only in Europeans (OR = 0.74; 95%CI, 0.60–0.90; p = 0.003), but not in Asians (OR = 0.86; 95%CI, 0.51–1.43; p = 0.56). Additionally, carrying the Ala12 allele was not associated with HOMA-IR in PCOS patients (OR = 0.29; 95%CI, 0.82–0.24; p = 0.29). This meta-analysis supported that PPAR-γ2 Pro12Ala polymorphism was capable of reducing polycystic ovary syndrome risk in Europeans, but not in Asians.

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1. Introduction

Polycystic ovary syndrome (PCOS) — one of the most common endocrine metabolic disorders among women of reproductive age, affects 4%–12% of this population (Setji and Brown, 2006; Sheehan, 2004). PCOS is characterized by hyperandrogenism, menstrual irregularity, chronic anovulation, and associated with insulin resistance (IR), pancreatic β-cell dysfunction, impaired glucose tolerance, type 2 diabetes, dyslipidemia, and visceral obesity (Tan et al., 2008; Vigil et al., 2007). Owing to this fact, the role of many genes involved in insulin action and secretion, energy metabolism and adipogenesis has been investigated in order to elucidate the pathogenesis of PCOS. Although many candidate genes have been studied, definite susceptible genes for PCOS remain still elusive (Deligeoroglou et al., 2009; Jakubowski, 2005; Wang et al., 2009).

Peroxisome proliferator–activated receptor (PPAR) γ is coded at chromosomal region 3p25 and is a ligand activated transcription factor that belongs to the nuclear hormone receptor superfamily, activated by certain fatty acids, rostanoids, and thiazolidinediones (TZDs), a class of insulin-sensitizing anti-diabetic agents (Stumvoll and Häring, 2002; Wang et al., 2006). It was reported that PPAR-γ variants are associated with PCOS in different ethnic backgrounds (Gu and Baek, 2009; Hahn et al., 2005; Yilmaz et al., 2006). The studies conducted to date in PCOS and gene polymorphism have been mostly confined to allele of the Pro12Ala in the PPAR-γ exon 2 (Unluturk et al., 2007). For PPAR-γ2, C→G substitution leads change of proline to alanine. Several studies have shown that the Pro12Ala polymorphism in the PPAR-γ2 is associated with a reduced risk for PCOS, and the effect of this polymorphism is probably mediated by improved insulin sensitivity, evaluated by decreased homeostasis model assessment-insulin resistance index (HOMA-IR). Moreover, the improvement in insulin sensitivity during treatment with TZDs is paralleled by amelioration of PCOS manifestations (Azziz et al., 2001; Hará et al., 2002; Narsing Rao et al., 2009). However, some studies have suggested that the Pro12Ala polymorphism of the PPAR-γ2 gene is not associated with the polycystic ovary syndrome (Chae et al., 2010; Christopoulos et al., 2010; Xita et al., 2009). We reported the findings of a meta-analysis of case–control trials examining the association between PPAR-γ2 Pro12Ala polymorphism and PCOS based on various ethnic groups from Asians and Europeans.
2. Methods

2.1. Search strategy

We systematically searched PubMed to identify studies to our research question in English language. We used Medical Subject Headings in the following search strategy: ‘polycystic ovary syndrome or PCOS’ and ‘peroxisome proliferator–activated receptor γ or PPAR-γ or peroxisome proliferator–activated receptor gamma or PPAR-gamma’ with either ‘gene’, ‘polymorphism’, ‘variant’, ‘mutation’, ‘allele’ or ‘genotype’. In addition, we reviewed reference lists of relevant articles to identify any additional studies overlooked by our research. Two reviewers independently assessed and abstracted pertinent data from trials in duplicate using a standardized, predefined criteria.

2.2. Selection criteria

Included trials met each of the following criteria: (1) the study was case-controlled trial; (2) the number of subjects with each allele or genotype was reported in cases and controls; (3) OR and 95% confidence intervals (CI) or raw data for each study can be provided. Exclusion criteria: (1) we were unable to obtain adequate details of study methodology or results from the article or study investigator; (2) When the same study group were included in more than one publication, data incomplete research was excluded; and (3) The studies that do not conform to the Hardy–Weinberg equilibrium in control group of each study were excluded (Ziegler et al., 2011). We also conducted a meta-analysis to identify HOMA-IR that probably contributed to heterogeneity.

