ASSOCIATION OF THE HUMAN PPAR γ 2 PRO12ALA POLYMORPHISM WITH OBESITY IN A POPULATION FROM TURKEY

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Abstract

Background. There have been a number of reports on the relationship between the PPAR γ 2 Pro12Ala genotype and the development of obesity.

Objective. A case-control survey was designed to investigate the potential association between a Pro12Ala polymorphism in the PPAR γ 2 gene and obesity and/or obesity-related phenotypes in a population from Turkey.

Materials and methods. The polymerase chain reaction and restriction enzyme digestion were used to genotype the Pro12Ala polymorphism of the PPAR γ 2 gene in 149 unrelated obese and 105 non-obese control subjects from Turkey. The data were analyzed statistically.

Results. We found that the overall minor allele frequency was 0.12 in cases and 0.095 in controls. In terms of genotype distribution and allele frequencies among the cases *versus* controls in the population studied, only the gender-stratified analysis revealed a significantly higher frequency of Pro/Ala genotype within males. The polymorphism was associated with significantly higher weight, height, waist circumference, central adiposity (waist-to-hip ratio, WHR), lean body weight as well as dry body weight, but not overall adiposity (total body fat percentage, TBF) in cases carrying Ala allele (Pro/Ala or Ala/Ala). However, in the subjects carrying Ala allele of the control group, WHR values were found significantly lower.

Conclusion. Our results showed that the Pro12Ala polymorphism in the PPAR γ 2 gene is associated with obesity in the studied adult population from Turkey. These data suggest that the Pro12Ala polymorphism in PPAR γ 2 may be a potential genetic risk factor for central obesity.

Key words: PPAR γ 2 gene, obesity, waist-to-hip ratio, central obesity, polymorphism.

INTRODUCTION

Obesity has become an important public health issue worldwide with its increasing prevalence and a risk factor for many illnesses such as Type 2 Diabetes and cardiovascular diseases (1). Obesity

is usually inherited in the polygenic, multifactorial mode of inheritance contributed by both genetic and environmental factors in the etiology of obesity, besides some rare monogenic forms, and characterized by a positive energy balance, and increase in the ratio of body fat (2). The current obesity pandemic is the result of an obesogenic environment with energy-dense foods and lack of physical activity in individuals who have a genetic susceptibility towards developing obesity (3).

Many candidate genes involved in the genetics of obesity have been linked with obesity-related phenotypes in different populations so far (2). One of them is a member of nuclear hormone receptor superfamily called Peroxisome Proliferator-activated Receptor γ (PPAR γ) gene expressed predominantly in adipose tissue. It modulates transcription of target genes involved in adipocyte differentiation, lipid oxidation and glucose metabolism (4). Given the importance of playing a role in adipose tissue metabolism, a variation at the genetic locus of PPAR γ could be involved in the aetiology of obesity trait. Several genetic variants in the human PPAR γ genes have been identified. The most prevalent one is the Pro12Ala polymorphism in PPAR γ -2 which is localized on chromosome 3 (3p25) (5). This variation, resulting from a cytosine to guanine substitution at nucleotide 34 of the exon B, leads to the synthesis of an alanine instead of proline in position 12 of PPAR γ -2 protein (5). This variant of the PPAR γ 2 gene has also been reported to be associated with obesity, but the available data on the relationship are inconsistent (8-9). In the early studies, although two studies have reported an association between the Ala allele, increased insulin sensitivity and decreased risk of type 2 diabetes (10, 11); later on, the reports studying the association between Pro12Ala polymorphism and obesity and relevant phenotypes have yielded controversial findings. Among those, some studies showed a positive association between the Pro12Ala variant of the PPAR γ 2 gene and obesity and/or obesity

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related phenotypes in a variety of populations (12-34), while the variant in question also yielded some negative results in other populations studied (35-40).

Considering the controversial results, we aimed at evaluating the impact of the Pro12Ala polymorphism of the human PPAR γ 2 gene on obesity and obesity related phenotypes as well as testing the hypothesis that the Pro12Ala variant is associated with measures of obesity in an adult sample from Turkey.

