Peroxisome proliferator-activated receptor Pro12Ala polymorphism and the risks of gestational diabetes mellitus

An updated meta-analysis of 12 studies

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Abstract

Background: Peroxisome proliferator-activated receptors-λ (PPAR-λ) is a member of nuclear receptor superfamily and acts as a ligand-dependent transcription factor often found in the adrenal gland, the spleen, and adipose tissue. The Pro12Ala polymorphism of PPAR-λ has been associated with the risks of gestational diabetes mellitus (GDM); however, association studies have provided conflicting results. The aim of this Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) compliant meta-analysis is to reach a more up-to-date and accurate estimation of the relationship between Pro12Ala genetic polymorphisms and the risks of GDM.

Methods: Eligible studies were retrieved by searching PubMed, EMBASE, Web of Science, Ovid, WanFang, and Chinese National Knowledge Databases and selected according to a pre-defined inclusion criterion. The risk of bias was assessed using the Newcastle-Ottawa quality assessment scale. The per-allele odds ratio (OR) of risk allele proline (Pro) was compared between cases and controls in each study to describe the association between the Pro allele and an individual’s risk of GDM. The ORs were pooled using both the random-effects model (the DerSimonian and Laird method) and the fixed-effects model (the Mantel-Haenszel method) and the 95% confidence interval (95% CI) was calculated using Woolf method.

Results: The final meta-analysis included a total of 11 articles of 12 data sets consisting of 7054 controls and 2980 GDM cases. Our results demonstrate that the Pro allele is not associated with GDM (OR: across multiple populations, 95% CI: 0.98–1.24; P|Z| = 0.01; P(Q) = 0.003). In the stratified analysis by ethnicity, significantly increased risks were found for the Chinese (OR = 2.36; 95% CI: 1.47–3.78) and Korean (OR = 1.39; 95% CI: 1.00–1.93) populations.

Conclusion: These data suggest the potential role of Pro allele in the pathogenesis of GDM in Asian populations. Although the funnel plot of included studies showed asymmetry, the results using the “trim and fill” method did not alter the conclusion of this study.

Abbreviations: Ala = alanine, BMI = body mass index, CI = confidence Interval, GDM = gestational diabetes mellitus, HWE = Hardy–Weinberg equilibrium, OR = odds ratio, PPAR-λ = peroxisome proliferator-activated receptors-λ, Pro = Proline.

Keywords: genetic polymorphism, gestational diabetes mellitus, gestational diabetes, meta-analysis, peroxisome proliferator-activated receptors, Pro12Ala

1. Introduction

Gestational diabetes mellitus (GDM) is defined as the intolerance of glucose that was not present or detected before pregnancy11 and often occurs when a woman’s pancreatic function is not sufficient to overcome the diabetogenic environment of pregnancy.2 GDM is the most common metabolic disorder during pregnancy,3 and its frequency has further increased in the past decade, with increases ranging from 10% to 100% in different groups of patients and ethnicities.4–6 Recent trends such as the decrease in physical activity,7 an epidemic of obesity,8 and adoption of unhealthy lifestyles may all contribute to the increasing prevalence of GDM.9

Although the exact disease etiology of GDM is still very much unknown, evidence to date suggests that it is a careful interplay between environmental factors and genetic background.10 Considerable research has been devoted to identifying potential genetic factors that contribute to GDM, and many genome-wide association studies have been conducted.11–12 The list of variants associated includes polymorphism within genes such as CDKAL1, IGF2BP2, KCNQ1, KCNF11, MTR1B, TCF7L2, PPARG, etc.13–18

