



Contribution of the Pro12Ala polymorphism of peroxisome proliferator-activated receptor γ 2 gene in relation to obesity



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ABSTRACT

Background and aims: Peroxisome proliferator-activated receptor γ 2 (PPAR γ 2) gene Pro12Ala polymorphism has been extensively studied in relation to obesity and its associated metabolic complications but the results were inconclusive. This study aimed to identify the genotypic and allelic frequencies of PPAR γ 2 gene and its association with anthropometric measurements, lipid profiles and the susceptibility for obesity in Malay subjects. **Methods and results:** This cross-sectional, comparative study involved 217 subjects (94 obese and 123 non-obese as controls). Anthropometric and lipid profiles were measured. Genotyping was performed by allele-specific PCR. Comparisons were made between the genotypes and the association of PPAR γ 2 Pro12Ala polymorphism with obesity was evaluated. The Pro12Pro, Pro12Ala and Ala12Ala genotypic frequencies were significantly different between groups (88.3%/11.7%/0.0% vs. 97.6%/2.4%/0.0% respectively, $P = 0.006$). The Ala12 allele was more frequent in obese than the non-obese (5.9% vs. 1.1%, $P = 0.007$). Ala12 carriers were associated with higher BMI ($P = 0.016$), BF% ($P = 0.019$) and a trend towards higher BAI ($P = 0.055$) than the non-carriers. Besides age, high level of triglycerides and LDL-cholesterol, Ala12 allele (Adjusted OR = 5.46, 95% CI = 1.27–23.40; $P = 0.022$) were regarded as independent risk factors for obesity.

Conclusions: This study indicates that the PPAR γ 2 gene Pro12Ala polymorphism predisposes to obesity in Malay subjects and Ala12 allele could predict alterations in adipocyte and lipid metabolism among them. Age, triglycerides, LDL-cholesterol and Ala12 allele conferred to increased risk for obesity in this ethnicity.

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1. Introduction

The prevalence of obesity has reached an alarming level and become the major health concern across the globe. Based on the recent WHO reports, over 600 million adults were obese in 2014 (World Health Organization, 2016). As for Malaysia, most reliable data for obesity prevalence were based on the reports from the Malaysian Adult Nutrition Survey (MANS) (Azmi et al., 2009) and National Health and Morbidity Survey (NHMS) (Nor et al., 2008; MOH, 2011). Obesity prevalence was reported at 12.2% in the 2003 MANS (Azmi et al., 2009), 14.0% in the 2006 NHMS III (Nor et al., 2008) and 15.1% in the recent 2011 NHMS IV (MOH, 2011). Given the adverse effects of obesity on multiple aspects of life, elucidating its pathogenesis remains an enigma from many standpoints, including the genetic analysis (Yao et al., 2015). Following the continuous developments in the human genome research,

numerous genes related to obesity have been successfully discovered (Rankinen et al., 2006). One of those genes, the peroxisome proliferator-activated receptor gamma (PPAR γ) gene has gained much attention in obesity research (Yao et al., 2015). PPAR γ , a nuclear hormone receptor, is a ligand activated transcription factor that is highly expressed in adipose tissue and plays a key role in adipocyte differentiation and lipid metabolism (Auwerx, 1999; Grygiel-Gorniak, 2014). PPAR γ regulates the transcriptional activity of numerous target genes via the PPAR-responsive elements (PPREs) in the target gene promoters (Auwerx, 1999; Meirhaeghe and Amouyel, 2004).

The human PPAR γ gene is located on chromosome 3p25 and has three protein isoforms, PPAR γ 1, PPAR γ 2 and PPAR γ 3 that differ at their 5'-end as a result from alternative promoter usage and differential splicing. In comparison to PPAR γ 1, PPAR γ 2 contains additional 28 amino acids at its NH₂-terminus encoded by the B exon (Desvergne and Wahli, 1999). Additionally, PPAR γ 2 is mainly found in adipose tissue and regulates the transcription of many adipocyte-specific genes (Auwerx, 1999), which therefore places PPAR γ 2 as a potential candidate gene for obesity. A number of genetic variants in the PPAR γ gene have been identified (Yen et al., 1997; Meirhaeghe et al., 1998; Ristow et al., 1998; Stumvoll and Haring, 2002). A CCA-to-GCA missense

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mutation resulting in a substitution of amino acid proline to alanine (Pro12Ala) is the most prevalent, with allelic frequencies varies across ethnicities; the highest frequency have been reported among the Caucasian Americans (12%), followed by Mexican Americans (10%), Samoans (8%), African Americans (3%), Nauruans (2%) while Chinese have the lowest frequency (1%) (Yen et al., 1997).

