ORIGINAL RESEARCH

Pro12Ala Polymorphism of the PPAR-γ Gene is not Associated with BMI and Energy Metabolism among Healthy Nigerian Males

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To cite this article: Olasore HSA, Adebisi AA, Ehima VO, James AB, Ojewunmi OO, Oguntona TS and Thompson VO. Pro12Ala Polymorphism of the PPAR-γ Gene is not Associated with BMI and Energy Metabolism among Healthy Nigerian Males. Journal of Basic and Social Pharmacy Research, 2020;1(4):43-53.
ISSN: 2705-3245

ABSTRACT

Background: Peroxisome proliferator-activated receptors (PPARs) are a nuclear hormone receptor superfamily of ligand-activated transcription factors while the PPAR-γ subtype regulates adipocyte differentiation, lipid metabolism and insulin sensitivity. The Pro12Ala (rs1801282) polymorphism of the PPAR-γ has been linked to obesity in some populations.
Objectives: The present study aimed to find the relationship between PPAR-γ Pro12Ala polymorphism, energy metabolism and obesity among Nigerians males.
Methods: One hundred and fifteen male participants were recruited and their physical parameters such as weight and height were measured. Blood samples were taken for the analysis of metabolic parameters (fasting blood sugar, electrolytes and lipid profile) and DNA extraction, while genotyping was done by restriction fragment length polymorphism (RFLP) method. Blood samples were also analysed for metabolic parameters, Deoxyribonucleic Acid extraction and genotyping by restriction fragment length polymorphism (RFLP) method.
Results: Results show that the frequencies of the C (Pro) and G (Ala) alleles were 74.8 % and 58.2 % while the genotype frequencies of Pro/Pro (CC), Pro/Ala (CG) and Ala/Ala (GG) were 54.8 %, 40.0 % and 5.2 % respectively within the population sampled. The genotype distribution was not in Hardy-Weinberg equilibrium (χ^2=0.422, $p<0.001$). The distribution of Pro and Ala alleles was not significantly different ($p>0.05$) between different BMI categories. The physical and metabolic parameters (except plasma sodium and chloride) measured were not also significantly different ($p>0.05$) between genotype groups as analyzed by ANOVA.
Conclusion: No association was found between the Pro12Ala polymorphism of the PPAR-γ gene and BMI in the Nigerian population sampled. Future studies involving females and a larger population are recommended.

Keywords: PPAR-γ, Pro12Ala gene polymorphism, Body mass index, Obesity.

INTRODUCTION

Being overweight or obese is part of a continuum which simply shows an individual has a weight too high for the given height. These individuals are said to have a body mass index (BMI) above the range considered normal (18.5 – 24.9)[1]. Having a BMI above normal range has been linked to a host of pathological conditions such as

