RESEARCH ARTICLE

Peroxisome proliferator activated receptor gamma 34C>G variant and anthropometric parameters in metabolic syndrome

Uzma Zafar, Saba Khaliq, Hafiz Usman Ahmad, Khalid Pervaiz Lone

Abstract

Objective: To determine the frequency of 34 Cytosine >Guanine (proline 12 alanine) variant of peroxisome proliferator activated receptor gamma, and to associate it with metabolic syndrome, insulin resistance and anthropometric obesity parameters.

Methods: The cross-sectional comparative study was conducted at the University of Health Sciences, Lahore, Pakistan, from September 2016 to 2017, and comprised patients of metabolic syndrome and healthy controls. Blood pressure and anthropometric measurements of all the subjects were recorded. Fasting blood sample of 4ml was taken for biochemical parameter and deoxyribonucleic acid extraction. The frequency of genetic variant was determined by amplification refractory mutation system polymerase chain reaction. Data was analysed using SPSS 22.

Results: Out of 400 subjects, 200 (50%) each were patients and controls. Overall, there were 308 (77%) males and 92 (23%) females. Patients had significantly higher blood pressure, body mass index, waist circumference, waist-to-hip ratio, mid-arm circumference and triceps skinfold thickness compared to the controls ($p<0.0001$). Insulin resistance was also significantly higher in the patients ($p<0.0001$) and showed significant correlation with body mass index, waist circumference, waist-to-hip ratio, mid-arm circumference and triceps skinfold thickness ($p<0.05$).Waist circumference and triceps skinfold thickness were significant predictors of homeostatic model assessment for insulin resistance. Overall, the frequency of homozygous dominant genotype CC of PPARγ 2 34C>G was 291 (72.75%), heterozygous CG was 93 (23.25%) and homozygous recessive GG was 16 (4%).There was no significant difference in frequency of genotypes between the groups ($p=0.216$). However, waist circumference and body mass index were significantly lower in GG genotype compared to the CC ($p=0.006$ versus $p=0.02$).

Conclusion: Waist circumference and triceps skinfold thickness were found to be the significant predictors of homeostatic model assessment for insulin resistance, while no association was found between 34 C>G variant of peroxisome proliferator activated receptor gamma and metabolic syndrome.

Keywords: PPARγ, Proline 12 alanine, Polymorphism, Metabolic syndrome. (JPMA 69: 1259; 2019)

Introduction

Metabolic syndrome (MetS) is a clustering of clinical and biochemical abnormalities including central obesity, hypertension (HTN), hyperglycaemia and impaired lipid profile. All these metabolic derangements lead to type 2 diabetes mellitus (T2DM), hepatic-steatosis, atherogenicity and inflammatory states.[1] MetS is a major public health issue with a 35-50% worldwide prevalence.[2] Its pathogenesis is multifactorial involving an interplay of genetic and environmental factors. Impedance to insulin action is a core defect in this entity and is the reason for the name insulin resistance syndrome (IRS).[3] Genetic studies have reported the association of various susceptibility genes with MetS.[4] One of the candidate genes, peroxisome proliferator activated receptor gamma (PPARγ) is a transcription factor and belongs to the class of nuclear receptor superfamily. Alternate splicing of PPARγ results in three functional isoformsγ 1, 2 and 3, differing in their tissue distribution;PPARγ2 dominates in adipocytes, while others have widespread tissue expression. PPARγ isoforms have three domains;

