Does Pro$^{12}$Ala Polymorphism Enhance the Physiological Role of PPAR$\gamma$2?

A. C. Pereira,$^{1,2}$ R. Oliveira,$^{3,4}$ A. C. Castro,$^1$ and R. Fernandes$^{1,2}$

$^1$ Unit of Molecular Mechanisms of Disease (CISA) and Chemical and Biomolecular Sciences, School of Allied Health Sciences, Polytechnic Institute of Porto (ESTSP-IPP), Portugal
$^2$ Center of Pharmacology and Chemical Biopathology (U38-FCT), Medical Faculty, University of Porto, Portugal
$^3$ Center for Research in Health Technologies and Information Systems (CINTESIS), Medical Faculty, University of Porto, Portugal
$^4$ Biomathematics, Biostatistics and Bioinformatics, ESTSP-IPP, Porto, Portugal

Correspondence should be addressed to R. Fernandes; rpf@estsp.ipp.pt

Received 5 April 2013; Accepted 29 June 2013

Obesity and type 2 diabetes mellitus (T2D) are two major public health problems that have motivated the scientific community to investigate the high contribution of genetic factors to these disorders. The peroxisome proliferator activated by gamma2 (PPAR$\gamma$2) plays an important role in the lipid metabolism. Since PPAR$\gamma$2 is expressed mainly in adipose tissue, a moderate reduction of its activity influences the sensitivity to insulin, diabetes, and other metabolic parameters. The present study aims to contribute to the elucidation of the impact of the Pro$^{12}$Ala polymorphism associated with T2D and obesity through a meta-analysis study of the literature that included approximately 11500 individuals, from which 3870 were obese and 7625 were diabetic. Statistical evidence supports protective effect in T2D of polymorphism Pro$^{12}$Ala of PPAR$\gamma$2 (OR=0.702 with 95% CI: 0.622; 0.791, $P<0.01$). Conversely the same polymorphism Pro$^{12}$Ala of PPAR$\gamma$2 seems to favor obesity since 1.196 more chance than nonobese was found (OR=1.196 with 95% CI: 1.009; 1.417, $P < 0.004$). Our results suggest that Pro$^{12}$Ala polymorphism enhances both adipogenic and antidiabetogenic physiological role of PPAR$\gamma$. Does Pro$^{12}$Ala polymorphism represent an evolutionary step towards the stabilization of the molecular function of PPAR$\gamma$ transcription factor signaling pathway?

1. Introduction

Peroxisome proliferator-activated receptors (PPARs) are transcription factors from nuclear receptor’s protein family that regulate target genes’ expression by connecting to response elements of peroxisomes proliferators (PPERs) in regulating sites of each gene. The signal transduction mechanism of these receptors involves retinol X receptor (RXR) and PPARs form heterodimers that regulate the transcription of several genes (Figure 1) [1–3].

Of all PPAR subtypes described, PPAR$\alpha$, PPAR$\beta$, and PPAR$\gamma$, the latter is the most studied [4]. The PPAR$\gamma$ gene, extended by a segment of over 150 kb, is localized in chromosome 3, region 3p25, and is constituted by 3 isoforms: PPAR$\gamma$1, PPAR$\gamma$2, and PPAR$\gamma$3, distinguished for generating different promoters and alternative splicing [1, 5, 6].

PPAR$\gamma$ is a transcriptional factor responsible for many metabolic and cellular processes, such as cellular growth, differentiation, and metabolism, in response to lipophilic hormones, fatty acids, and its metabolites [1, 2]. It has high affinity to fatty acids specific proliferators and is almost exclusively expressed in the adipose tissue in humans, where it modulates target genes expression involved in adipocytes expression, insulin sensitivity, angiogenesis, and inflammatory processes, among others (Figure 2) [1, 5–7].

To date, several PPAR$\gamma$2 polymorphisms have been described and the Pro$^{12}$Ala polymorphism has been associated with variations of BMI and insulin sensitivity, depending on the ethnicity, although the reasons for this heterogeneity remain unclear [1, 7–9].

Given that PPAR$\gamma$2 is mainly expressed in adipose tissue and taking in consideration the role of free fatty acids
Figure 1: PPARγ transduction pathway. PPARγ has several extracellular and intracellular ligands that include dietary and bioactive lipids. Given its antidiabetogenic role, some PPARγ ligands include antidiabetic drugs such as thiazolidinediones. PPARγ is also modulated by several growth factor transduction pathways such as Jnk/Erk/MKP. It is also known that cyclin-dependent kinase 5 (Cdk5) bond to p25 (a product of the cleavage of p35 in obesity environment) inhibits PPARγ pathway by its phosphorylation. As a transcriptional factor, PPARγ binds to RXR (retinol X receptor) in order to transcribe several genes related to adipocyte differentiation and lipid storage in adipose tissue and increase insulin sensitivity in peripheral tissues by indirect increase of AMP kinase activity as well as several other antidiabetogenic effects. Moreover, PPARγ blocks NFκB signaling, thus reducing proinflammatory cytokines and inflammation.