cardiovascular diseases, diabetes and cancer[2,3,4]. Besides, being overweight or obese is a leading cause of problems with self-image among youths who are inundated with images of celebrities in entertainment industry and on social media[5,6]. These psychosocial problems lead to the youths engaging in practices which further endanger their physical and psychological health. Excess weight gain is believed to be caused by an imbalance in energy intake and energy expenditure. High caloric intake and sedentary lifestyle as found in the developed societies contributes greatly to high incidence of obesity. However globally, the outbreak of COVID-19 pandemic has led to a less active lifestyle in many societies due to lockdown which has greatly increased the incidence of obesity[7,8]. Excess calorie is often stored in the form of fat especially triglycerides, in the adipose tissue[9]. Molecular machinery involved in the fat synthesis, transport and storage is essential in this process[10]. Peroxisome proliferator-activated receptors (PPARs) are a nuclear hormone receptor superfamily of ligand-activated transcription factors. They regulate gene expression by binding with retinoid X receptor as a heterodimeric partner to specific DNA sequences termed PPRA response element located in the 5'-flanking region of target genes[11]. The gamma subtype of PPARs (PPAR-*γ*), is pharmacologically important as it is a molecular target of the insulin sensitizers, thiazolidinediones (glitazones) used in treatment of type 2-diabetes. PPAR-γ has been suggested to play important roles in obesity, adipogenesis and the development of type 2 diabetes and it influences insulin sensitivity and lipid metabolism[12,13]. As a biomolecule involved in regulation of energy metabolism, PPAR-γ is a potential target for weight control drugs. The human *PPAR-γ* gene has been mapped to chromosome 3 (3p25)[14,15]. Quite a number of genetic variations in the human *PPAR-γ* gene have been discovered and the most studied one is the functional Pro12Ala polymorphism which has been found to result in the reduction of the activity of PPAR-γ[16,17]. The physiological effect of this functional polymorphism has also been confirmed experimentally[18]. This polymorphism results from substitution of cytosine to guanine at nucleotide 34 of exon B, changing the codon for proline to alanine and leading to the synthesis of alanine rather than proline at position 12 of PPAR-γ2 protein[19]. The location of this amino acid substitution is in the PPAR-γ2 AF-1 domain, which controls the ligand-independent ability to activate target gene expression. It has been demonstrated by in-vitro studies that the presence of the PPAR-γ 12Ala allele is related to a diminished affinity of PPAR-γ for the peroxisome proliferator response element (PPRE) sequence in target gene promoters[20]. This has resulted in a decrease of their expression level, confirmed by in-vivo studies[21,22]. Since the PPAR-γ is a drug target for thiazolidinediones, the functional Pro12Ala polymorphism of the PPAR-γ is therefore of major pharmacogenomic and pharmacogenetic importance.

The Ala12 variant of the *PPAR-γ*2 gene has been shown to influence the risk for obesity in various ethnic populations worldwide[23-25]. However, there have been some discrepancies in the findings from various populations especially in the association of the Pro12Ala polymorphism and adiposity[24,25]. The aim of the present work was to determine the relationship between the Pro12Ala polymorphism, BMI and some metabolic parameters among Nigerian males. The male population only was considered in the present study to eliminate the possible influence of the female hormonal changes on the metabolic parameters.

MATERIALS AND METHODS

Ethical Considerations and Participants

Before the commencement of the study, the approval of the Human Research Ethics Committee (HREC) of the College of Medicine of the University of Lagos was sought. The ethical approval with number CMUL/HREC/10/18/447, was obtained.

One hundred and fifteen healthy male participants were recruited for the study. Only males were recruited for the study in order to rule out the effect of female hormonal changes on the metabolic parameters. The participants were students and their peers who were willing to participate and they all provided written informed consent prior to enrolment. The weight and height of the participants were checked using a standard weighing scale and stadiometer. The body mass index was also calculated by dividing the weight (in kilograms) by the square of height (in metres). Venous blood was taken in the morning after an overnight fast for DNA extraction and assays for plasma biochemistry.

Biochemical assays

Assays for fasting blood sugar (FBS), plasma total cholesterol (TC), high density lipoprotein-cholesterol (HDL-chol) and triglycerides (TG) analyses as well as plasma sodium and chloride were performed using Hitachi 704 auto-analyzer serviced by Roche Diagnostics (Indianapolis, IN, USA).

DNA extraction and genotyping

High molecular weight genomic DNA was prepared from peripheral blood samples using a Quick- DNA™ Miniprep Plus Kit (Zymo Research, CA, USA) based on spin column method of DNA extraction. The DNA yield was determined using Nanodrop 1000 (Thermo Scientific, USA). The yield was found to be between 45-55 µg/ml of whole blood.

The PPAR-γ Pro12Ala polymorphism (rs180128) was genotyped by polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) analysis. The sequences of the primer set used are as follows: forward-5'-GCCAATTCAAGCCCAGTC-3'; reverse-5'-GATATGTTTCGAGACAGTGTATCAGT GAAGGAATCGCTTTCCG-3'. PCR was carried out in a reaction volume of 25 µl, containing 12.5µl of 2x master mix with standard buffer (New England Biolabs, USA), 1µl each of forward and reverse primer, 8.5 µl of nuclease free water and 2 µl of genomic DNA. The PCR cycling profile was: initial denaturation at 94ºC for 1min followed by 35 cycles of denaturation at 94 ºC for 30 s, annealing at 55 ºC for 30 s, and extension at 68 ºC for 30 s and then a final extension at 68 ºC for 5 min. The PCR products were digested for 65 min at 60 ºC with *Bst*UI. The products were electrophoresed on 2.5 % agarose gels stained with ethidium bromide and visualized using Alpha Imager HP (Alpha Innotech, USA).