Department of Physiology & Cell Biology, University of Health Sciences, Lahore.
Correspondence: Uzma Zafar. e-mail: uzma.zargham@gmail.com

deoxyribonucleic acid (DNA) binding, ligand-activated, and ligand-independent domain. PPARγ2 on hetero-dimerization with Retinoid X Receptor is involved in transcription of multiple genes involved in lipolysis, lipogenesis, insulin sensitivity and tissue metabolism.[5] PPARγ2 is also a target of drug class thiazolidinediones which are insulin sensitizers. These transcription factors on ligand activation improve insulin sensitivity by upregulating adiponectin and Glucose transporter type 4(GLUT4) expression, and suppressing tumour necrosis factor-alpha (TNFα) and interleukin-6 (IL6) production. PPARγ gene is located on chromosome 3p25, extending over 100Kb of genomic DNA and consisting of 9 exons. A missense mutation 34CCA>GCA (rs1801282) at exon 2 of PPARγ2 gene results in proline12 alanine substitution. This amino acid change is associated with a conformational change in protein structure and altered transcriptional activity of this nuclear receptor.[5,6] PPARγ2 is a highly addressed, insulin sensitising transcription factor and understanding the distribution of its polymorphism and associated factors might help us in early prediction and better therapeutic approach towards MetS.Numerous studies have been conducted to determine the possible association of 34C>GPPARγ polymorphism with T2DM and insulin resistance (IR).[7,8] Proline, the wild type allele of PPARγ2,showed increased susceptibility to IR traits.[9,10] However, studies conducted on Asian Indians and French populationshowed that alanine, and not proline, is associated with higher body mass index (BMI), obesity and IR.[11,12] Although this polymorphism has been studied in various populations, its association with IR, T2DM and obesity is still controversial and inconsistent.[7-12] The current study was planned to determine the frequency of PPARγ2 34C>G polymorphism in subjects with and without MetS, and to check its association with MetS, IR and selected anthropometric obesity parameters.

Subjects and Methods

The cross-sectional comparative study was conducted at the Department of Physiology and Cell Biology, University of Health Sciences (UHS), Lahore, Pakistan, from September 2016 to 2017, and comprised MetS patients and healthy controls. The sample size was calculated using World Health Organisation (WHO) calculator using the following formula:[13]

$$n = \frac{\left(Z_{1-\alpha/2}\sqrt{2\bar{p}(1-\bar{p})} + Z_{1-\beta}\sqrt{p_1(1-p_1)p_2(1-p_2)}\right)^2}{(p_1-p_2)^2}$$

$$Where\ \bar{p} = \left(\frac{p_1+p_2}{2}\right)$$

The desired power of the study taken was 90%, level of significance was set at 0.05, proportion of major and minor allele frequency was taken as 0.79 and 0.21 in controls and 0.92 and 0.08 for the cases.[9]

The subjects were recruited using convenience sampling. Patients of MetS were recruited from the Diabetic Clinic of Sheikh Zayed Hospital, Lahore, as per the International Diabetes Federation (IDF) guidelines.[1,2] All MetS patients having evidence of end-stage renal or liver disease and chronic illness were excluded. Controls matched for age and gender were taken from the general population. For the selection of controls, detailed history was taken regarding T2DM, HTN drug intake, and clinical examination was also done. Those subjects were selected who were apparently healthy and free of MetS as per IDF guidelines.[1,2] Approval was taken from the institutional review board and informed consent was taken from all the subjects.

The weight, height, waist circumference (WC), hip circumference (HC), mid-arm circumference (MAC), triceps skin fold thickness(TSFT) and blood pressure (BP) were recorded by standard methods. Body weight was taken on a standard hospital scale bare footed with minimal clothing. Height was measured by wall-mounted stadiometer. WC was measured by non-stretchable tape midway between iliac crest and lower border of last rib at the end of expiration. MAC was measured midway between acromion and olecranon process at left arm. TSFT was taken by Harpenden caliper over triceps at posterior aspect of arm midway between acromion of scapula and olecranon process of ulna. Body mass index (BMI) was calculated by dividing the weight in kg by height in square meters. Waist-to-hip ratio (WHR) was calculated by dividing WC with HC.[14] Fasting blood sample of 4 ml was taken from subjects having overnight fast of 8-10 hours to study different biochemical parameters. Blood for DNA extraction was stored at -80^{O}C and serum was separated and stored at -80^{O}C. Fasting serum glucose and lipid profile, including serum triglyceride (TG), high-density lipoprotein (HDL) and cholesterol were measured by colorimetric method

