Biomedical Research 2017; 28 (13): 5671-5677 *ISSN 0970-938X*
www.biomedres.info

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.

Accepted on May 09, 2017

Introduction

Obesity is a complex condition resulting from interplay between behavior, environment, and genetic factors. A growing body of evidence have identified that variants in a variety of genes involved in regulating of food intake, energy expenditure and body weight, may contribute in a polygenic manner in predisposing to obesity and of course only in a few cases are genes the primary cause of obesity [1]. Depending on the population examined, the contribution of genetic factors in the development of obesity is estimated to be 40-70% and the search for identification of polymorphisms in various genes contributing to susceptibility can be helpful to clarify the etiology of obesity and its metabolic complications or consequences, as well as identifying at-risk individuals [2,3]. Despite the identification of more than 227 genetic variants associated with obesity and fat distribution, considering the greater number of genes involved in different biological pathways has been yet of major interest [2]. Identification of those genes will help clarify a genetic profile with the goal of developing personalized prevention and treatment strategies.

Peroxisome proliferator-activated receptor gamma (PPAR-γ), a ligand-inducible transcription factor expressed predominantly in adipose tissue, is known to be involved in regulating a number of genes associated with adipogenesis, lipid metabolism, inflammation, and energy homeostasis [4]. Because of direct activation of PPAR-γ receptors by dietary fatty acids or related metabolites, they are also known as lipid sensors and are able to markedly redirect metabolism [5]. Previous studies have revealed some sever mutations in PPAR-γ gene especially in its ligand-binding domain, may cause extreme metabolic condition included insulin resistance, diabetes, dyslipidemia and hypertension, while common mild variants of this gene may contribute to the common, multifactorial forms of metabolic disorders [6,7].

Regarding to its functional role in metabolic pathways, *PPAR-γ2* has been one of the most commonly studied candidate gene for metabolic disorders, such as obesity. Among several polymorphisms reported in the *PPAR-γ2* gene, two common variant including a missense *Pro12Ala* polymorphism in exon B and a silent mutation *C1431T* in exon 6 have been studied in

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/m^2, and 150 as nonobese with BMI <30 kg/m^2. 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 wor. 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 χ^2 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.*

Characteristics	nonobese (n=150)	obese (n=83)	p value
Gender (M/F)	59/91	20/63	0.022
Age (years)	47 ± 7.76	48 ± 7.55	0.258
Weight (kg)	69.10 ± 10.29	82.68 ± 9.18	<0.001
Height (cm)	162.3 ± 8.9	158.9 ± 8.4	0.005
BMI (kg/m^2)	26.14 ± 2.40	32.71 ± 2.56	<0.001
Waist circumference (cm)	89.38 ± 10.2	103.13 ± 9.8	<0.001
Hip circumference (cm)	99.7 ± 5.8	110.6 ± 8.4	<0.001
W/H ratio	0.89 ± 0.08	0.93 ± 0.07	<0.001
Glucose (mmol/l)	4.52 ± 1.00	5.14 ± 1.50	0.002
HDL-C (mmol/l)	1.05 ± 0.17	1.05 ± 0.26	0.870
LDL-C (mmol/l)	3.10 ± 0.80	3.22 ± 0.84	0.350
TC (mmol/l)	4.89 ± 1.00	5.08 ± 0.87	0.165
TG (mmol/l)	1.74 ± 1.09	1.95 ± 1.25	0.002
SBP (mmHg)	118.4 ± 14.9	124.3 ± 21.5	0.016
DBP (mmHg)	77.6 ± 10.7	82.1 ± 11.5	0.004
Smoking (n (%))			
Yes	30 (20)	20 (24)	0.950
No	105 (70)	54(65)	
Former	15 (10)	9 (11)	
HSCRP	1.72 (0.85-3.37)	1.39 (0.83 ± 3.21)	0.168

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; W/H: 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

The genotypes distribution and allelic frequency and association between these two polymorphisms of *PPAR-γ2* gene and risk of obesity are summarized in Table 2. Genotype frequencies of two SNPs for the total sampled population and for each group separately were in accordance with Hardy-Weinberg equilibrium (P>0.05). In both SNPs due to the small number of homozygous genotypes comprised of rare alleles Ala and T respectively, data from Pro/Ala and Ala/Ala individuals and also from CT and TT subjects were combined and analyzed together. Both PPAR- *Pro12Ala* and *C1431T* variants showed no statistically significant differences between obese and nonobese subjects neither in crude nor after adjustment for covariates like age and gender.

