Effect of two common variants in PPAR-γ2 gene on susceptibility to obesity

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Abstract

Background/Aims: Association of genetic variants in the PPAR-γ2 gene with obesity and related phenotypes is widely scrutinized in recent years. However, there have been inconsistent findings in different populations. This study aimed to explore the association of PPAR-γ2 gene Pro12Ala and C1431T polymorphisms and their haplotypes with susceptibility to obesity and related traits in an Iranian population.

Methods: A total of 233 unrelated subjects, including 83 obese and 150 nonobese controls, were enrolled in this study. Anthropometric indices, fasting plasma glucose, and lipid profile were measured using standard protocols. Genotyping was done by TaqMan® SNP Genotyping Assay.

Results: Waist/hip ratio, systolic and diastolic blood pressure, triglyceride and fasting blood glucose showed a significant difference between groups. The genotype distributions were not significantly different between obese and non-obese subjects. However, in the obese group, the carriers of 12Ala risk allele had higher body weight (P=0.019), waist circumference (P=0.003), and waist/hip ratio (P=0.048) compared to the wild-type homozygotes. Haplotype analyses identified four common haplotypes with frequency greater than 1% but none of them was associated with the risk of obesity.

Conclusions: The present study suggests that Pro12Ala but not C1431T polymorphism of PPAR-γ2 gene is correlated with predisposition to obesity related markers.

Keywords: Peroxisome proliferator-activated receptor-γ, Obesity, Single nucleotide polymorphism.
several populations and the results have been inconsistent. Many epidemiological studies reported these two common variants of PPAR-γ2 are associated with obesity risk and measures of it, however, some other studies have showed no association with obesity [8-13].

On the basis of the proposed involvement of PPAR-γ2 in several signaling pathways important for lipid and carbohydrate homeostasis, and, conflicting results regarding the association between obesity and these two common variants, present study was designed to investigate their association with susceptibility to obesity or related markers in an Iranian population.

Material and Methods
A total of 233 unrelated adult subjects aged at 18 to 60 years were randomly selected using a population-based cluster sampling from Mashhad as a second largest city in Iran. Of these 83 subjects were classified as obese with a BMI ≥ 30 kg/m², and 150 as nonobese with BMI <30 kg/m². All study participants were attended a complete clinical examination that included assessment of a detailed personal and family history, determination of anthropometric indices, measurement of various biochemical parameters and self-reported smoking status. Subjects with a history of coronary artery disease, congestive heart failure, stroke, diabetes mellitus, endocrinological abnormalities, liver and/or renal disease, and alcohol consumption or those under medications that altered blood pressure, lipid or glucose metabolism, as well as pregnant women were excluded. Data concerning the smoking habit were also collected by a self-reported questionnaire designed for the purposes of this work. Informed consent was obtained from each participant and the study was carried out in accordance with the protocols approved by the Ethics Committee of the Mashhad University of Medical Science (MUMS).

Anthropometric and laboratory measurements
In all individuals, anthropometric indices included body weight, height, waist circumferences (WC), and hip circumferences (HC) were measured for calculation of BMI and waist/hip ratio respectively. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured by the trained investigators using standard methods of measurement in accordance with WHO standards [14]. Venous blood samples were collected in the morning after an overnight fast and plasma and serum samples were stored frozen at 80°C until measurements. Standard quality controlled enzymatic methods were used for determining fasting plasma glucose and serum lipid profile including total cholesterol (TC), high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL) and triglyceride (TG).

Genotyping
Genomic DNA was extracted from whole blood using the FlexiGene DNA isolation Kit (FlexiGene DNA isolation Kit, Qiagen) according to the manufacturer’s protocol. The analysis of Pro12Ala, C1431T PPAR- polymorphisms were determined by allelic discrimination assays using TaqMan probes (Applied Biosystems, Foster City, USA). The reaction was performed on an ABI 7500 Real Time PCR System (Applied Biosystems) as described in our previous study [15]. Briefly the conditions for the polymerase chain reaction were 95°C for 10 minutes and 40 cycles of 92°C for 15 seconds and 60°C for 1 minute. Individual genotypes identification was analyzed by SDS software version 1.3 (Applied Biosystems). To assess genotyping quality control, genotyping was repeated for ten percent of randomly selected samples with 100% reproducibility. Nuclease-free water was used as negative control.