(Randox Kits, United Kingdom). Insulin was measured by human insulin enzyme-linked immunosorbent assay (ELISA) kit (Elabscience, Germany) followed by calculation of insulin resistance(IR) by homeostatic model assessment (HOMA-IR).[15] DNA was extracted using Favor Prep Blood Genomic DNA Extraction Kit (Taiwan, China) according to the manufacturer's guidelines, and the yield was checked by nanodrop followed by gel electrophoresis. PPARγ genotyping was carried out by amplification refractory mutation system (ARMS-PCR) in25µl reaction mixture containing 2µl (50 ng/µl) of DNA, 2X polumerase chain reaction (PCR) master mixture (Fermetas, USA) and two Forward and two Reverse allele-specific primer sets (Macrogen, Inc. Korea).[16] The product was run on 3% agarose gel, the resultant outer PCR product was of 362 bp, product for C allele was 221bp and G allele was 195bp. Primer sequences used were:

PPPAR inner F-5'-
GAAACTCTGGGAGATTCTCCTATTGTCC -3' (28mer),

PPARG inner R-5'-
GTATCAGTGAAGGAATGGCTTTCAGC-3' (26mer),

PPARG outer
F5'GAAACTGATGTCTTGACTCATGGGTGTA-3' (28mer)

PPARG outer R-
5'GCAACGAGCTAAGCATTAAAATACTGGA-3' (28mer)

Data was analysed using SPSS 22. Normal distribution of the data was checked by Shapiro-Wilk's statistics. Mean ± standard deviation(SD) were given for normally distributed quantitative variables and median with interquartile range (IQR) for non-normally distributed quantitative variables. Student's 't' test and Mann-Whitney U test were applied to compare normally and non-normally distributed quantitative variables between the cases and the controls. Spearman correlation and multiple regression analysis were applied to see the relation between anthropometric parameters and HOMA-IR.

Genotypic and allelic frequencies were calculated and Hardy Weinberg equilibrium was determined.[17] In order to study the frequency and association of polymorphisms with study groups, Co-dominant, Dominant, and Recessive genetic models were constructed. Genotype frequencies of the two groups were compared by chi-square test and odds ratio (OR) was calculated. Allelic frequencies of the two groups were compared by Soft pad graphics. Anthropometric parameters of study population in three genotypes were compared by one-way analysis of variance (ANOVA) and comparison between the two models was done by 't' test.

Results

Out of 400 subjects, 200(50%) each were patients and controls. Overall, there were 308(77%) males and 92(23%) females.Among the patients, 176(88%) were diabetics, 156(78%) were hypertensive and dyslipidaemia was found in 186(93%) subjects or they were on lipid-lowering drugs. The patients had significantly higher BP, BMI, WC, WHR, MAC and TSFT along with serum glucose and insulin levels compared to the controls ($p<0.0001$ each). IR was also significantly higher in the patients than controls ($p<0.0001$) (Table 1).

HOMA-IR significantly correlated with anthropometric parameters, including BMI, WC, WHR, MAC and TSFT ($p<0.05$ each). Multiple regression analysis showed that WC and TSFT were significant predictors of HOMA-IR (Table 2; Figures 1-2).On comparison of mean values of the two genders in the group of patients, systolic BP

Table-1: Comparison of clinical and anthropometric parameters among the study groups and on gender basis.