Peroxisome proliferator-activated receptors-λ (PPAR-λ) is a member of nuclear receptor superfamily and acts as a ligand-
dependent transcription factor often found in the adrenal gland, the spleen, and adipose tissue.\textsuperscript{[19–21]} PPAR-\(\lambda\) forms heterodimers with the retinoid X receptors and regulates various genes involved in metabolism and adipocyte differentiation.\textsuperscript{[22,23]} Furthermore, PPAR-\(\lambda\) has been shown to have diverse functions such as negatively regulates macrophage activation,\textsuperscript{[24]} inhibits the production of monocytes inflammatory cytokines,\textsuperscript{[25]} adipogenesis, and insulin desensitization.\textsuperscript{[26]} Mutations in the PPAR-\(\lambda\) gene have been associated with obesity and diabetes-related phenotypes, such as improved insulin sensitivity and plasma leptin levels.\textsuperscript{[27–29]} The polymorphism of a proline (Pro) substituted with an alanine (Ala) at Amino acid 12 is a common polymorphism. The Ala allele is associated with reduced activity of PPAR-\(\lambda\).\textsuperscript{[27]} The Pro12Ala has been heavily researched for its role in obesity and type 2 diabetes and is considered one of the most common genetic risk factors for human diabetes.\textsuperscript{[30–32]} However, studies have found conflicting results in Pro12Ala’s role in GDM. For example, some studies have reported such a correlation, while other studies have found otherwise. To clarify the in-conflict findings reported so far as well as heterogeneity and publication bias that exists between studies, we have conducted a meta-analysis of genetic association studies of the PPAR-\(\lambda\) Pro12Ala polymorphism to assess its effect on the risk of GDM.

Table 1
The Newcastle–Ottawa quality assessment scale for studies included in this meta-analysis.

| Ref.          | Adequacy of case definition | Representative of the cases | Selection of controls | Definition of controls | Comparability of cases/controls | Ascertainment of exposure | Same method of ascertainment |
|---------------|-----------------------------|-----------------------------|-----------------------|------------------------|-------------------------------|---------------------------|-------------------------------|
| Cheng et al\textsuperscript{[35]} |                         |                             |                        |                        |                               |                           | NA                           |
| Cho et al\textsuperscript{[36]} |                         |                             |                        |                        |                               |                           | NA                           |
| Chon et al\textsuperscript{[39]} |                         |                             |                        |                        |                               |                           | NA                           |
| Du et al\textsuperscript{[30]}  |                         |                             |                        |                        |                               |                           | NA                           |
| Heude et al\textsuperscript{[41]} |                         |                             |                        |                        |                               |                           | NA                           |
| Lauenborg et al\textsuperscript{[42]} |                         |                             |                        |                        |                               |                           | NA                           |
| Pappa et al\textsuperscript{[43]} |                         |                             |                        |                        |                               |                           | NA                           |
| Shaat et al\textsuperscript{[43]} |                         |                             |                        |                        |                               |                           | NA                           |
| Shaat et al\textsuperscript{[42]} |                         |                             |                        |                        |                               |                           | NA                           |
| Tok et al\textsuperscript{[44]}  |                         |                             |                        |                        |                               |                           | NA                           |
| Zhu et al\textsuperscript{[37]}  |                         |                             |                        |                        |                               |                           | NA                           |

Figure 1. PRISMA flowchart of study selection.
### Table 2

| Ref. | Year | Ethnicity | Genotyping method | Diagnostic criteria | Number of cases/control | Genotype distribution | Mean age of cases/control | Mean BMI of cases/control | P (HWE) for controls | Number of controls |
|------|------|-----------|-------------------|---------------------|-------------------------|-----------------------|---------------------------|--------------------------|---------------------|------------------|
| Wang et al. Medicine (2016) 95:44 www.md-journal.com | | | | | | | | | | |
| | | | | | | | | | | |
| ref1 | 2010 | Chinese | PCR-RFLP | OGTT confirmed | 55/173 | 52/3/0 | 157/16/0 | 27.0/29.6 | NA/NA | 0.52 |
| ref2 | 2010 | Korean | TaqMan | OGTT confirmed | 94/41 | 89/5/0 | 34/7/0 | 32.6/34.2 | 26.77/29.2 | 0.55 |
| ref3 | 2012 | Chinese | PCR-RFLP | GDM per WHO criteria | 66/69 | 59/7/0 | 57/12/0 | 29.24/28.2 | NA/NA | 0.43 |
| ref4 | 2011 | French | TaqMan | OGTT confirmed | 148/107 | 143/5/0 | 100/7/0 | 32.5/26.7 | 26.0/24.3 | 0.73 |
| ref5 | 2009 | Danish | TaqMan | OGTT confirmed | 265/2383 | 201/60/4 | 1790/542/51 | 43.1/46.2 | 28.9/25.0 | 0.19 |
| ref6 | 2004 | Arabian | PCR-RFLP | OGTT confirmed | 400/428 | 286/111/3 | 317/105/6 | 32.4/NA | 28.9/NA | 0.41 |
| ref7 | 2007 | Swedish | TaqMan | OGTT confirmed | 637/1232 | 468/158/11 | 918/298/16 | 32.3/30.5 | NA/NA | 0.13 |
| ref8 | 2009 | Turkish | PCR-RFLP | OGTT confirmed | 100/122 | 91/9/0 | 106/15/1 | 31.9/NA | 30.9/NA | 0.57 |
| ref9 | 2004 | Scandinavian | TaqMan | OGTT confirmed | 179/180 | 165/14/0 | 155/20/5 | 28.1/27.4 | 24.6/23.4 | 0.00 |