MATERIALS AND METHODS

Subjects

For the present association study, a total of 149 obese subjects (78 women and 71 men) having BMI values of 30 kg/m² and over (obese study group) and 105 non-obese control subjects (50 women and 55 men) whose BMI values were between 18 and 25 were included in this study. All participants were recruited from Obesity Clinic at Afyonkarahisar State Hospital and Medical Genetics Department in the Medical Faculty at Afyon Kocatepe University. The exclusion criteria for the study were Type 2 Diabetes, Cushing syndrome, hypo and hyperthyroidism and pregnancy. The study protocol was approved by the Local Ethics Committee at Afyon Kocatepe University and adhered to the Declaration of Helsinki. Informed consent was obtained from each participant.

Anthropometric measurements

All anthropometric measurements were carried out in the Physiology lab of the Medical Faculty at Afyon Kocatepe University. Anthropometric measurements (namely weight, height body mass index (BMI), waist circumference (WC), hip circumference (HC), waist-to-hip ratio (WHR) for each subject were determined by standard protocols. BMI was calculated as body weight (kg) divided by the square of the height (m²). Circumference measurements were taken with a 7 mm wide tape measure to calculate the WHR which reflects central obesity. On the other hand, body composition parameters such as body fat percentage, total body fat (TBF), lean body mass, body water percentage, total body water and dry lean weight, were determined by bioelectrical impedance analysis (BIA) system (Bodystat 1500, Bodystat Ltd., Douglas, Isle of Man, UK).

Sample Preparation and Genotyping

Peripheral blood samples were collected from all participants, and genomic DNA was isolated using a commercially-available Nucleospin DNA extraction

kit (Macherey-Nagel). The method used was essentially as described (5) with the following modifications. PCR amplification using a perfect match upstream primer (F: 5'-GCCAATTCAAGCCCAGTC-3') and a downstream mismatched primer (R:5'-GAC AGTGTATCAGTGAAGGAATCGCTTTCCG-3') (mismatched base is underlined) was used to create a BstUI restriction site in the PCR product only when the variant Ala allele (12Ala) is present. This pair of PCR primers encompassed the Pro12Ala polymorphism (rs1801282) and was used to genotype the Pro12Ala variant of PPAR- γ 2 following a polymerase chain reaction (PCR)-based enzymatic digestion (RFLP) strategy (5). Briefly, PCR was carried out in a 15-mL volume containing all sufficient ingredients for PCR. The conditions used for PCR amplification were initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, 60°C for 30 s, and 72°C for 60 s and a final elongation at 72°C for 10 min. Then the PCR products were digested with restriction enzyme BstUI (NEB) for 2 h followed by agarose gel electrophoresis (2%) stained with ethidium bromide. The ones having only one band of 258 bp were genotyped as CC (Pro/Pro) and two bands of 227 and 31 bp as GG (Ala/Ala) and the ones that have both 258 bp as well as 227 and 31 bp bands were genotyped as CG (Pro/Ala) as shown in Figure 1. Afterwards genotype distributions and allele frequencies were calculated for both obese and non-obese subjects.

Figure 1. PCR/RFLP analysis of PPAR γ 2 Pro12Ala genotype. PPAR γ 2 genotypes were determined by the PCR products digested with BstUI after separation on a 2.5% agarose gel followed by ethidium bromide staining and UV transillumination. The wild-type (Pro) allele results in 1 band of 258 bp (upper arrow), while the mutant (Ala) allele results in 2 bands of 227 bp and 31 bp. The representative genotypes are as follows: CC: Pro/Pro genotype (lanes 1, 3, 4, 5, 6, 7, 9, 10, 12), GG: Ala/Ala genotype (lanes 8), CG: Pro/Ala genotype (lanes 2), the 100-bp DNA ladder (lanes 11) (Axygen Biosciences).