Clinical and anthropometric parameters	Metabolic syndrome	Healthy group	*p*-value	Male vs Females		*p*-value
	Mean ± SD Median (IQR)	Mean ± SD Median (IQR)		Mean ± SD Median (IQR)		
Age	47.26±8.15	46.2±8.26	0.532	46.65±8.30	47.42±7.10	0.444
Systolic BP	124.92±16.5	115±13.32	<0.0001*	119±14.80	125±16.10	0.001*
Diastolic BP	81±10.39	75±7.99	<0.0001*	77±9.60	79±10.20	0.154
BMI	28.9±4.98	23.48±2.38	<0.0001*	25±5.06	27±5.85	0.011*
Waist circumference (cm)	101.5±9.01	79.56±8.64	<0.0001*	90±14.11	92±13.60	0.361
Waist to hip ratio	0.98±0.15	0.85±0.06	<0.0001*	0.92±0.086	0.93±0.26	0.569
MAC in cm	29.87±3.71	26.08±3.44	<0.0001*	23.7±10.87	28.1±11.50	0.001*
TSFT in mm	31.90±8.94	16.94±7.62	<0.0001*	27.67±3.74	29.36±4.99	0.001*
HOMA-IR	10.50(5.8-17.8)	1.95(1.32-2.96)	<0.0001**	6 (2.2-15)	10 (5.3-14)	0.004**

p-value is generated by t* test and Mann Whitney U** test, BP (blood pressure), BMI (body mass index), MAC (mid arm circumference), TSFT (triceps skin fold thickness), HOMA-IR (homeostatic model assessment for insulin resistance), SD (standard deviation), IQR (interquartile range)

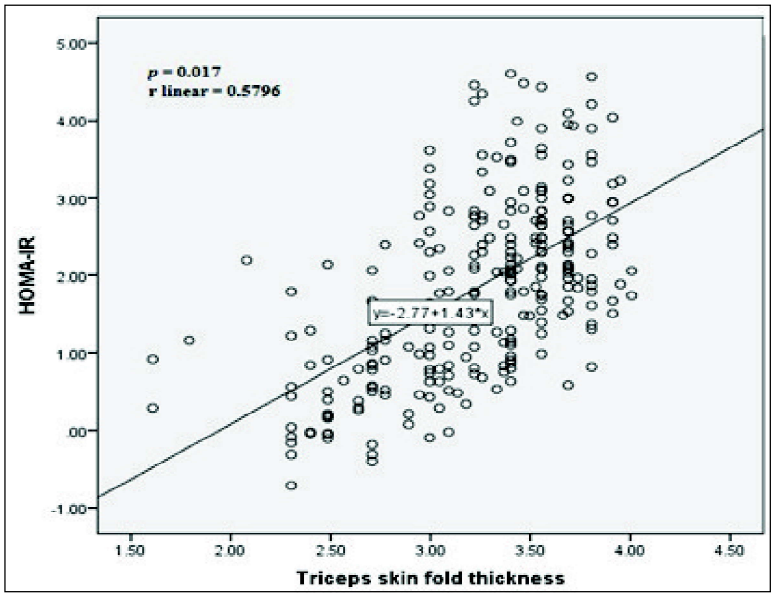

Figure-1: Scatter plot between Triceps skin fold thickness and homeostatic model assessment for insulin resistance(HOMA-IR).