Statistical analysis
Genotypic and allelic frequencies, the association between genotypes and the risk of obesity and analysis of deviation from the Hardy-Weinberg equilibrium were assessed using χ² test or Fisher’s exact tests. Association analyses were performed assuming general, additive, dominant and recessive models. All values were presented either as the mean ± SD for normally distributed variables or as the median and interquartile range for nonnormally distributed variables. Continuous variables without normal distribution such as TG, and LDL were logarithmically transformed before entering the analysis. The student t test was used to compare the clinical characteristics and baseline demographics between the groups. The Mann-Whitney U test was used for continuous variables if they are not normally distributed. Binary logistic regression analysis used to adjust odds ratios and its 95% confidence intervals (CI), for age and gender as confounding variables. The Bonferroni procedure was used for the correction of multiple comparisons. cubeX software was used to calculate haplotype frequencies and D’ and r² values for linkage disequilibrium (LD) [16]. All statistical analysis was performed using the Statistical Package for Social Science (SPSS for windows, version 16) (SPSS Inc., Chicago, IL, and U.S.A). Two-tailed tests were performed with the significance level of 0.05.

Results
Characteristics of subjects
Demographic and clinical characteristics of the study subjects are summarized in Table 1. Systolic blood pressure, diastolic blood pressure, body weight, height, BMI, waist circumference, hip circumference, waist/hip ratio, the ratio of female to male and serum levels of fasting glucose and TG showed significant differences between obese and nonobese subjects (P<0.05), while other factors of lipid profile like TC, HDL-C and LDL-C did not show any significant differences.

Table 1. Comparison of demographic and clinical characteristics between obese and non-obese subjects.
Association of PPAR-γ2 polymorphisms with obesity

The independent sample t-test was used to compare demographic and clinical characteristics between Obese and non-Obese Subjects. Data are expressed as mean ± SD. BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; WH: Waist/Hip; TC: Total Cholesterol; HDL-C: High Density Lipoprotein Cholesterol; LDL-C: Low Density Lipoprotein Cholesterol; TG: Triglycerides; HSCRP: High-Sensitivity C-Reactive Protein.

Genotypic and allelic frequencies

To improve the chance to detect an association between the SNPs and the obesity, we also computed haplotype frequencies and degree of linkage disequilibrium by D' and r² according to combination of PPAR- Pro12Ala and C1431T variants (Table 3). Results showed that two polymorphisms were in a weak linkage disequilibrium (D'=0.332, r²=0.294). On the basis of haplotype analysis, four possible haplotypes including PC, PT, AC and AT with estimated frequency more than 1% were
observed in the both obese and nonobese subjects. There was no significant difference in the distribution of haplotypes between two groups. These analyses revealed no further association between the SNPs and obesity.

**Table 3.** Haplotype frequencies associating the PPAR-γ2 polymorphisms in the study population and linkage disequilibrium statistics.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Haplotype</th>
<th>Frequency among non-obese</th>
<th>Frequency among obese</th>
<th>OR (95% CI) Obese vs. non-obese</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPAR-γ2</td>
<td>Pro-C</td>
<td>0.82</td>
<td>0.83</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Pro-T</td>
<td>0.08</td>
<td>0.09</td>
<td>1.02 (0.53 - 1.97)</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>Ala-C</td>
<td>0.06</td>
<td>0.05</td>
<td>0.90 (0.36 - 2.27)</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>Ala-T</td>
<td>0.04</td>
<td>0.03</td>
<td>0.55 (0.16 - 1.84)</td>
<td>0.33</td>
</tr>
</tbody>
</table>

The degree of linkage disequilibrium (LD) between the two variants is shown as $D'$ and $r^2$ for study population.

To evaluate the association of different genotypes of PPAR-γ2 variants with obesity related markers, further related analysis were done in the content of obese group and the result showed PA+AA genotype of Pro12Ala variant was associated with higher values of body weight, WC and waist/hip ratio in obese subjects only (Table 4). This association was not observed within nonobese group (Data not shown). There was also a significant association between CC genotype of C1431T gene variant and higher level of HDL-C in obese subjects ($P=0.019$).