Statistical analysis

Deviation of the genotypes from Hardy-Weinberg equilibrium was tested online. Statistical differences in genotypic and allelic distribution between obese subjects and non-obese controls were evaluated by χ^2 and Fisher's exact tests respectively. Kolmogorov-Smirnov and the Shapiro-Wilk tests were used to test the normality of data distribution (with $p > 0.05$ considered as a non-significant departure from normality). Since the data were found to be non-normally distributed, the Mann-Whitney U test was used to compare different variables and to evaluate if there is any significant difference using the Statistical Package for Social Sciences software version 18.0 (SPSS Inc., Chicago, IL, USA). Results were considered statistically significant at $p < 0.05$ between compared groups.

RESULTS

A total of 254 subjects including 149 obese and 105 non-obese controls were recruited in this study to conduct an association analysis between the Pro12Ala polymorphism in PPAR γ 2 gene and obesity and obesity related phenotypes. To carry out a case-control survey, genotype and allele frequencies for the Pro12Ala polymorphism of PPAR γ 2 gene were compared in obese cases *versus* non-obese controls first. None of the genotype distributions of the polymorphism studied in cases ($p = 0.965$) and controls ($p = 0.28$) deviated from Hardy-Weinberg equilibrium. The genotype frequencies of the Pro12Ala polymorphism in the study and control groups were not different as shown in Table

1 ($p = 0.454$). Likewise, the allele frequencies were not different among obese subjects as compared with controls ($p = 0.12$). The rare (variant) allele frequencies were 12% in cases and 9.5% in controls. Interestingly, separate analysis by gender revealed that obese men (but not women) had significantly higher frequency of the Pro/Ala genotype (29.6% *vs.* 12.7%; $\chi^2 = 6.077$, $p = 0.048$) compared to control men (Table 1).

In addition, some obesity related phenotypes (BMI, waist circumference, hip circumference, percentage fat, etc.) were also analyzed in groups in regard to genotypes. The data from Pro/Ala and Ala/Ala individuals were pooled and analyzed together due to the very low number of Ala/Ala genotypes at PPAR γ 2 Pro12Ala polymorphism. Pro/Ala+Ala/Ala genotype was associated with higher values of height, weight, WC, WHR, lean body mass, total mass as well as dry lean mass in obese study group (Table 2) while no association was observed with other obesity related phenotypes such as BMI, % body fat, and fat mass in obese study group. That is, compared to the carriers of Pro/Pro (CC) homozygotes, WC and WHR were significantly higher for the carriers of the Pro/Ala (CG) and Ala/Ala (GG) genotypes in the obese subjects (96.36 ± 10.58 *vs.* 103.05 ± 10.40 cm, $P = 0.018$; 0.86 ± 0.1 *vs.* 0.92 ± 0.9 , $P = 0.045$). Similarly, height and weight were significantly higher for the carriers of the Pro/Ala (CG) and Ala/Ala (GG) genotypes in the obese subjects (161.94 ± 8.15 *vs.* 167.58 ± 10.06 cm, $P = 0.02$; 93.32 ± 14.02 *vs.* 99.67 ± 14.54 , $P = 0.015$) compared to non Ala allele carriers. Also, lean body mass, total mass and dry lean mass were significantly higher for the carriers of the Pro/Ala (CG) and Ala/Ala

Table 1. PPAR γ 2 gene Pro12Ala Polymorphism Genotype Distribution and Allele Frequencies in Obese Cases *versus* Population-Matched Non-obese Controls in a population from Turkey