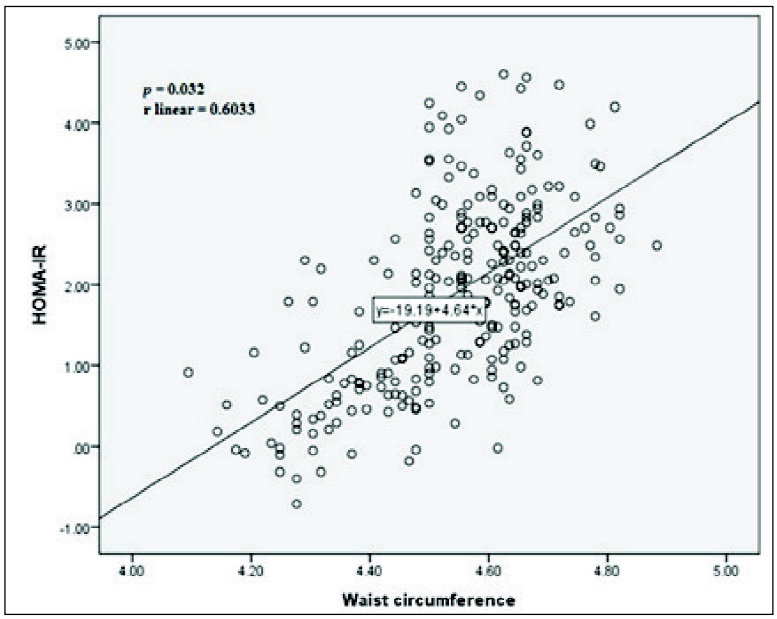

Figure-2: Scatter plot between waist circumference and homeostatic model assessment for insulin resistance(HOMA-IR).

(p=0.004), BMI (p=0.015), TSFT (p=0.003) and MAC (p=0.001) were significantly higher in females compared to the males. Among the healthy group, TSFT was significantly higher in females than males (p=0.037). There was no significant difference regarding other measurements between the two genders in controls (p>0.05). HOMA-IR was significantly higher in females than males in the healthy group (p=0.035), while difference between the two was not significant in the patients group (p=0.316).

Overall, the frequency of homozygous dominant genotype CC of PPARγ2 34C>Gwas 291(72.75%), heterozygous CG was 93(23.25%) and homozygous recessive GG was 16 (4%). The frequency of C allele was 675(84.37%) and G was 125(15.63%).Genotype frequencies in cases and controls were in Hardy Weinberg equilibrium(p>0.05). On comparison of allelic, co-dominant (CC: CG: GG), dominant (CC:

Table-3: Comparison of genotypes between the diseased and healthy group (n=200).

Genotypes of , PPARγ 34 C>G variant	Metabolic Syndrome n (%)	Healthy group n (%)	*p*-value, OR & CI
CC	147 (73.5)	144 (72)	p = 0.216
CG	46 (23)	47 (23.5)	
GG	7 (3.5)	9 (4.5)	
Dominant model			
CC vs	147 (73.5)	144 (72)	0.135
CG+GG	53 (26.5)	56 (28)	0.13 (0.89-2.2)
Recessive model			
GG vs	7 (3.5)	9 (4.5)	0.481
CG+CC	193 (96.5)	191 (95.5)	0.81 (0.35-2.2)
Allelic frequency			
C	340(85)	335(83.7)	p = 0.136
G	60 (15)	65(16.25)	

p-value is generated by Chi-square test, OR (Odds ratio); CI (Confidence interval).

Table-2: Correlation between HOMA-IR and anthropometric parameters.

Spearman's rho correlation coefficient of HOMA-IR	BMI	WC	Waist to hip ratio	MAC	TSFT
Study population	0.490, <0.0001*	0.589, <0.0001*	0.517, <0.0001*	0.460, <0.0001*	0.574, <0.0001*
Metabolic syndrome	0.091, 0.229	0.167, 0.025*	0.125, 0.097	0.122, 0.105	0.044, 0.056
Healthy group	0.375, 0.001*	0.466, <0.0001*	0.348, 0.002*	0.409, <0.0001*	0.293, <0.0001*
After controlling for confounders such as age and sex					
Spearman's rho correlation coefficient of HOMA-IR	---	0.183, 0.004*	---	---	0.195, 0.002*
Linear regression model taking HOMA-IR as dependent variable					
HOMA-IR	0.043, 0.670	0.257, 0.032*	0.026, 0.732	-0.027, 0.763	0.188, 0.017*

BMI (body mass index), WC (waist circumference), MAC (mid arm circumference), TSFT (triceps skin fold thickness).

Table-4: Comparison of anthropometric parameters in different genotypes.