**Table 4.** Comparison of anthropometric, clinical and biochemical characteristics between different genotypes of Pro12Ala and C1431T polymorphisms in obese subjects.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Pro12Ala</th>
<th>C1431T</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro/Pro</td>
<td>70 (84.3)</td>
<td>13 (15.7)</td>
<td>-</td>
</tr>
<tr>
<td>Pro/Ala+Ala/Ala</td>
<td>81.6 ± 9.1</td>
<td>88.4 ± 11.8</td>
<td>0.019</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.019</td>
<td>82.3 ± 10.0</td>
<td>0.642</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>158.5 ± 8.1</td>
<td>161.2 ± 9.7</td>
<td>0.274</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>32.5 ± 2.6</td>
<td>33.9 ± 2.3</td>
<td>0.058</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>101.7 ± 9.4</td>
<td>104.9 ± 9.3</td>
<td>0.003</td>
</tr>
<tr>
<td>HC (cm)</td>
<td>110.0 ± 8.6</td>
<td>114.1 ± 6.7</td>
<td>0.094</td>
</tr>
<tr>
<td>W/H ratio</td>
<td>0.91 ± 0.07</td>
<td>0.98 ± 0.07</td>
<td>0.048</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.17 ± 1.70</td>
<td>4.95 ± 1.04</td>
<td>0.628</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.04 ± 0.19</td>
<td>1.12 ± 0.41</td>
<td>0.224</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>3.23 ± 0.84</td>
<td>3.17 ± 0.86</td>
<td>0.796</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>5.11 ± 0.85</td>
<td>5.01 ± 1.06</td>
<td>0.709</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>1.10 ± 1.32</td>
<td>1.79 ± 0.78</td>
<td>0.599</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>123.2 ± 21.0</td>
<td>130.1 ± 23.67</td>
<td>0.297</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>81.8 ± 11.5</td>
<td>83.7 ± 11.9</td>
<td>0.585</td>
</tr>
<tr>
<td>hs-CRP (mg/dL)</td>
<td>1.37 (0.83-3.11)</td>
<td>1.51 (0.88-3.50)</td>
<td>0.741</td>
</tr>
</tbody>
</table>

The independent sample t-test was used to compare anthropometric and clinical characteristics between different genotype carriers. Values are expressed as mean ± SD. *Adjusted for age and gender.

**Discussion**

Obesity as a chronic medical disease has shown increases in prevalence during recent years worldwide and requires enhanced research, treatment and prevention efforts. A growing body of evidence have demonstrated not only environmental factors including lifestyle of bad diet, inactivity as well as metabolic and endocrine abnormalities are the most important players in the etiology of obesity, but genetic factors also have a major role. Genome-wide association studies have clearly revealed that there is a genetic contribution to obesity [17]. Until now several candidate genes, including PPAR-γ2 gene have been searched in term of their involvement in the etiology of obesity and body fat accumulation. The PPAR-γ2 which has a key role in adipocyte differentiation, energy storage, insulin resistance and lipid metabolism, has gained interest as a good candidate gene for the metabolic related disorders [18].
Association of PPAR-γ2 polymorphisms with obesity

In recent years many studies have focused on the association between PPAR-γ2 gene variants and obesity to may clarify the molecular mechanism of obesity, but the results has been inconsistent. The present study set out to assess the association of two most common variants of PPAR-γ2 gene with obesity and obesity related markers in an Iranian population. Our results showed no significant association of PPAR-Pro12Ala and C1431T polymorphisms with risk of obesity, but suggest that Ala risk allele of Pro12Ala polymorphism is correlated with predisposition to obesity related markers. The results also did not detect any significant interaction between two polymorphisms so that all four possible haplotypes including Pro-C, Pro-T, Ala-C and Ala-T were in the same distribution frequency in both obese and nonobese groups, indicating no further association between the SNPs and the obesity.