OGTT = oral glucose tolerance testing, PCR = polymerase chain reaction, RFLP = restriction fragment length polymorphism.
The $\chi^2$ test was used to evaluate whether there is a significant deviation from HWE among the control subjects of the study. The per-allele OR of risk allele proline (Pro) was compared between cases and controls in each study to quantitatively describe the presence of the Pro allele and an individual’s risk of GDM. The ORs were pooled using both the random-effects model (the DerSimonian and Laird method) and the fixed effects model (the Mantel–Haenszel method) as previously described,\(^\text{[45,46]}\) and 95% CI was calculated using Woolf method.\(^\text{[47]}\) The results of the random effects model were reported in this article because it takes into consideration the variation between studies. A prespecified stratified analysis was conducted to explain the heterogeneity between each study and to investigate the relationship present in a subgroup. Stratified analysis was performed for ethnicity (Caucasian, Chinese, Korean, and Middle Eastern).

Heterogeneity across individual studies was examined using Cochran $\chi^2$ Q test.\(^\text{[48]}\) Q test was also performed to detect the heterogeneity within each subgroup. Publication bias was assessed using the linear regression approach to measure funnel plot asymmetry on the natural logarithm of OR, as described by Egger et al.\(^\text{[49]}\) All statistical analysis were carried out with Stata statistical software version 13.0 (Stata Corporation, College Station, TX). Type I error rate was set at 0.05, and all $P$ values were for 2-sided analysis.

### 3. Results

#### 3.1. Study characteristics

The search yielded a combined 69 references. Study selection process is shown in Fig. 1. The final meta-analysis included a total of 11 articles of 12 data sets,\(^\text{[14,35–44]}\) The 12 data sets included 7054 controls and 2980 GDM cases. The detailed characteristics of included studies are summarized in Table 2. Of the GDM cases, 300 were Chinese, 959 were Korean, 1559 were Caucasian, and 162 were Middle Eastern.

#### 3.2. Meta-analysis results

Overall, there was no evidence of an association between the Pro12Ala variant and increased risks of GDM when all data sets were combined. The forest plot of GDM risk associated with the Pro allele at amino acid position 12 is shown in Figure 2. The forest plot included a total of 12 data sets with 2980 cases and 7054 controls. The OR and 95% CI for each subgroup are presented in Table 3.

### Table 3

| Total/Subgroup | Number of data sets | Number of cases/controls | OR (95% CI) | $P$ (Z) | $P$ (Q) |
|---------------|---------------------|--------------------------|-------------|--------|---------|
| Total         | 12                  | 2980/7054                | 1.10 (0.98–1.24) | 0.10   | 0.003   |
| Chinese       | 3                   | 300/422                  | 2.36 (1.47–3.70) | 0.01   | 0.22    |
| Korean        | 2                   | 959/673                  | 1.39 (1.00–1.93) | 0.05   | 0.12    |
| Caucasian     | 5                   | 1559/5737                | 1.00 (0.88–1.14) | 0.99   | 0.41    |
| Middle Eastern| 2                   | 162/222                  | 1.11 (0.63–1.97) | 0.71   | 0.25    |

95% CI = 95% confidence interval, OR = odds ratio.
were pooled together. The per-allele OR of Pro using the random
effects models was 1.10 [95% CI: 0.98–1.24; P(Z)=0.01; P(Q)=
0.003; Fig. 2]. The main results of the meta-analysis are listed in
Table 3.