Table 2. *Genotype distributions and Allele frequencies of the PPAR-γ2 polymorphisms in subjects.*

Genotype	Non-obese	Obese	Crude OR (95% CI) Obese *vs.* non-obese	p-value	†OR (95% CI) Obese *vs.* non-obese	P-value
Pro12Ala	150	83				
Pro/Pro	117 (78.7)	70 (84.3)	1.00	-	1.00	-
Pro/Ala	31 (20.7)	13 (15.7)	0.72 (0.35-1.46)	0.360	0.77 (0.37-1.59)	0.57
Ala/Ala	1 (0.7)	0 (0)				
Pro/Ala+Ala/Ala	32 (21.5)	13 (15.7)	0.67 (0.34-1.41)	0.315	0.75 (0.36-1.55)	0.44
Pro allele	267 (89.0)	153 (92.0)	1.00	-		
Ala allele	33 (11.0)	13 (8.0)	0.68 (0.35-1.32)	0.252		
C1431T	150	83				
CC	119 (79.0)	66 (80.0)	1.00	-	1.00	-
CT	24 (16.0)	15 (18.0)	1.14 (0.56-2.33)	0.711	1.10 (0.53-2.28)	0.550
TT	7 (5.0)	2 (2.0)	0.52 (0.11-2.59)	0.427	0.45 (0.09-2.26)	0.391
C allele	262 (87.3)	147 (89.0)	1.00	-		
T allele	38 (12.7)	19 (11.0)	0.62 (0.32-1.19)	0.154		

Data are reported as numbers with frequencies in parentheses. Because there were too fewer individuals with Ala/Ala genotype, Ala/Ala genotype was combined with Pro/Ala genotype in the chi-square/Fisher's exact test and Logistic regression analysis. OR, odds ratio; CI, confidence interval. †Adjusted for age and gender.

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 r^2 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, r^2=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.*

Gene	Haplotype	Frequency among non-obese	Frequency among obese	†OR (95% CI) Obese vs. non-obese	p-value
PPAR-γ^2	Pro-C	0.82	0.83	1	-
	Pro-T	0.08	0.09	1.02 (0.53 - 1.97)	0.95
	Ala-C	0.06	0.05	0.90 (0.36 - 2.27)	0.83
	Ala-T	0.04	0.03	0.55 (0.16 - 1.84)	0.33
LD	D'	0.332			
	r^2	0.294			

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.*

Characteristics	*Pro12Ala*			*C1431T*		
	Pro/Pro	**Pro/Ala+Ala/Ala**	**P***	**CC**	**CT+TT**	**P***
N (%)	70 (84.3)	13 (15.7)	-	66 (80.0)	17 (20.0)	-
Weight (kg)	81.6 ± 9.1	88.4 ± 11.8	0.019	82.3 ± 10.0	84.2 ± 9.4	0.451
Height (cm)	158.5 ± 8.1	161.2 ± 9.7	0.274	158.7 ± 8.6	159.6 ± 7.4	0.686
BMI (kg/m^2)	32.5 ± 2.6	33.9 ± 2.3	0.058	32.6 ± 2.6	33.0 ± 2.4	0.557
WC (cm)	101.7 ± 9.4	110.4 ± 9.3	0.003	102.9 ± 9.9	103.7 ± 10.0	0.746
HC (cm)	110.0 ± 8.6	114.1 ± 6.7	0.094	110.1 ± 8.5	112.2 ± 8.3	0.366
W/H ratio	0.91 ± 0.07	0.98 ± 0.07	0.048	0.94 ± 0.07	0.93 ± 0.08	0.66
Glucose (mmol/l)	5.17 ± 1.70	4.95 ± 1.04	0.628	5.19 ± 1.49	4.98 ± 1.751	0.636
HDL-C (mmol/l)	1.04 ± 0.19	1.12 ± 0.41	0.224	1.06 ± 0.22	1.04 ± 0.26	0.803
LDL-C (mmol/l)	3.23 ± 0.84	3.17 ± 0.86	0.796	3.31 ± 0.85	2.88 ± 0.73	0.089
TC (mmol/l)	5.11 ± 0.85	5.01 ± 1.06	0.709	5.14 ± 0.84	4.85 ± 0.99	0.241
TG (mmol/l)	1.10 ± 1.32	1.79 ± 0.78	0.599	2.00 ± 1.34	1.8 ± 0.79	0.542
SBP (mmHg)	123.2 ± 21.0	130.1 ± 23.67	0.297	125.9 ± 22.9	117.8 ± 12.7	0.177
DBP (mmHg)	81.8 ± 11.5	83.7 ± 11.9	0.585	82.6 ± 11.4	80.33 ± 8.7	0.594
hs-CRP (mg/dL)	1.37 (0.83-3.11)	1.51 (0.88-3.50)	0.741	1.37 (0.83-3.21)	1.41 (0.89-4.10)	0.417

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].

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.

Acknowledgments

Authors are very grateful to all participants for taking part in this study, all fieldworkers, data and laboratory staffs.

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