These results are in agreement with our recent study in which PPAR-C1431T polymorphism in combination with the Pro12Ala polymorphism were associated with the risk of metabolic syndrome but, no association was found in genotypes distribution regard to BMI and central obesity as a metabolic syndrome related parameter [19]. On the other hand another research conducted in different region of Iran showed that although the PPAR-Pro12Ala allelic frequency in both obese and nonobese Iranian subjects is nearly similar to our results but on the whole the SNP was associated with obesity, and the presence of the Ala allele predicted a higher BMI [20].

These controversial results may be due to differences in ethnicity, geographic region, number of samples and inadequate statistical power, and environment. This inconsistency supports the notion of a potential contribution of ethnic or regional differences in modulating the eect of genetic polymorphism on various phenotypes [21]. Consistent with our results some studies revealed no significant associations [22-26].

Moreover, present study showed a significant association between Pro12Ala polymorphism genotypes containing Ala allele and some obesity related phenotypes including higher body weight, waist circumference and waist/hip ratio especially among obese subjects. These data supports the notion that genetic variability in PPAR-γ2 gene may influence body weight control and lipid homeostasis and individuals carrying the Ala12 allele may be more prone to develop obesity. These results were controversial compared to studies in other populations; they were in line with a study conducted by Meirhaeghe et al. in a sample of French subjects [27], however, studies on French Caucasian, Qatari and Cameroonian population reported different results [10,28-30].

Given to being at the center of many controversies, some meta-analysis has been conducted to acquire a more accurate assessment of the association between Pro12Ala polymorphism and obesity [6,11,31]. Although the result of a former meta-analysis performed by Paracchini et al. indicated no evidence of any association between the Pro12Ala polymorphism and obesity, but according to two other meta-analysis the Pro12Ala polymorphism may be one of the genetic factors in predisposing to obesity [6,11]. It was also reported that in healthy adults, Ala12 was associated with increased BMI under a dominant model of inheritance [32].

In line with our results many of previous studies showed no significant association between PPAR-C1431T polymorphism and obesity [8,33-35]. However, it was suggested that the polymorphism may be a modulator of insulin resistance in especially in T carriers [8]. As such, we also found obese individuals with T allele had lower level of HDL-C than those without T allele and the difference was also significant when adjusted by gender and age. This was inconsistent with the result of Yang’s Chinese population study in which T-allele carriers in metabolic syndrome relatives had higher HDL-C levels [36]. This means T allele may have the potential risk effect from developing obesity which is in conflict with the silent nature of synonymous nucleotide C to T substitution resulting no change in protein sequence. One possible explanation for the impact of this silent polymorphism in PPAR-γ2 activity is that it may be in linkage disequilibrium with another possible variant. However, this needs to be clarified in further studies.

It seems to be hard to explain these discrepancy results in PPAR-γ2 polymorphisms and obesity, but our result may have some explanations; one important reason is that these two polymorphisms are correlated with some markers of obesity, while some are protective and others are harmful. Others, as previously mentioned may be due to differences in ethnicity, geographic region, number of samples, and environment. The small sample size was an important limitation of our study, so some groups had fewer subjects and the statistical power might be insufficient to detect a weak genotype–phenotype association. This may lead to some bias in results. Further research with larger population should be done to confirm the results presented here. The last but not the least, the other variants of this gene may be associated with this phenotype and further studies are needed to clarify the fact that genetic variability in PPAR-γ2 may be associated with the obesity or its related phenotypes

Conclusion

In conclusion PPAR- C1431T and Pro12Ala polymorphisms did not show any significant association with the risk of obesity but Pro12Ala variant genotype was associated with some obesity related phenotypes including higher values of body weight, waist circumference and waist/hip ratio especially in obese subjects. This indicated, individuals carrying the Ala12 risk allele may be more prone to develop obesity. On the other hand individual carrying T allele at the C1431T variant also showed lower level of HDL-C, indicating the T allele may therefore be considered as a predisposing risk factor for obesity. Because of limited sample size, it is clear our findings should be interpreted within the context of its limitations and viewed as a preliminary for future studies in larger sample sizes and different ethnic groups to investigate a more subtle effect of this gene in this serious phenotype.
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References


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