2.3. Data abstraction

Independently and in duplicate, two authors (H. Zhang and Y. Bi) of this article abstracted the data. Any discrepancies were resolved through discussion and arbitration by a third author if necessary. We abstracted first author’s name, publication year, sample size, ethnicity of the study population, diagnosis criteria of PCOS, characteristics of the cohorts, the number of cases and controls with each of three genotype, allele frequencies and the most completely adjusted estimate (OR and 95% CI). Furthermore, information on Hardy–Weinberg equilibrium test was also tracked or calculated manually if unavailable.

2.4. Statistical analysis

We calculated OR and 95% CIs between Pro12Ala polymorphism and PCOS, Subjects who carry the variant genotypes (Pro/Ala + Ala/Ala) in spite of homozygous and heterozygous were calculated as a whole and were compared with the Pro/Pro genotype. The Z-test was used to determine the statistical significance of the pooled OR, and $p<0.05$ was considered statistically significant. We performed stratified analysis on ethnicity (Europeans, Asians and Americans) separately. Additionally, meta-analysis was conducted between carrying Ala12 alleles of Pro12Ala variant and HOMA-IR in PCOS patients.

We estimated the degree of heterogeneity among trial results using Q test ($p<0.10$ was considered significant) and the I² statistic, which quantifies the proportion of the variability in trial results that is due to heterogeneity rather than chance and uses a value greater than 50% to indicate meaningful heterogeneity (Higgins and Thompson, 2002). If significant heterogeneity existed, the pooled OR estimate of each study was calculated by the random-effects model, otherwise the fixed-effects model was used (Desimini and Laird, 1986). We evaluated the presence of publication bias by means of visual inspection of the funnel plot and using the fail-safe number (Nfs) with the significance set at 0.05. Calculation formula is $N_{fs0.05} = (\sum Z/1.64)^2 - k$ (k is the number of articles included in the meta-analysis) (Niu et al., 2010). The meta-analysis was conducted with open-source Review Manager software (version 4.2.10) (http://www.cc-ims.net/RevMan/download/revman-4).

3. Results

3.1. Literature search

Through the electronic database search, we initially identified 45 potentially eligible studies after the primary literature search, 25 articles were excluded after screening the titles and abstracts, because they were either review articles or irrelevant to our current study. The left nineteen articles were for full-text review. Further, three articles, in which one lacked control (Hara et al., 2002), other two (Orio et al., 2003; San-Millán and Escobar-Morreale, 2010) share the same authors, were excluded. Therefore, there were a total of 17 studies including 2176 cases and 2373 controls in the current meta-analysis on Pro12Ala polymorphism in relation to susceptibility to PCOS (Fig. 1). The genotypic distributions in the control group of each study were in agreement with Hardy–Weinberg equilibrium with $\chi^2<3.84$ ($p>0.05$) (Table 1).

3.2. Study characteristics

Table 1 provides the characteristics of the 17 case–control trials that met inclusion criteria. Trials were conducted in a diverse array of countries, most often at a single center. Study size ranged from 105 to 599 patients. Among the studies, 3 studies were Asian descendents (Chae et al., 2010; Gu and Baek, 2009; Wang et al., 2006), 2 were European origin non-Hispanic Caucasians from America (Antoine et al., 2007; Guzman et al., 2007) and the remaining 12 were European descendents (Biddini-Spiechert et al., 2011; Christopoulos et al., 2010; Haap et al., 2005; Hahn et al., 2005;}

![Fig. 1. Study selection for inclusion in the meta-analysis of Pro12Ala polymorphism in PCOS.](http://www.cc-ims.net/RevMan/download/revman-4)
Table 1
Characteristics of studies included in the meta-analysis.