Diabetes mellitus is a metabolic disorder of multiple etiologies, characterized by chronic hyperglycemia due to deficient secretion or action of insulin and, like obesity, is considered one of the main health threats [16].

The present study pretends to contribute to the understanding of the role of the PPARγ2 gene Pro12Ala polymorphism in two metabolic conditions, obesity and diabetes mellitus 2 (T2D), through a systematic review and meta-analysis.

2. Materials and Methods

We performed a systematic review and meta-analysis of published works about the presence of particular PPARγ polymorphism such as Pro12Ala, as a risk factor for obesity and for T2D, according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

2.1. Selection Criteria and Identification of Studies. The study was conducted according to PRISMA directives. In order to
identify the studies, an electronic search, with no linguistic restriction, was conducted, researching two databases, the ScienceDirect and Medline (PubMed). The Boolean search was conducted in both databases according to the following keywords: (1) PPARγ [AND] Pro¹² Ala, (2) Pro¹² Ala [AND] obesity, and (3) Pro¹² Ala [AND] diabetes mellitus 2.

Two investigators evaluated, independently, the titles and abstracts of the papers identified as of potential relevance in the inclusion criteria. Where a title or abstract could not be included for certainty, a third corrector would help decide whether to reject or not.

The papers that satisfied the initial inclusion criteria and that were identified as of randomized or quasi-randomized clinical trial conducted in human, and used the Pro¹² Ala polymorphism as a comparison between control groups and obese or diabetic groups, were then used for systematic revision.

After the papers’ analysis, the data of interest was used based on the place where the study was conducted, year of publication, outcome, and number of individuals.

2.2. Statistics. The statistical analysis included false positives, false negatives, true positives, and true negatives to establish sensitivity and specificity and correlate both in a Roc curve, P value, and odd ratios (OR), with and with a confidence interval of 95%.

For the data analysis was used the program RevMan version 5.1 and the SPSS Statistics version 17.0.

3. Results and Discussion

The research identified 11 studies, from which 6 were related to the presence of Pro¹² Ala polymorphism in obesity and 8 to the presence of Pro¹² Ala polymorphism in T2D.
### Table 1: Included studies in the meta-analysis for obesity and respective genotype of the studied population.

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Metabolic disorder</th>
<th>Participants</th>
<th>No. of cases (O)</th>
<th>No. of control (NO)</th>
<th>Genotype</th>
<th>Pro/Pro</th>
<th>Pro/Ala</th>
<th>Ala/Ala</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ghoussaini et al., 2005</td>
<td>French (children)</td>
<td>Obesity</td>
<td>591</td>
<td>396</td>
<td>195</td>
<td></td>
<td>304</td>
<td>156</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>French (adults)</td>
<td>Obesity</td>
<td>1713</td>
<td>1102</td>
<td>611</td>
<td></td>
<td>857</td>
<td>478</td>
<td>231</td>
</tr>
<tr>
<td></td>
<td>Spanish</td>
<td>Obesity</td>
<td>459</td>
<td>145</td>
<td>314</td>
<td></td>
<td>119</td>
<td>264</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Korean</td>
<td>Obesity</td>
<td>229</td>
<td>111</td>
<td>118</td>
<td></td>
<td>103</td>
<td>108</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Palestinian</td>
<td>Obesity (T2D individuals)</td>
<td>202</td>
<td>121</td>
<td>81</td>
<td></td>
<td>106</td>
<td>73</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Tunisian</td>
<td>Obesity</td>
<td>675</td>
<td>387</td>
<td>288</td>
<td></td>
<td>348</td>
<td>271</td>
<td>39</td>
</tr>
<tr>
<td><strong>Summary</strong></td>
<td></td>
<td></td>
<td><strong>3869</strong></td>
<td><strong>2262</strong></td>
<td><strong>1607</strong></td>
<td></td>
<td><strong>1837</strong></td>
<td><strong>1350</strong></td>
<td><strong>402</strong></td>
</tr>
</tbody>
</table>

Legend: T2D: type 2 diabetes mellitus; O: obese; NO: non-obese.

The studies related to obesity investigated a total of 3870 individuals, with 2260 being obese and 1610 nonobese (Table 1).