Subjects	n	Genotype*			χ^2	P- value	Allele*		P-value
		CC (Pro/Pro)	CG (Pro/Ala)	GG (Ala/Ala)			C (Pro)	G (Ala)	
Obese Cases	149	116 (0.779)	31 (0.208)	2 (0.134)			263/298 (0.88)	35/298 (0.12)	
Controls	105	85 (0.773)	20 (0.182)	0 (0.00)	1.58	0.454**	190/210 (0.905)	20/210 (0.095)	0.20***
Obese Men	71	49 (0.69)	21 (0.296)	1 (0.014)			119/142 (0.838)	23/142 (0.162)	
Control Men	55	48 (0.873)	7 (0.127)	0 (0.00)	6.077	0.048**	96/110 (0.873)	14/110 (0.127)	0.26***
Obese Women	78	67 (0.859)	10 (0.128)	1 (0.013)			144/156 (0.923)	35/298 (0.077)	
Control Women	50	37 (0.74)	13 (0.26)	0 (0.00)	4.12	0.128**	87/100 (0.87)	13/100 (0.13)	0.28***

* Genotype and allele data are presented as counts and frequency.

** Genotype P-values were calculated by χ^2 (2 df).

*** Allele frequency P-values were calculated by Fisher's exact test, 2-tailed.

Table 2. Comparisons of physical characteristics (anthropometric measurements) and body compositions of obese study group according to PPAR γ 2 genotype

Variable	Pro/Pro (n=116)	Pro/Ala+Ala/Ala (n=33)	P value**
Age	41.65 $\pm$ 9.99	43.15 $\pm$ 10.46	0.45
Height (cm)	161.94 $\pm$ 8.15	167.58 $\pm$ 10.06	0.02
Weight (kg)	93.32 $\pm$ 14.02	99.67 $\pm$ 14.54	0.015
BMI (Kg/m ²)	35.59 $\pm$ 4.89	35.55 $\pm$ 5.03	0.537
Waist (cm)	96.36 $\pm$ 10.58	103.05 $\pm$ 10.40	0.018
Hip (cm)	112.75 $\pm$ 9.8	111.5 $\pm$ 10.34	0.841
WHR	0.86 $\pm$ 0.1	0.92 $\pm$ 0.9	0.045
Body fat (%)	40.35 $\pm$ 9.27	38.05 $\pm$ 8.9	0.252
Body fat (kg)	38.15 $\pm$ 11.6	37.82 $\pm$ 10.49	1
Lean body mass	54.87 $\pm$ 12.62	61.84 $\pm$ 12.89	0.015
Total mass	93.28 $\pm$ 14.02	99.7 $\pm$ 14.62	0.039*
Dry lean mass	15.29 $\pm$ 4.75	17.95 $\pm$ 5.47	0.035
Water (%)	43.35 $\pm$ 5.9	44 $\pm$ 5.54	0.596
Water (kg)	40.37 $\pm$ 7.58	43.88 $\pm$ 8.36	0.054

*Phenotypic data are presented as counts $\pm$ standard deviation.

**P values were calculated using Mann-Whitney U test and P values of <0.05 between compared groups were regarded as statistically significant.

Table 3. Comparisons of physical characteristics (anthropometric measurements) and body compositions of non-obese control group according to PPAR γ 2 genotype

Variable	ProPro (n=85)	ProAla + AlaAla (n=20)	P value**
Age	30.11 $\pm$ 10.01	31.25 $\pm$ 12.52	0.75
Height (cm)	166.87 $\pm$ 9.32	163.47 $\pm$ 7.77	0.26
Weight (kg)	63.49 $\pm$ 10.49	61.18 $\pm$ 10.37	0.71
BMI (Kg/m ²)	22.68 $\pm$ 2.32	22.81 $\pm$ 3.06	0.674
Waist (cm)	75.58 $\pm$ 8.2	72.38 $\pm$ 7.63	0.29
Hip (cm)	93.34 $\pm$ 5.08	94.74 $\pm$ 7.53	0.578
WHR	0.81 $\pm$ 0.08	0.77 $\pm$ 0.08	0.034
Body fat (%)	25.27 $\pm$ 8.49	25.96 $\pm$ 8.37	0.629
Body fat (kg)	15.8 $\pm$ 5.27	16 $\pm$ 6.33	0.99
Lean body mass	47.77 $\pm$ 10.58	45.18 $\pm$ 8.89	0.313
Total mass	63.56 $\pm$ 10.28	61.18 $\pm$ 10.37	0.528
Dry lean mass	15.2 $\pm$ 4.7	12.56 $\pm$ 3.53	0.123
Water (%)	53.03 $\pm$ 7.36	53.67 $\pm$ 6.55	0.711
Water (kg)	33.77 $\pm$ 7.27	32.61 $\pm$ 5.89	0.62

*Phenotypic data are presented as counts $\pm$ standard deviation.