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Ethnicity (country)</th>
<th>PCOS criteria</th>
<th>Characteristics of cohort</th>
<th>Cases/controls</th>
<th>Genotypes (cases&lt;sup&gt;a&lt;/sup&gt;/controls&lt;sup&gt;b&lt;/sup&gt;)</th>
<th>HWE&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Korhonen</td>
<td>2003</td>
<td>European (Finland)</td>
<td>Other criteria</td>
<td>Hospital-based study</td>
<td>135</td>
<td>104/28/3</td>
<td>0.207</td>
</tr>
<tr>
<td>Orio</td>
<td>2004</td>
<td>European (Italy)</td>
<td>NIH 1990</td>
<td>Hospital-based study</td>
<td>120</td>
<td>115/7/0</td>
<td>0.0026</td>
</tr>
<tr>
<td>San-Millán</td>
<td>2004</td>
<td>European (Spain)</td>
<td>NIH 1990</td>
<td>Hospital-based study</td>
<td>120</td>
<td>115/5/0</td>
<td>0.113</td>
</tr>
<tr>
<td>Hahn</td>
<td>2005</td>
<td>European (Germany)</td>
<td>NIH 1990</td>
<td>Population-based study</td>
<td>102</td>
<td>79/22/1</td>
<td>0.343</td>
</tr>
<tr>
<td>Haap</td>
<td>2005</td>
<td>European (Germany)</td>
<td>Rotterdam 2004</td>
<td>Population-based study</td>
<td>53</td>
<td>43/9/1</td>
<td>2.621</td>
</tr>
<tr>
<td>Tok</td>
<td>2005</td>
<td>European (Turkey)</td>
<td>Other criteria</td>
<td>Hospital-based study</td>
<td>69</td>
<td>54/6/0</td>
<td>0.164</td>
</tr>
<tr>
<td>Wang</td>
<td>2006</td>
<td>Asian (China)</td>
<td>Rotterdam 2004</td>
<td>Hospital-based study</td>
<td>201</td>
<td>183/18/0</td>
<td>0.665</td>
</tr>
<tr>
<td>Yilmaz</td>
<td>2006</td>
<td>European (Turkey)</td>
<td>Rotterdam 2004</td>
<td>Hospital-based study</td>
<td>147</td>
<td>136/10/1</td>
<td>0.285</td>
</tr>
<tr>
<td>Antoine</td>
<td>2007</td>
<td>European origin (Los Angeles)</td>
<td>NIH 1990</td>
<td>Population-based study</td>
<td>267</td>
<td>213/52/2</td>
<td>2.589</td>
</tr>
<tr>
<td>Guzmán</td>
<td>2007</td>
<td>European origin (Chile)</td>
<td>NIH 1990</td>
<td>Hospital-based study</td>
<td>50</td>
<td>42/7/1</td>
<td>0.679</td>
</tr>
<tr>
<td>Knebel</td>
<td>2008</td>
<td>European (Germany)</td>
<td>NIH 1990</td>
<td>Not available</td>
<td>21</td>
<td>17/4</td>
<td>2.579</td>
</tr>
<tr>
<td>Gu</td>
<td>2009</td>
<td>Asian (Korea)</td>
<td>Rotterdam 2004</td>
<td>Hospital-based study</td>
<td>238</td>
<td>222/16/0</td>
<td>0.157</td>
</tr>
<tr>
<td>Xita</td>
<td>2009</td>
<td>European (Greece)</td>
<td>NIH 1990</td>
<td>Population-based study</td>
<td>125</td>
<td>125/0/0</td>
<td>0.003</td>
</tr>
<tr>
<td>Koika</td>
<td>2009</td>
<td>European (Greece)</td>
<td>Other criteria</td>
<td>Not available</td>
<td>180</td>
<td>150/30/0</td>
<td>2.867</td>
</tr>
<tr>
<td>Christopoulos</td>
<td>2010</td>
<td>European (Greece)</td>
<td>Rotterdam 2004</td>
<td>Population-based study</td>
<td>183</td>
<td>166/14/3</td>
<td>1.968</td>
</tr>
<tr>
<td>Chae</td>
<td>2010</td>
<td>Asian (Korea)</td>
<td>Rotterdam 2004</td>
<td>Population-based study</td>
<td>184</td>
<td>171/11/2</td>
<td>1.484</td>
</tr>
<tr>
<td>Bidzińska-Speichert</td>
<td>2011</td>
<td>European: Poland</td>
<td>Rotterdam 2004</td>
<td>Population-based study</td>
<td>54</td>
<td>35/13/5</td>
<td>2.079</td>
</tr>
</tbody>
</table>

<sup>a</sup> Top row.
<sup>b</sup> Bottom row.
<sup>c</sup> HWE: Chi-square value of Hardy–Weinberg equilibrium test in controls.

Knebel et al., 2008; Koika et al., 2009; Korhonen et al., 2003; Orio et al., 2004; San Millán et al., 2004; Xita et al., 2009; Yilmaz et al., 2006. Insulin resistance was considered as the potential pathogenesis of PCOS in most of the studies. In several studies, women with all Ala carriers (Pro/Ala + Ala/Ala) had lower homeostasis model assessment insulin resistance (HOMA-IR) compared with Pro/Pro women in PCOS (Antoine et al., 2007; Chae et al., 2010; Orio et al., 2004; Tok et al., 2005; Yilmaz et al., 2006), so we attempted to take this into account by meta-analysis, however, we failed to find any significant results in PCOS patients.