The studies regarding the correlation between Pro\textsuperscript{12}Ala polymorphism and T2D investigated a total of 7625, with 4464 being diabetic and 3161 nondiabetic (Table 2).

The major characteristics of the investigated individuals were similar in all included studies, as well as the methodology.

The sensitivity analysis to evaluate population characteristics was not possible due to the small number of included studies.

Ali and collaborators studied the effect of Pro\textsuperscript{12}Ala polymorphism on obesity risk in a Tunisian population, in 2009. They notice a significant difference between male obese individuals and control and pointed out an association between the polymorphism and obesity in nondiabetic male. These individuals also displayed elevated BMI. However this study did not proceed to body fat measurement or its distribution in order to confirm the polymorphism contribution in obesity. Still, it is supported the hypothesis that Pro\textsuperscript{12}Ala polymorphism is a relevant marker in nondiabetic Tunisian man obesity, despite a noneffect on the individuals metabolic characteristics [20].

Ereqat and collaborators studied obese Palestinian individuals with T2D. They notice the polymorphism was associated with high plasmatic levels of total cholesterol, with a tendency to increase LDL cholesterol levels. However, there was no significant impact on BMI, triglycerides, or arterial pressure. Hence, they concluded that the Ala12 allele may influence cardiovascular risk, through effect on lipidic metabolism, in obese Palestinian patients with T2D [19].

Ghoussaini and collaborators studied the French population with T2D and obesity. Regarding obesity, they demonstrated no association with Pro\textsuperscript{12}Ala polymorphism, in children or adults. Still they concluded that the polymorphism confers a reduction in obesity risk [17].

Oh and collaborators conducted their study in the Korean population. They found no significant association between the Pro\textsuperscript{12}Ala polymorphism and obesity, hypertension, or dyslipidemia. However, despite the statistical nonsignificance, there is, according to the authors, the possibility of PPAR\gamma mutation having a minor effect on obesity, assuming a significant effect if it occurs simultaneously with other genes' mutations or environmental factors [1].

González Sánchez and collaborators were the firsts to conduct a population-based nationwide multicenter study in Spain, suggesting that Pro\textsuperscript{12}Ala polymorphism may promote peripheral deposition of adipose tissue. The Ala12 allele frequency was higher on obese male individuals than in lean ones. Men carrying the Ala12 allele had a higher BMI than noncarriers (38.9% versus 21.3%), despite possessing a lower abdominal diameter [18].

Ghoussaini and collaborators studied the French population with obesity and T2D, in 2005. Regarding the T2D study, they affirmed that the polymorphism has a risk role, since a significant association between the polymorphism and T2D was found, with a $P$ value of 0.04 and OR = $1.37$ (95% CI: 1.02; 1.85) [17].

Lindi and collaborators studied a diabetic Finnish population. They were the first to try an association between Pro\textsuperscript{12}Ala polymorphism and T2D incidence, in a high-risk population with diminished glucose tolerance, in a longitudinal study. No significant association of the Ala12 allele and T2D incidence in the studied group [21] was found.

Chistiakov and collaborators analyzed Russian individuals with T2D. Their results suggest that the polymorphism reduces T2D risk, performing a protective role in this pathology. In the studied population, the Pro12 allele contributes to higher risk in developing T2D, according to OR = 1.69 (95% CI: 1.02; 3.03) [22].

Oh and collaborators analyzed the association between Pro\textsuperscript{12}Ala polymorphism and T2D, in diabetic Koreans. They compared individuals with normal and diminished glucose
tolerance and with diabetes. No significant differences were found among Ala12 allele frequency in the study groups, concluding an inexistent significant association between Pro12 Ala polymorphism and T2D. However, despite these results, they affirm that there is the possibility of an effect, at minor scale, of the polymorphism on the pathology, when associated with other mutations on other genes, as well with environmental factors [1].

Bouassida and collaborators studied the association of Pro12 Ala polymorphism and Tunisian T2D carriers. They concluded that there was no significant differences between diabetic and control groups and that, therefore, the polymorphism does not display a role in the pathology [23].

Malecki and collaborators studied the Pro12 Ala polymorphism with T2D incidence in a polish population. They compared the polymorphism incidence in a diabetic and a nondiabetic (control) group and notice that the Pro12 and Ala12 alleles’ frequency were similar (83.5% and 16.5% versus 84.5% and 15.5%, resp., $P = 0.607$), as well as the genotypic distribution. They could not conclude that the polymorphism confers higher risk to T2D susceptibility, as in numerous European studies in Caucasians [24].