Statistical Analysis

All statistical analyses were carried out using the SPSS version 25 software (SPSS Inc. USA). The descriptive statistics as well as the allele and genotype distribution was done using frequency estimation. Analysis of variance was used to estimate the mean differences in the physical and metabolic parameters across the genotype groups. Student's t-test was used for pairwise analysis of parameters across genotype groups. Hardy-Weinberg equilibrium (HWE) was estimated using Chi-square test. Value of $p<0.05$ were considered statistically significant.

RESULTS

Table 1 show the description of the study population. Most of the participants (46.1 %) were between 1.60 m and 1.69 %. Only 5.2 % of them were below 1.6 m, while 13.9 % were 1.8 m and above. Similarly, the weights of most participants (39.1 %) was between 60.0-69.0 kg with 14.8 % weighing 80.0 kg and above while only a few (3.5 %) weighed 50.0 kg and below. By BMI, only 5.0 % in each case met the criteria for being underweight or obese. Majority (63.5 %) were within normal BMI range while 27.8 % were overweight.

Table 1: Descriptive statistics of the study population

Age (mean ± SD) years	20.57 ± 2.34
Height n (%)	
Below 1.6 m	6 (5.2)
1.60-1.69 m	53 (46.1)
1.70-1.79 m	40 (34.8)
1.8 m and above	16 (13.9)
Weight n (%)	
Below 50 kg	4 (3.5)
50-59 kg	14 (12.2)
60-69 kg	45(39.1)
70-79 kg	35 (30.4)
80 kg and above	17 (14.8)
Obesity status n (%)	
Underweight (BMI <18.5)	5 (4.3)
Normal (BMI 18.5-24.99)	73 (63.5)
Overweight (BMI 25-29.99)	32 (27.8)
Obese (BMI 30 or higher)	5 (4.3)