3.3. The association of the PPAR-γ2 Pro12Ala polymorphism with PCOS women

The results indicated that Pro12Ala polymorphism was related to reduced risks of PCOS (OR = 0.80; 95% CI: 0.66–0.96; p = 0.02). However, obvious heterogeneity was presented in the meta-analysis (p = 0.07, I<sup>2</sup> = 36.1%). After excluded one study by Gu et al. in Asian groups (Gu and Baek, 2009) that caused the heterogeneity, these studies were homogeneous and could be combined. The results of the meta-analysis showed statistically significant association between Pro12Ala polymorphism and PCOS (OR = 0.75; 95% CI: 0.62–0.91; p = 0.003). So the article was excluded in following analysis.

We also performed subgroup analyses stratifying trials by ethnicity (Asian and European), statistically significant association was presented only in European groups (OR = 0.74; 95% CI: 0.60–0.90; p = 0.003), but not in Asian groups (OR = 0.86; 95% CI: 0.51–1.43; p = 0.56) (Fig. 2, Table 2).

3.4. The differences in HOMA-IR among PCOS women with (Pro/Ala + Ala/Ala) genotype and Pro/Pro genotype

When HOMA-IR with all Ala carriers (Pro/Ala + Ala/Ala) was compared with those with Pro/Pro genotype, no significant difference was found in PCOS women. The pooled ORs for 8 studies were −0.29 (95% CI: −0.82–0.24; p = 0.29) for the random-effects model (Fig. 3).

3.5. Publication bias

We found that funnel plot is symmetry (Fig. 4), and N<sub>f=0.05</sub> values for all the contrasts were greater (120) than the number of studies included (n = 17) in the meta-analysis.

4. Discussion

The current meta-analysis including 17 case-control studies and 4549 subjects were conducted to explore the association of the PPAR-γ2 Pro12Ala polymorphism with PCOS. The results indicated that individuals with the Ala allele had a statistically significant protective effect on the development of PCOS than participants with the Pro/Pro homozygotes. The pooled OR on the analysis by ethnicity indicated that the reduced risk existed only in Europeans, but not in Asians. A recent meta-analysis also showed that there was protective effect of PPAR-γ2
Pro12Ala polymorphism on PCOS women, however, the author did not undertake further subgroup analysis by ethnicity, and the number of articles included was not comprehensive, only 9 were included in their study (San-Millán and Escobar-Morreale, 2010).

A number of studies have suggested that the Ala allele of the Pro12Ala polymorphism in the isoform PPAR-γ2 is associated with reduced risk for type 2 diabetes, obesity, cardiovascular disease, which is probably mediated by increased insulin sensitivity (Buzzetti et al., 2005; Gouda et al., 2010; Huguenin and Rosa, 2010; Legro, 2003; Tellechea et al., 2009). Insulin resistance is also a feature of PCOS (Katsiki and Hatzitolios, 2010; Xita et al., 2009). Some studies reported significant improvement in insulin sensitivity in PCOS women with Pro12Ala Ala allele (Hahn et al., 2005; Hara et al., 2002), whereas others reported no changes in HOMA-IR in those with PCOS (Antoine et al., 2007; Haap et al., 2005; Orio et al., 2003; Orio et al., 2004). In the present meta-analysis, the results showed that HOMA-IR had no statistically significant difference between Ala carriers and Pro/Pro homozygotes in PCOS women. Discrepancies may be explained by differences in different ethnic groups. Interactions with other genetic variants are the possible reasons. A previous study indicated that the rare alleles of the P2 −689C>T and Pro12Ala SNPs were associated with an increased risk of the metabolic syndrome when combined to the 1431CC genotype. When taken individually, none of the polymorphisms was significantly associated with the metabolic syndrome (Meirhaeghe et al., 2005); In addition, gene-environmental factors may also explain the discrepancies. Studies suggested that when the dietary polyunsaturated fatty acid to saturated fatty acid ratio is low, the insulin levels and BMI in Ala carriers are higher than those in Pro/Pro homozygotes (Luan et al., 2001). Kahara et al. found that the Ala allele of Pro12Ala polymorphism is associated with improvement in insulin resistance after exercise (Kahara et al., 2003). Thus, further studies are required to clarify the role of Pro12Ala polymorphism and other confounding factors.