Anthropometric parameters in different genotypes of PPARγ 34 C>G						
Parameters	**CC**	**CG**	**GG**	***p*-value**	**Post hoc Tukey**	***p*-value**
BMI	26.6±5.4	25.35±4.9	25.2±5.3	0.014*	CC vs GG	0.026*
WC	91.88±13.7	87.76±14.78	86.22±17.13	0.007*	CC vs GG	0.025*
Waist/hip	0.92±0.15	0.89±0.07	0.89±0.1	0.410	--	
MAC	28±4.09	27.24±4.38	26.05±3.5	0.366	--	
TSFT	26.05±3.5	23.39±11.7	21.27±8.6	0.162	--	

p-value generated by ANOVA followed by Post hoc Tukey in above comparison

Comparison of anthropometric parameters in dominant and recessive genotype model

Parameters	**CC vs CG+GG**		***p*-value**	**GG vs CG+CC**		***p*-value**
	CC	**CG+GG**		**GG**	**CG+CC**	
BMI	26.6±5.4	24.13±5	0.006*	25.2±5.3	26±5.28	0.018*
WC	91.88±13.7	87.43±14.98	0.020*	86.22±17.13	90±14.11	0.015*
Waist/hip	0.92±0.15	0.89±0.08	0.182	0.89±0.1	0.91±0.14	0.219
MAC	28±4.09	27.41±4.16	0.233	26.05±3.5	27±4.1	0.060
TSFT	26.05±3.5	22.92±11.09	0.202	21.27±8.6	24±11.1	0.090
HOMA-IR	6.4(2.3-14)	5.3(2.2-16)	0.778	6.4(2.3-15)	7.6(2.6-15)	0.980

p-value generated by "t" test and Mann Whitney U test.
BMI (body mass index), MAC (mid arm circumference), TSFT (triceps skin fold thickness), HOMA-IR (homeostatic model assessment for insulin resistance).

CG+GG) and recessive models (GG: CC+CG), there was no significant difference in frequency of genotypes between the groups (p>0.05) (Table 3). On comparison of anthropometric parameters in different genotypes, BMI and WC were significantly lower in GG genotypes compared to the others (Table 4).

Discussion

The study found a significant correlation of HOMA-IR with anthropometric obesity markers, including WC, BMI, WHR, MAC and TSFT. In the MetS group, IR and WC correlation persisted, but was not observed with other obesity parameters, while in the healthy group, correlation was there with all anthropometric parameters. This weaning off can be justified as normal body glucose homeostasis disappears in the diseased group. Most of the patients were on anti-diabetics and lipid-lowering agents, and these might interfere with insulin release, circadian rhythm and glucose homeostasis.[18] In multiple regression model, among all the co-variates, significant regressers of HOMA-IR were WC and TSFT. These two measures of adiposity were best predictors of IR and were found to be superior compared to the others. Studies using magnetic resonance imaging (MRI) and computed tomography (CT) for measurement of total body fat have also revealed that upper body and central fat are the major contributors of metabolic derangements in IR leading to T2DM and related complications.[14] As per IDF guidelines, central adiposity is mandatory in the diagnosis of MetS and in Asian Indians, cut-off levels of WC, a predictor of central obesity, are lower compared to Euripides as they are more susceptible to metabolic derangements resulting from abdominal fat accumulation leading to insulin impedance.[1,2] Adipose-derived cytokines i.e. TNFα, IL6 and adiponectin, establish the connection between central fat and IR by modulating insulin secretion and its action at target areas.[19] Central adipocytes are metabolically more active with preponderance of beta2 receptors, resulting in increased release of free fatty acids in portal venous system, thus creating resistance to insulin action at the level of hepatocytes.[20] When anthropometric parameters were compared on gender basis, females had significantly higher obesity-related measurements than males. These findings were supported by another study in Karachi which depicted significantly higher central obesity and body fat in T2DM females compared to the males.[21]