Pintéróvá and collaborators, in 2004, wanted to understand the association between the Pro12 Ala polymorphism and T2D. In order to do so, they studied the Czech population, comparing a study group of 133 diabetic individuals to a nondiabetic control group of 97 individuals. In the study group, 3 individuals (2.26%) were identified as homozygotes for the Ala/Ala genotype, 99 individuals (74.44%) were identified as homozygotes for the Pro/Pro genotype, and 31 individuals (23.31%) were identified as heterozygotic. In the control group, 6 individuals (6.19%) were identified as homozygotes for the Ala/Ala genotype, 61 individuals (62.89%) were identified as homozygotes for the Pro/Pro genotype, and 30 individuals (30.93%) were identified as heterozygotic. The allelic frequency for the Ala allele was lower in the diabetic group (13.91% versus 21.43% for control group). They concluded that the polymorphism is associated with reduced risk of T2D, assuming a protective factor in the pathology [26].

Morí and collaborators analyzed the polymorphism and T2D in a Japanese population. They included in their study 2201 individuals with T2D (study group) and 1212 individuals without the pathology (control group). The allelic frequency of Ala12 was higher in in control group (4.13% versus 2.39% for the study group). They concluded that the polymorphism is associated with reduced risk of T2D development, acting out protectively factor in the pathology [26].

3.1. Meta-Analysis Results. The combination of the studies that analyzed the presence of Pro12 Ala polymorphism in obesity is showed on Table 3.
Regarding the presence of Pro^{12}Ala polymorphism in T2D, the combination of all included studies is presented in Table 4.

Eleven studies that investigated the Pro^{12}Ala polymorphism were included in the present meta-analysis. From these, 6 studies investigated the hypothetical association of the polymorphism and obesity and 8 investigated the hypothetical association of the polymorphism, Tables 1 and 2. The data was then aggregated according to presence/absence of the polymorphism and presence/absence of the pathology. Individuals with the genotype Pro/Pro did not possess the polymorphism and individuals with genotype Pro/Ala (heterozygotes) and genotype Ala/Ala (homozygotes) possess the polymorphism. This way, it was possible to assort all individuals in TN, FN, TP and FP and, with these data, obtain the sensitivity and specificity graphics (Tables 3 and 4). The analysis of these tables allowed concluding that the studies are not sensitive but very specific.

In Table 3, Ghoussaini and collaborators’ study [17] conducted in obese children represented the most sensitive study, with a value of 0.23 (95% CI: 0.19; 0.28). On the other hand, Oh and collaborators [1] had the least sensitive study, with 0.07 (95% CI: 0.03; 0.14). The study with the highest average specificity belongs to Ben Ali and collaborators [20], conducted in 2009, with a specificity value of 0.94 (95% CI: 0.91; 0.97) and the one with the lowest average specificity was conducted by González Sánchez and collaborators, 0.75 (95% CI: 0.68; 0.80).

In Table 4, Lindi and collaborators’ study [21] represented the most sensitive study, with a value of 0.34 (95% CI: 0.28; 0.41). On the opposite side, Mori and collaborators [26] had the least sensitive study, with 0.05 (95% CI: 0.04; 0.06). The latter also had the study with the highest average specificity, with 0.92 (95% CI: 0.90; 0.93) and the one with the lowest average specificity was conducted by Chistiakov and collaborators [22], 0.59 (95% CI: 0.55; 0.63).

The results from these tables allowed constructing the respective ROC curve (not shown) and we conclude that the Pro^{12}Ala polymorphism is not a good test (regarding sensitivity and specificity) to predict the presence of obesity, since the test line is very close to the random line. The same conclusion can be taken from the analysis of the Pro^{12}Ala polymorphism which is not a good test to predict the presence of T2D.

### Table 5: Pro^{12}Ala polymorphism and obesity.

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Obese</th>
<th>Non-obese</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>423</td>
<td>260</td>
<td>683</td>
<td></td>
</tr>
<tr>
<td>Non-present</td>
<td>1837</td>
<td>1350</td>
<td>3187</td>
<td>P &lt; 0.004</td>
</tr>
<tr>
<td>Total</td>
<td>2260</td>
<td>1610</td>
<td>3870</td>
<td></td>
</tr>
</tbody>
</table>

### Table 6: Pro^{12}Ala polymorphism and T2D.

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Diabetic</th>
<th>Non-diabetic</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>667</td>
<td>633</td>
<td>1300</td>
<td></td>
</tr>
<tr>
<td>Non-present</td>
<td>3797</td>
<td>2528</td>
<td>6325</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Total</td>
<td>4464</td>
<td>3161</td>
<td>7625</td>
<td></td>
</tr>
</tbody>
</table>