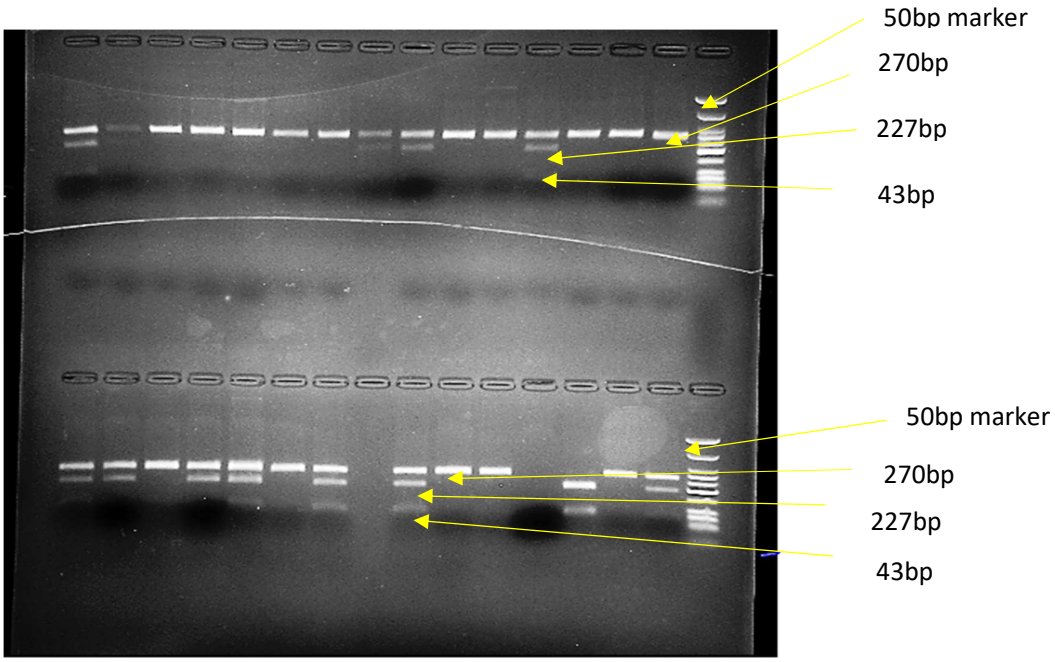

Figure 1: A two-tier Gel electropherogram of *Bst*UI digested PCR products - From right to left, the first lane of each tier shows the 50 bp marker, while the 15 other lanes are sample lanes. The presence of a whole uncut 270 bp fragment means *PPAR-γ2* site absent (Pro12 allele) while the presence of 227 bp and a 43 bp fragments means *PPAR-γ2* site present (Ala12 allele)

Figure 1 shows gel electropherogram of the amplicons, resolved on 2.5 % agarose.

As presented in Table 2, the Pro12 (C) allele was present in 74.8 % while 25.2 % of the participants had and Ala12 (G) allele. The majority of the participants making up 54.8 % were homozygous for the Pro12 allele while only 5.2 % were homozygous for the Ala12 allele. Most of the Ala12 allele existed in the heterozygous (Pro/Ala) form accounting for 40.0 %. The genotype

frequency was not in Hardy-Weinberg equilibrium (χ^2 =0.422, p>0.05).
Allelic distribution across the BMI categories is shown in Table 3. The same pattern was seen for both Pro12 and Ala12 alleles in their distribution. The highest frequencies for both Pro12 and Ala12 (61.5 % and 71.5 % respectively) were found among the participants with normal BMI. The lowest frequencies of both Pro12 and Ala12 allele (4.6 % and 3.8 % respectively) were found among the underweight with 4.6% and 5.8% respectively among the overweight. These distributions show that there was no association between the two allelic variants and BMI.

Table 2: Allele and genotype distribution within the study population

	Frequencies (%)		
Alleles	**Pro (C)**	**Ala (G)**	
	172 (74.8)	58 (25.2)	
Genotypes	CC (Pro/Pro)	CG (Pro/Ala)	GG (Ala/Ala)
	63 (54.2)	46 (40.0)	6 (5.2)
	χ^2	df	p
Hardy –Weinberg Equilibrium	0.422	1.000	>0.05

Table 3: Allele distribution among BMI categories

	Allele distribution n(%)	
BMI Category	**C (n=109)**	**G (n=52)**
Underweight (BMI <18.5)	5 (4.6)	2 (3.8)
Normal (BMI 18.5-24.99)	67 (61.5)	37 (71.2)
Overweight (BMI 25-29.99)	32 (29.4)	10 (19.2)
Obese (BMI 30 or higher)	5 (4.6)	3 (5.8)

Table 4: Association between PPAR-γ Pro12Ala genotypes and physical and plasma metabolic parameters

Genotype	Pro/Pro (Mean ± S.D)	Pro/Ala (Mean ± S.D)	Ala/Ala (Mean ± S.D)	F	p
Weight (kg)	68.90 ± 10.46	68.68 ± 11.40	61.08 ± 5.78	1.488	0.230
Height (m)	1.69 ± 0.08	1.71 ± 0.07	1.68 ± 0.11	0.537	0.588
BMI (kg/m)	23.99 ± 3.38	23.57 ± 3.59	21.82 ± 2.15	1.161	0.317
Sodium (mmol/L)	144.60 ± 4.78	144.10 ± 5.06	151.50± 9.72	5.451	0.006**
Chloride (mmol/L)	102.70 ± 7.92	104.40 ± 7.51	95.65 ± 12.32	3.272	0.042*
FBS (mg/dL)	94.89 ± 15.27	97.22 ± 17.09	88.83 ± 15.55	0.825	0.441
HDL-c (mmol/L)	1.41 ± 0.32	1.36 ± 0.40	1.035 ± 0.32	3.054	0.051
TC (mmol/L)	4.11 ± 0.92	4.21 ± 0.79	3.75 ± 1.48	0.715	0.492
TG (mmol/L)	1.01 ± 0.41	1.01 ± 0.42	1.54 ± 1.62	2.820	0.064