This polymorphism has been extensively studied in relation to obesity and obesity related phenotypes (Gonzalez Sanchez et al., 2002; Kim et al., 2004; Vaccaro et al., 2007; Bhatt et al., 2012; Bener et al., 2013; Carlos et al., 2013) and blood lipid profile (Beamer et al., 1998; Meirhaeghe et al., 2000; Kim et al., 2007; Mirzaei et al., 2009; Bhatt et al., 2012) with inconsistent results. The aim of this study was to determine the genotypic and allelic frequencies of this polymorphism and its association with anthropometric measurements, lipid profile and the risk for obesity in Malay subjects.

2. Materials and methods

2.1. Subjects

This was a cross-sectional, comparative study involving a total of 217 Malay adult subjects recruited by a convenience volunteer sampling from educational establishments around Kuala Terengganu. By definition, Malay ethnic group is comprised of ten sub-ethnicities including the Kelantan, Minang, Jawa, Bugis, Banjar, Aceh, Kedah, Champa, Pattani and Rawa (Halim-Fikri et al., 2015). However, all these sub-ethnicities were categorized as Malay ethnic group as a whole. These were 94 obese and 123 non-obese with an age mean \pm SD of 36.01 ± 10.64 years. They were selected by primary obesity screening based on body mass index (BMI) that divides them into obese (BMI ≥ 30 kg/m²) and non-obese (BMI 18.50–24.99 kg/m²) as per standard criteria (WHO, 2000). Subjects with cardiovascular and respiratory illness, diabetic patients, underweight (BMI < 18.5 kg/m²) and overweight ($25.0 \text{ kg/m}^2 \leq \text{BMI} \leq 29.99 \text{ kg/m}^2$) individuals were excluded from the study. This study was approved by University Human Research Ethics (UHREC) (UniSZA): UniSZA.N/1/628-1 and all subjects gave their informed consent prior to the commencement of this study.

2.2. Anthropometric measurements

Weight, height, waist and hip circumference were measured according to the previous established protocol (Darmawan and Irfanuddin, 2007). The waist-hip ratio (WHR) and waist-height ratio (WhtR) were calculated by dividing the measurement of waist circumference to that of the hip circumference and the height, respectively. The assessment of body fat percentage (BF %) was done using a Slim Manager N40 (AIIA communications Inc., South Korea). Other indicators for body adiposity such as body adiposity index (BAI) (Bergman et al., 2011), abdominal volume index (AVI) (Guerrero-Romero and Rodriguez-Moran, 2003) and conicity index (CI) (Valdez, 1991) that employs mathematical formula using the existing anthropometric data were also calculated.

2.3. Blood collection and biochemical assays

Blood samples were drawn after an overnight fast into plain and EDTA-coated tubes (Becton Dickinson, Franklin Lakes, NJ). After serum separation, total cholesterol, triglycerides and HDL-cholesterol were analysed using an Olympus-AU400 chemistry analyser (Olympus, Tokyo, Japan). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald's formula (Friedewald et al., 1972).

2.4. Blood extraction and genotyping

Genomic DNA was isolated from the whole blood using the GF-1 blood extraction kit (Vivantis, CA, USA). The Pro12Ala variant was detected by allele-specific PCR with specific primers designed according

to the guidelines described by Pasternak (2005). The primers (Integrated DNA Technologies Inc., Malaysia) were as follows: forward (wild-type), 5'-GGG AGA TTC TCC TAT TGA CC-3' and forward (mutant), 5'-GGG AGA TTC TCC TAT TGA CG-3'; reverse, 5'-GAA CGC GAT AGC AAC GAG C-3'. The underlined bases indicate that the primers were specific for the C and G nucleotides (translated as Pro12 and Ala12 allele), respectively. PCR was carried out in a total volume of 20 μ L containing 1 \times PCR buffer, 1.7 mM MgCl₂, 0.34 mM dNTPs, 0.8 μ M of each primer and 1 U *Taq* polymerase (Promega, Madison, WI). PCR products at 222 bp fragment formed from the wild-type primer, both wild-type and mutant primers and the mutant primer were identified as Pro12Pro, Pro12Ala and Ala12Ala, respectively, when resolved on a 2% agarose gel by electrophoresis. A site-directed mutagenesis PCR was carried out to generate positive control for Ala12Ala genotype in case of no homozygous Ala12 allele detected from the previous amplification. The reproducibility of each genotype from a few representative samples was verified by DNA sequencing (1st Base Laboratories, Selangor, Malaysia).