Literature on the association of PPARγ C>G variant with IR, T2DM and related traits has depicted conflicting relations. Risk allele of PPARγ 34C>G (rs1801282) also varies in different ethnic groups.9-12In this study, PPARγ C>G(proline 12 alanine) variant was evaluated and there was no significant association found with MetS. However, BMI and WC were significantly lower in CG/GG genotypes compared to the prevalent CC type of PPARγ2. In a previous study on Pakistani diabetics, alanine was found to have a protective role in diabetic retinopathy whereas in another study on Pakistanis with rheumatoid arthritis, the results were contradictory; frequency of GG genotype (alanine)

was significantly high in cases compared to controls, and G allele was associated with the disease.[22,23] In a study, alanine was found to be associated with obesity markers like BMI, central fat and skinfold thickness in obese non-diabetic subjects in Asian Indians.[11] Two large meta-analysis concluded that Caucasians and Chinese with GG (alanine) genotype were more sensitive to insulin than those with CC (proline). Hence, the presence of the minor allele G of PPARγ2 34 C>G variant was found to be protective andbeneficial.[24,25] The transcription factor PPARγ2 is highly expressed in adipose tissue and master regulator of energy homeostasis.[26] In this study, BMI and WC were significantly less in GG and CG (minor variants) genotypes of PPARγ but the intervening connection between the two is missing i.e. levels of PPAR proteins and messenger ribonucleic acid (mRNA) expression were not checked to see the effect of this missense mutation on their levels.

Conclusion

The 34 C>G variant of PPARγ was not found to be associated with MetS, but subjects with CG+GG genotype had significantly lower obesity parameters, including WC and BMI, compared to the CC one. Best anthropometric predictors of HOMA-IR were WC and TSFT. Central and upper body fat reduction is to be emphasised as it might result in improving insulin sensitivity and postpone metabolic derangements resulting from insulin impedance.

Disclaimer: The study is part of a PhD thesis.
Conflict of interest: None.
Source of Funding: None.