**P values were calculated using Mann-Whitney U test and P values of <0.05 between compared groups were regarded as statistically significant.

(GG) genotypes in the obese subjects (54.87 $\pm$ 12.62 vs. 61.84 $\pm$ 12.89 cm, P = 0.015; 93.28 $\pm$ 14.02 vs. 99.7 $\pm$ 14.5462, P = 0.039; 15.29 $\pm$ 4.75 vs. 17.95 $\pm$ 5.47 P = 0.035) compared to non Ala allele carriers.

In non-obese subjects, carriers of Pro/Ala+Ala/Ala had significantly lower WHR values compared to the subjects with Pro/Pro genotype. (0.81 $\pm$ 0.08 vs. 0.77 $\pm$ 0.08, P = 0.034) (Table 3). However, no association was observed in all other anthropometric variables such as BMI, hip circumference, % body fat, fat mass, etc. studied in non-obese group (Table 3).

Overall, these results show a significant association between the Pro12Ala polymorphism in PPAR γ 2 gene and obesity, particularly with central obesity and body mass.

DISCUSSION

PPAR γ 2 is one of the candidate genes in obesity. This gene is expressed predominantly in adipose tissue and plays a role in adipocyte differentiation, lipid oxidation as well as glucose metabolism (4). A number of association studies have been carried out to assess the association of the PPAR γ 2 gene Pro12Ala polymorphism with obesity and obesity related phenotypes. Human studies regarding the association of the PPAR γ 2 gene with obesity have shown contradicting results among different populations. Many of them showed positive associations with the risk of obesity and obesity related phenotypes such as BMI, fat percentage, fat mass, waist circumference and waist-to-hip ratio (12-34), while other studies have

failed to detect any significant associations in several populations (35-40). The main aspect of the current study was to investigate the association of PPAR γ 2 gene Pro12Ala polymorphism with obesity and obesity related phenotypes like BMI, circumferences of waist and hip, WHR, body fat percentage and total body fat, etc. As a result, this study mainly showed a gender-specific significant higher frequency of ProAla genotype in obese men only together with higher weight, height, WC and WHR mean values in all obese cases carrying Ala allele than the subjects having homozygous Pro allele. Thus, it indicated a significant association of PPAR γ 2 gene Pro12Ala polymorphism with risk of obesity, in particular, central obesity and body weight. In general, our results are in agreement to the previous studies in which PPAR γ 2 gene Pro12Ala polymorphism was associated with obesity.

Moreover, even association studies having positive results have shown associations with different aspects of obesity phenotypes. For instance, in a number of studies carried out in various populations, the Ala variant was associated with increased body mass index (BMI) [12-22], while in Finns, this variant was associated with decreased BMI and the Ala allele was associated with higher BMI and a higher weight gain over time in obese men, but a lower BMI and a slower weight gain in controls in Danish and Portuguese studies (23-24, 34). It was also found to be associated with higher hip circumference and skin fold thickness (25) as well as higher body fatness, waist circumference and central obesity (13, 26, 18, 27, 21). In this study, we observed that the Ala-allele carriers in the obese group were found to possess a significantly higher mean of WC and WHR than non-carriers showing a significant association of PPAR γ 2 Pro12Ala polymorphism with risk of obesity. In particular, individuals carrying the Pro/Ala or Ala/Ala genotype had higher values of waist circumference and waist-to-hip ratio among obese subjects, thus supporting the role of PPAR γ 2 Pro12Ala polymorphism on central obesity. Additionally, the finding of significantly higher values of waist circumference and waist-to-hip ratio values among only among obese subjects carrying the Ala allele shows that this particular allele constitutes a relative risk for central obesity in obese subjects. On the other hand, the fact that the individuals carrying the Ala allele had lower levels of waist-to-hip ratio among non-obese group was considered interesting and the details of these different effects of the PPAR γ variant on the obese and non-obese groups must be further explored in other studies as also observed and discussed