### Table 7: Estimated risk of the Pro^{12}Ala polymorphism with obesity.

<table>
<thead>
<tr>
<th>Value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>OR for polymorphism Pro^{12}Ala (0/1)</td>
<td>1.196 (1.009; 1.417)</td>
</tr>
<tr>
<td>OR for variable non-obese = 0</td>
<td>1.113 (1.003; 1.235)</td>
</tr>
<tr>
<td>OR for variable obese = 1</td>
<td>0.931 (0.871; 0.994)</td>
</tr>
</tbody>
</table>

### Table 8: Estimated risk of the Pro^{12}Ala polymorphism with T2D.

<table>
<thead>
<tr>
<th>Value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>OR for polymorphism Pro^{12}Ala (0/1)</td>
<td>0.702 (0.622; 0.791)</td>
</tr>
<tr>
<td>OR for variable non-diabetic = 0</td>
<td>0.940 (0.920; 0.961)</td>
</tr>
<tr>
<td>OR for variable diabetic = 1</td>
<td>1.340 (1.214; 1.479)</td>
</tr>
</tbody>
</table>

We also proceed to the determination of the odds ratio (OR) and P values, by the Chi-squared test. Tables 5 and 6 show the P value for obesity and T2D, respectively, and Tables 7 and 8 show the risk estimative (OR) for the variables used in this meta-analysis, with a 95% CI, for obesity and T2D, respectively.
Regarding the association between the polymorphism and obesity, obese individuals possess a 1.196 more chance of carrying the polymorphism than non-obese (OR = 1.196 with 95% CI: 1.009; 1.417, \( P < 0.004 \)).

Concerning the association between the polymorphism and T2D, diabetic individuals possess a 0.702 less chance of carrying the polymorphism than nondiabetic (OR = 0.702 with 95% CI: 0.622; 0.791, \( P < 0.001 \)).

Therefore, the Pro12Ala polymorphism from gene PPARγ2 acts out as a risk factor in obesity (\( P < 0.05 \) and OR = 1.196 with CI values that never include 1) and as a protective factor for T2D (\( P < 0.05 \) and OR = 0.702 with CI values that never include 1), by the evident statistical significance.

4. Conclusions

Comparing results obtained from this meta-analysis and each of the included studies, we can verify some differences. There are studies that conclude that Pro12Ala polymorphism is a risk factor in obesity or T2D, while others conclude the opposite for both pathologies. There are also studies that do not have statistically significant data to support either conclusion.

Ben Ali et al. [20], Ghoussaini et al. [17], Oh et al. [1], and respective collaborators could not associate, from their studies, the polymorphism presence in obesity. Meanwhile González Sánchez and collaborators [18] associated the polymorphism with higher risk of obesity. Ereqat and collaborators [19] associated the polymorphism to lipidic metabolism in obese Palestinian individuals with T2D, concluding that the Ala allele presence may influence cardiovascular risk.

Results regarding the role of the polymorphism in T2D, Boussida et al. [23], Lindi et al. [21], Malecki et al. [24], Oh et al. [1], and their respective collaborators, could not obtain significative results and, consequently, could not associate the polymorphism to the T2D presence. Chistiakov et al. [22], Mori et al. [26], Pintérová et al. [25], and collaborators had agreeing results with the ones obtained in this meta-analysis, pointing out the polymorphism's protective effect in T2D. Contrariwise Ghoussaini et al. [17] and collaborators sustained that the polymorphism plays a risk factor in the pathology.

Association studies clearly show this gene's regulation in adipose tissue is a complex process. Several studies suggest that genetics or the environment (such as diet) participates in the formation of association patterns of Pro12Ala polymorphism with body mass in different human populations. Numerous studies have also clearly showed the heterogeneous effects of Pro12Ala polymorphism in decreased risk of T2D several populations. Considering that the two pathologies are multifactorial it is difficult to clarify the real contribution that the polymorphism makes in these metabolic disorders.

The present study includes approximately 11500 individuals, from which 3870 are obese and 7625 are diabetic. From our results we concluded that the Pro12Ala polymorphism from PPARγ2 gene has a protective role in T2D but is a risk factor for obesity accordingly with what we should expected substitute to for about to the physiological role of PPARγ itself. It seems that the Pro12Ala polymorphism may be a transitory state towards the structural and functional stabilization of the gene role in human kind, resembling a genetic drift at the molecular level.

Therefore, this study supports the need of investment for further research, mainly by population genetics, in order to fully understand the role of this polymorphism in the PPARγ cascades.

References