References

1. Ford ES. Prevalence of the metabolic syndrome defined by the International Diabetes Federation among adults in the U.S. Diabetes Care 2005; 28: 2745-9.
2. Grundy SM. Metabolic syndrome pandemic. Arterioscler Thromb Vasc Biol 2008; 28: 629-6.
3. Brown AE, Walker M. Genetics of insulin resistance and the metabolic syndrome. Curr Cardiol Rep 2016; 18: 75.
4. Gao L, Wang L, Yun H, Su L, Su X. Association of the PPAR gamma 2 gene Pro 12 Ala variant with primary hypertension and metabolic lipid disorders in Han Chinese of Inner Mongolia. Genet Mol Res 2010; 9: 19295-308.
5. Tyagi S, Gupta P, Saini AS, Kaushal C, Sharma S. The peroxisome proliferator-activated receptor: A family of nuclear receptors role in various diseases. J Adv Pharm Technol Res 2011; 2: 236-40.
6. Sokkar S, El-Sharnouby JA, Helmy A, El-Bendary A, Ahmad LS, Okasha K. Role of peroxisome proliferator-activated receptor gamma2 (PPAR-γg2) gene polymorphism in type 2 diabetes mellitus. Eur J Gen Med2009; 6: 78-86.
7. Ruchat SM, Rankinen T, Weisnagel SJ, Rice T, Rao DC, Bergman RN, et al. Improvements in glucose homeostasis in response to regular exercise are influenced by the PPARG Pro12Ala variant: results from the HERITAGE Family Study. Diabetologia 2010; 53: 679-89.
8. Montagnana M, Fava C, Nilsson PM, Engström G, Hedblad B, Lippi G, et al. The Pro12Ala polymorphism of the PPARG gene is not associated with the metabolic syndrome in an urban population of middle-aged Swedish individuals. Diabet Med 2008; 25: 902-8.
9. Majid M, Masood A, Kadla SA, Hameed I, Ganai BA. Association of Pro12Ala polymorphism of peroxisome proliferator-activated receptor gamma 2 (PPARγ2) gene with type 2 diabetes mellitus in ethnic Kashmiri population. Biochem Genet 2017; 55: 10-21.
10. Regieli JJ, Jukema JW, Doevendans PA, Zwinderman AH, van der Graaf Y, Kastelein JJ, et al. PPAR gamma variant influences angiographic outcome and 10-year cardiovascular risk in male symptomatic coronary artery disease patients. Diabetes Care 2009; 32: 839-44.
11. Bhatt SP, Misra A, Sharma M, Luthra K, Guleria R, Pandey RM, et al. Ala/Ala genotype of Pro12Ala polymorphism in the peroxisome proliferator-activated receptor-γ2 gene is associated with obesity and insulin resistance in Asian Indians. Diabetes Technol Ther 2012; 14: 828-34.
12. Meirhaeghe A, Cottel D, Amouyel P, Dallongeville J. Association between peroxisome proliferator-activated receptor γ haplotypes and the metabolic syndrome in French men and women. Diabetes 2005; 54: 3043-8.
13. Lwanga SK, Lemeshow S. Two sample situations. Hypothesis tests for two population proportions. In:Sample size determination in health studies a practical manual.Geneva: World Health Organization (WHO), 1991: pp 7.
14. Anjana M, Sandeep S, Deepa R, Vimaleswaran KS, Farooq S, Mohan V. Visceral and central abdominal fat and anthropometry in relation to diabetes in Asian Indians. Diabetes Care 2004; 27: 2948-53.
15. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and γ-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412-9.
16. Medrano RF, de Oliveria CA. Guidelines for the tetramer primers ARMS-PCR technique development. Mol Biotechnol 2014; 56: 599-608.
17. Rodriguez S, Gaunt TR, Day IN. Online Genetic Epidemiology Tool (OEGE). [Online] [Cited 01 Oct 2017]. Available from: URL: http://www.oege.org/software/hwe-mr-calc.shtml.
18. Muscogiuri G, Sarno G, Gastaldelli A, Savastano S, Ascione A, Colao A, et al.The good and bad effects of statins on insulin sensitivity and secretion. Endocr Res 2014; 394: 137-43.
19. Makki K, Froguel P, Wolowczuk I. Adipose tissue in obesity-related inflammation and insulin resistance: cells, cytokines, and chemokines. ISRN Inflamm 2013; 2013: 139239.
20. Westphal SA. Obesity, abdominal obesity, and insulin resistance. Clin Cornerstone 2008; 9: 23-31.
21. Fatima SS, Rehman R, Chaudhry B. Body mass index or body fat! which is a better obesity scale for Pakistani population? J Pak Med Assoc 2014; 64: 1225-8.
22. Tariq K, Malik SB, Ali SH, Maqsood SE, Azam A, Muslim I, et al. Association of Pro12Ala polymorphism in peroxisome proliferator activated receptor gamma with proliferative diabetic retinopathy. Mol Vis 2013; 19: 710-7.
23. Jalil SF, Ahmed I, Gauhar Z. Association of Pro12Ala (rs1801282) variant of PPAR gamma with Rheumatoid Arthritis in a Pakistani population. Rheumatol Int 2014; 34: 699-703.
24. Wang L, Teng Z, Cai S, Wang D, Zhao X, Yu K. The association between the PPARγ2 Pro12Ala polymorphism and nephropathy susceptibility in type 2 diabetes: a meta-analysis based on 9,176 subjects. Diagn Pathol 2013; 8: 118.

25. Huguenin GV, Rosa G. The Ala allele in the PPAR-γ2 gene is associated with reduced risk of type 2 diabetes mellitus in Caucasians and improved insulin sensitivity in overweight subjects. Br J Nutr 2010; 104: 488-97.

26. Afzal N, Hassan M, Fatima S, Tariq S, Qayum I. Expression of peroxisome-proliferator activated receptors- in diabetics, obese and normal subjects. J Ayub Med Coll 2016; 28: 130-34.