in another report by Ek *et al.* (24). However, it suggests that the variant may interact with various combinations of genetic and environmental factors in lean and obese subjects to cause divergent modulating effects on WHR and body weight control as also suggested in a Danish study for BMI (24).

Some studies also reported that this polymorphism had a gender-specific effect on different obesity measures (24, 153, 28-29), besides overall obesity risk (30, 22). Our study also supports that notion only with a significantly higher frequency of ProAla genotypes in males (showing a male-specific effect for the risk of obesity), not with all obesity measures. This gender-specific effect may be attributed not only to the modulation of the action of PPAR γ variants by some hormonal, genetic and environmental factors but also to the differences in fat-distribution patterns among genders as well as sex-specific lifestyle components such as differences in smoking habits, physical activity, diet or alcohol consumption acting possibly as modifiers of the effect of gene variants in determining the risk of obesity as also suggested by several studies (41-42, 18, 28-29, 31).

We also observed in this study that the carriers of Ala/Ala genotype had a higher weight and height values without any significant difference in BMI, and body fat percentage as well as total body fatness, than Pro/Pro homozygotes in the obese group. In addition, in this study, the observation that Ala allele carriers (Pro/Ala and Ala/Ala) had a higher height, weight, lean fat mass and lean body mass than Pro/Pro homozygotes while having normal level of BMI and fat mass among obese subjects suggests that PPAR γ 2 Pro12Ala polymorphism might have an impact on body size (mass) independent of its effect on body fat distribution (32, 14, 16, 33, 19).

Finally, since obesity has a multi-factorial inheritance, more studies must be done to clarify the role of different genes and SNPs. In this study, the associations between the PPAR γ 2 gene and obesity along with obesity-related phenotypes were analysed. To our knowledge, this is the first association study between the PPAR γ 2 Pro12Ala polymorphism and central obesity in an adult population of Western Turkey. This finding is quite important because central obesity is considered to cause many cancers, diabetes liver adiposity and cardio-vascular diseases compared to other types of obesity. It has been established that waist circumference and WHR is an indicator of central obesity (80 cm and 85 cm and over in women and 94 cm and 95 cm and over in men respectively)

and also constituting a risk for many disorders such as cardiovascular system diseases and diabetes (43). Therefore, these results suggest that Pro12Ala gene Pro12Ala polymorphism, Ala allele in particular, might be a risk factor for central obesity in obese cases in the studied population. It also suggests that “Ala” allele might be associated with having a protective effect in non-obese controls for central obesity.

One of the limitations of this study is relative small sample size. Therefore, one must be careful to point out that this study will have to be repeated in other centres with larger numbers before the results are accepted as conclusive and it needs to be confirmed further to reveal its exact role in obesity.

In conclusion, we show that the Pro12Ala polymorphism in the PPAR γ 2 gene is associated with obesity. In fact, obese men had significantly higher frequency of Pro/Ala genotype compared to controls. In addition we have identified several associations with obesity-related phenotypes related to central obesity and body size that might constitute a risk for obesity. Besides, this is the first report showing its allelic frequency and association with obesity in an adult population from Turkey. Moreover, this study presents evidence which supports the role of PPAR γ 2 gene 45-bp Pro12Ala polymorphism in obesity. These results are very interesting and they can contribute to a substantial improvement for elucidating the genetic factors involved in the development of obesity in Turkish population. However, these results require confirmation in other populations and additional studies are required to elucidate the role of PPAR γ 2 in the pathophysiology of obesity and metabolic disorders.

Conflict of interest

The authors declare that they have no conflict of interest concerning this article.

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