Key: *=significant; **=very significant

Table 4 shows the comparison of the mean values of the physical and metabolic parameters evaluated across all the three genotype groups. There was no significant difference (p>0.05) in all the parameters presented across all the genotype groups. This indicates that the genotypes had no influence on these metabolic parameters in the study population. However, in contrast to other, the mean plasma values of sodium and chloride were found to be significantly different (p>0.05) across all the genotype groups.

A Student's t test was carried out for a pairwise comparison of all the genotype groups with respect to all the parameters measured. It was found that only HDL-cholesterol was significantly different ($p<0.04$) when the homozygous Pro12 group was compared to the homozygous Ala12 group. The result for this analysis alone is presented in Table 5 while others are not shown because the pairwise comparison of other parameters did not show significantly different results across the various genotype groups.

Table 5: **Pairwise comparison of HDL-cholesterol levels across the PPAR-γ2 genotype groups**

PPAPR- γ2 Genotype	Mean values	Adjusted P value
CG/CC	1.363/1.407	0.7999
CG/GG	1.363/1.035	0.0870
CC/GG	1.407/1.035	0.0400*

DISCUSSION

Many genes have been linked to energy metabolism especially the storage of excess calorie in the form of fat deposit in the adipose tissue and these include the leptin and ghrelin genes[26,27,28]. The present study examined the relationship between PPAR-γ Pro12Ala polymorphism, physical and metabolic parameters associated with obesity. The allele and genotype frequencies of the Pro12Ala polymorphism found in this population were very different from what have been previously reported in most other populations. In most of these populations, the frequency of the Pro12 allele ranged between 88.0% among the Indians[29], to 97.8% among some Asian populations[30]. However, the frequency of the Pro12 allele in this study population was close to what was reported in a Ukrainian study with a frequency of 72.0 %[31]. The Pro12 allele is the major allele and the frequency is far higher than the Ala12 allele in most populations studied so far including African populations[29,30,31]. A study conducted among the Greeks seems to present the lowest occurrence of the Pro12 allele at 32.0 %[32]. It was noted among the populations cited above that while the most frequent genotype was the Pro/Pro, most of the few Ala12 existed in heterozygous form (Pro12Ala) while the homozygous Ala12 was not present in many of the populations as it is considered a fairly rare allele[29,30].

In this study, no significant association was found between the Pro12Ala polymorphism and the physical parameters such as height, weight and BMI. This is in contrast to many studies that have reported a significant influence of the polymorphism on weight and BMI. Mansoori and colleagues[33] found that presence of Ala12 variant was associated with increased BMI. Another study reported an association between this polymorphism and weight gain among short children born small for gestational age during growth hormone treatment[34]. An association was found between polymorphism and obesity among coronary artery disease and type 2 diabetes patient, in a previous study[35].

Contrary to the above studies and in line with our results, some authors have reported no association between the physical parameters mentioned above and the Pro12Ala polymorphism. A Nigerian investigation of this association among type 2 diabetes patients found a lack of significant association between this gene polymorphism and obesity[36]. Cardoso and colleagues[25] also reported no significant

association between the Pro12Ala gene polymorphism and weight among participants undergoing aerobic training further confirming our results that although PPAR-γ Pro12Ala is a functional polymorphism, it may not influence fat metabolism in a way that leads to weight changes.

Although in the study conducted by Engwa and colleagues[36], cited above, there was an association between the PPAR-γ gene polymorphism and lipid profile, we did not find such association in our study. We also found no correlation between this polymorphism and fasting blood sugar contrary to our expectation as PPAR-γ is known to regulate both lipid and glucose metabolism[12]. In line with our results, a Turkish study could not find any correlation between plasma fasting blood sugar, lipid profile and the Pro12Ala polymorphism[37]. Arko-Bonham and colleagues[38] also reported no correlation between plasma fasting glucose and Pro12Ala polymorphism, while Orion and colleagues[39] found no association with plasma lipids and fasting blood sugar.