In the stratified analysis by ethnicity, significantly increased
risks were found for the Chinese (OR = 2.36; 95% CI: 1.47–3.78)
and Korean (OR = 1.39; 95% CI: 1.00–1.93) population (See Fig.
2). However, no significant associations were detected for the
Caucasian (OR = 1.00; 95% CI: 0.88–1.14) and Middle Eastern
(OR = 1.11; 95% CI: 0.63–1.97) populations.

3.3. Sensitivity analysis

Sensitivity analyses using single-study omission demonstrated
that this meta-analysis was stable (Fig. 3). Statistical signifi-
cance of the summary ORs was not modi-
cated (data not shown). Therefore, the results of this study are stable.

3.4. Publication bias

Begg’s and Egger’s funnel plots were constructed using the
standard error and compared against the OR of each study (Figs.
4 and 5). The plots suggest the possibility of publication bias
toward positive findings in smaller studies. The Duval and
Egger’s test using nonparametric “trim and fill” method was utilized to adjust for publication bias[50] and its results did show different
conclusions (data not shown). Thus, this indicates that this meta-
analysis is statistically robust.

4. Discussion

PPAR-α is a ligand-dependent transcription factor involved in
many body functions, including adipogenesis and also regulates
immune responses.[20,25] The substitution of a Pro to Ala at site
12 is associated with reduced PPAR-α activities[27] and has been
identified as a possible polymorphism involved obesity and type 2
diabetes.[30–32]

Our up-to-date meta-analysis summarizes the evidence to
date regarding the association between PPAR-α Pro12Ala and
GDM using a total of 7054 controls and 2980 GDM cases. Our
study suggests that Pro12Ala is not associated with the risks of
GDM.

In our stratified analysis by ethnicity, a strong association was
observed for both the Chinese (OR: 2.36, 95% CI: 1.47–3.78)
and Korean (OR: 1.39, 95% CI: 1.00–1.93) population but not
for the Caucasian (OR = 1.00, 95% CI = 0.88–1.14) and Middle Eastern
(OR = 1.11, 95% CI = 0.63–1.97) populations. These
results indicate that the association of the polymorphism has a
genetic and possibly environmental background factor in
contributing to the pathology of GDM. Other factors such as
differences in matching criteria and selection bias could also play
a role in the difference between ethnic groups. It should also be
noted that the analysis only included 3 Chinese studies and 2
Korean studies. This suggests the possibility that the observed
differences may be due to chance. Thus, additional studies are
required to increase the statistical power and validate the racial
difference of the Pro12Ala polymorphism and GDM risk.

The preferential publication of studies with positive results is a
significant source of bias in many meta-analyses. However, the
included studies in our meta-analysis also consist of studies with
negative conclusions. Although our funnel plots showed
asymmetry, the results using the “trim and fill” method did not alter the conclusion of this study. This suggests that the bias
may not be caused by publication bias but by potential
difference between each study’s population, language bias,
citation bias, or simply by chance.
Several limitations should be noted in interpreting our results. We were not able to adjust for potential confounding effects conferred by gender, environmental factors, and lifestyle due to the lack of data. Our results were based on unadjusted estimates—a more precise analysis could be conducted if all raw data were available. The lack of individual health and metabolic data, such as fasting plasma glucose levels, β-cell function, and indices for insulin sensitivity also forbid us from performing a more sensitive analysis.

In conclusion, the pooled results of our meta-analysis indicate that Pro12Ala is not associated with the risks of GDM. However, in the Chinese and Korean populations, the Pro allele is strongly associated with the risks for GDM. Larger association studies with strict selection criteria are required to validate this result.

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