Analysis showed that obvious heterogeneity was observed in the meta-analysis; the results should be interpreted with caution when heterogeneity is presented, however, after exclusion of one study that caused the heterogeneity (Gu and Baek, 2009), no evident heterogeneity was observed ($p=0.18$). When we analyzed the association between Pro12Ala polymorphism and HOMA-IR, heterogeneity also existed. This implies that different genetic backgrounds, possible interactions with other genetic variants and uncontrolled environmental factors, may explain the discrepancies in genotype distribution. Additionally, a smaller study sample and the recruiting of the cohorts were also the source of heterogeneity, therefore, we performed subgroup analyses stratifying trials by ethnicities, diagnostic criteria of PCOS, and the cohorts. Two diagnostic criteria of PCOS, National Institutes of Health (NIH) 1990 and Rotterdam 2004, are commonly used. The Rotterdam Criteria is wider, including many

### Table 2

The association of PPAR-γ2 Pro12Ala polymorphism with PCOS according to ethnicity.

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Number of study</th>
<th>OR (95%CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asian</td>
<td>18</td>
<td>0.86 (0.51–1.43)</td>
<td>0.56</td>
</tr>
<tr>
<td>European</td>
<td>14</td>
<td>0.74 (0.40–1.39)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

OR = odds ratio; 95%CI = confidence interval.

**Fig. 2.** Subgroup analysis pooled odds ratios of PCOS and PPAR-γ2 CG + GG genotypes with the CC genotype.
more patients, most notably patients without androgen excess (Azziz, 2005). In the current analysis, we found in Europeans that statistically significant association between Pro12Ala polymorphism and reduced risk of PCOS was presented only in the studies of Rotterdam 2004 Criteria (OR = 0.61; 95% CI, 0.41–0.90; p = 0.01), but not in the studies of NIH 1990 Criteria (OR = 0.98; 95% CI, 0.73–1.30; p = 0.88) (data not shown). Similarly, when we differentiated hospital-based case-control studies from population-based case-control studies (Jing et al., 2011), there was a statistically significant association in the hospital-based case-control study groups (OR = 0.58; 95% CI, 0.41–0.82; p = 0.002), and a negative result was observed in the population-based case-control study groups (OR = 0.90; 95% CI, 0.69–1.18; p = 0.44) (data not shown).

Meta-analysis itself has its own limitations, they must be considered when interpreting the results. Firstly, the key limitation to any literature-based review and meta-analysis is that of reporting bias. Although no obvious publication bias was observed in our study, it is not possible to rule it out entirely (Colhoun et al., 2003). Secondly, study numbers were relatively small in Asians, however a recent genome-wide association study from 4082 Han Chinese PCOS study numbers were relatively small in Asians, however a recent genome-wide association study from 4082 Han Chinese PCOS women further supported our finding, in which they reported no significant associations between 3 loci on chromosome 2p16.3 (rs13405728), 2p21 (rs13429458), and 9q33.3 (rs2479106) with PCOS (Chen et al., 2011). Nonetheless, another study indicated no association between the Pro12Ala polymorphism and PCOS in Caucasian women (Lerchbaum et al., 2011). These results support our finding that the Pro12Ala polymorphism appears to be a modifier of insulin resistance. To make diagnostic, preventive, or therapeutic use of the polymorphism, it is necessary to understand how and in which metabolic, genetic, or environmental context the genotype influences the phenotype (Stumvoll and Haring, 2002). However, further large sample prospective studies on different ethnic groups are warranted to verify the role of Pro12Ala polymorphism of PCOS in women with PCOS. In addition, comprehensive interactions on gene–gene and gene–environment should also be evaluated in future studies.

In conclusion, our study demonstrated significant association between the PPAR-γ2 Pro12Ala allele and the decreased risk of PCOS in Europeans, but not in Asians. The Pro12Ala polymorphism did not appear to be a modifier of insulin resistance. To make diagnostic, preventive, or therapeutic use of the polymorphism, it is necessary to understand how and in which metabolic, genetic, or environmental context the genotype influences the phenotype (Stumvoll and Haring, 2002). However, further large sample prospective studies on different ethnic groups are warranted to verify the role of Pro12Ala polymorphism of PPAR-γ2 gene in women with PCOS. In addition, comprehensive interactions on gene–gene and gene–environment should also be evaluated in future studies.

Acknowledgments

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