However, Bercer and Çırakoğlu[40] found an association between the polymorphism under study and lipid profile, despite finding no direct association between this polymorphism and obesity. Similarly, Alves and colleagues[41] found a relationship between plasma lipid profile and the PPAR-γ gene variation. In addition, a correlation was found between the Pro12Ala gene polymorphism and dyslipidemia[42], while a Palestinian study also found a lower fasting blood glucose among individuals carrying the Ala12 allele[43]. The Ala12 allele has been reported to have an insulin-sensitizing effect on the liver and skeletal muscles which can lead to the suppression of lipolysis in adipocytes and diminish the release of free fatty acids. This reduced availability of fatty acids causes a specific shift of energy metabolism to anaerobic metabolism with a simultaneous increase in glucose consumption in active skeletal muscles[44,45]. In spite of these expected effects of the Ala12 allele, inconsistencies have been found in the reports of the relationship between this genetic locus and lipid and glucose metabolism.

Variations in the association between the plasma metabolites and the PPAR-γ Pro12Ala polymorphisms as discussed above indicate that this genetic locus may be acting with other loci to produce different effects. This may especially be true considering the fact that the studies were carried out at different locations with populations that may have different genetic traits. The physical and metabolic parameters studied in this work and the related works presented above are not monogenic traits that are controlled by a single gene locus, but rather multigenic. Environmental factors could also be a major reason, while it must also be noted that some of the studies were conducted using study populations with different physiological and pathological correlation such as physical exercise and diabetes mellitus[25,35-37]. These conditions may influence the interactions of these genetic changes and the measured parameters. In the present study, healthy male volunteers were used to eliminate the effect of adapted physiological and pathological conditions. Males were used to eliminate effect due to hormones and differences in measured physical and metabolic parameters.

In most of the African populations cited[35,36,38], the Ala12 allele were not found. Where a few were found, they existed in the heterozygous form. Increasing the sample size above what was used in most of the studies referenced above would increase the statistical power of the study thus increasing the possibility of finding more Ala12 allele with more pronounced effect. Student's t-test in a pairwise analysis was done to show the difference between the physical and metabolic parameters across various genotype groups. We noticed a significant difference in the homozygous Pro/Pro genotype group when compared to the Ala/Ala group in the levels of HDL-cholesterol.

Interestingly and contrary to the findings on other metabolic parameters, a significant association was found between PPAR-γ Pro12Ala polymorphism and plasma sodium and chloride. Searching the literature, no work was found linking this polymorphism to plasma sodium and chloride. However, it has been reported that PPAR-γ modulates the expression of angiotensin converting enzyme (ACE)[46]. This leads to the reduction in the level of angiotensin II and the angiotensin-induced aldosterone secretion resulting in decreased water and sodium reabsorption[47,48]. We found plasma sodium to be higher in homozygous Ala12 group and this variant of the PPAR-γ has been reported to have reduced affinity for the peroxisome proliferator response element (PPRE) sequence in target gene promoters[20]. This may lead to disinhibition of the ACE expression to ultimately result in increased sodium reabsorption.

Limitations of the study

The size of the population used for this study was just over a hundred participants. The study focused only on male subjects. Future studies should consider including females and increasing the sample size in order to increase statistical power thus enhancing the probability of having more Ala12 alleles to be captured.

CONCLUSION

Although this study found no association between the PPAR-γ Pro12Ala gene polymorphism, BMI, plasma lipid profile and fasting blood sugar, there was a correlation between this polymorphism and plasma sodium and chloride levels. This study was also able to show that when the homozygous forms of the two alleles were compared, there was significant reduction in plasma HDL-cholesterol.

ACKNOWLEDGEMENTS

The authors are grateful to all those that consented to participate in the study. We declare no conflict of interest as regards this research work.

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