2.5. Statistical analysis

Power and Sample Size Calculation version 3.0, 2009 was used for sample size calculation using two proportion formula (Naing, 2011) adopting 15.1% as expected proportion of population with obesity as per previous literature (MOH, 2011); 80% power of study, 0.05 significance (α) level at 95% confidence interval; and taking into consideration the 20% of included subjects who withdrew when the study was conducted. All calculations were carried out using SPSS version 20.0 (IBM Corporation, Armonk, NY). Data normality was assessed by use of the Kolmogorov-Smirnov test, and expressed as mean \pm SD or as median (interquartile range) for the skewed variables. Genotypic and allelic frequencies were determined by gene counting method, and assessed for Hardy-Weinberg equilibrium using chi-squared χ^2 -test. Comparisons of anthropometrics and clinical characteristics between obese and non-obese were assessed by independent *t*-test and Mann-Whitney *U* test. A binary logistic regression was used to determine the association of PPAR γ 2 genotypes with obesity, while controlling for other predictor variables. In univariate logistic regression analysis, PPAR γ 2 genotypes and gender were analysed as categorical variables, while age, triglycerides, total cholesterol, HDL-C and LDL-C were included as continuous variables. Crude odds ratios (ORs), 95% confidence intervals (CI) together with the *P*-values were evaluated. The factors with *P* < 0.05 were further analysed in a stepwise multiple logistic regression analysis. Their adjusted odds ratios (ORs) and 95% confidence intervals (95% CI) were evaluated for the strength of the association between the variables and obesity risk. *P*-value = 0.000 was demonstrated as *P* < 0.001. Both *P* < 0.001 and *P* < 0.05 were considered as statistically significant.

3. Results

3.1. Characteristics of the study subjects

The anthropometrics and clinical characteristics of all subjects included in this study are summarized in Table 1. Compared to the non-obese, obese subjects were significantly older (39.18 ± 9.97 vs. 33.59 ± 10.54 year, *P* < 0.001). Besides, all variables except for height, triglycerides and total cholesterol showed significant differences in obese compared to the non-obese.

3.2. Genotypic and allelic frequencies of PPAR γ 2 gene Pro12Ala polymorphism

The PPAR γ 2 gene Pro12Pro, Pro12Ala and Ala12Ala genotype distributions were 88.3%, 11.7% and 0.0% in obese subjects with Hardy Weinberg Equilibrium (HWE) *P* value of 0.55 and 97.6%, 2.4% and 0.0% with HWE *P* value of 0.89 in non-obese subjects, respectively. Significant

Table 1
Anthropometrics and clinical characteristics of the study group.

	Obese (n = 94)	Non-obese (n = 123)	P-value
Age (year)	39.18 ± 9.97	33.59 ± 10.54	<0.001 ^a
Weight (kg)	84.47 ± 12.58	55.40 ± 7.33	<0.001 ^a
Height (m)	1.60 ± 0.08	1.58 ± 0.08	0.067
Body mass index (kg/m ²)	32.87 ± 3.34	22.11 ± 1.86	<0.001 ^a
Waist circumference (cm)	95.22 ± 11.06	71.95 ± 7.95	<0.001 ^a
Hip circumference (cm)	109.03 ± 10.61	92.04 ± 5.73	<0.001 ^a
Waist-hip ratio	0.88 ± 0.08	0.78 ± 0.07	<0.001 ^a
Waist-height ratio	0.59 ± 0.06	0.46 ± 0.05	<0.001 ^a
Conicity index	1.20 ± 0.10	1.12 ± 0.09	<0.001 ^a
Abdominal volume index	18.60 ± 4.24	10.79 ± 2.24	<0.001 ^a
Body adiposity index	35.96 ± 5.71	28.44 ± 4.07	<0.001 ^a
Body fat (%)	33.71 ± 6.35	24.16 ± 7.12	<0.001 ^a
Triglycerides (mmol/L) ^b	1.20 (0.50–14.30)	0.90 (0.30–7.90)	0.967
Total cholesterol (mmol/L)	5.72 ± 1.46	5.41 ± 1.31	0.094
HDL-C (mmol/L)	1.21 ± 0.30	1.37 ± 0.33	<0.001 ^a
LDL-C (mmol/L)	4.13 ± 1.22	3.76 ± 1.15	0.024 ^a

Data are mean ± SD values for the indicated number of subjects (n) in each group unless indicated otherwise.

HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.

^a Independent *t*-test; *P*-value = 0.000 was demonstrated as *P* < 0.001. Both *P* < 0.001 and *P* < 0.05 are considered significant.

^b Median (minimum–maximum) for the skewed data.

difference was observed between groups (*P* = 0.006). The Pro12 allele frequency in obese and non-obese subjects was 94.1% and 98.9%, and the Ala12 allele frequency was 5.9% and 1.1%, respectively. The Ala12 allele frequency was higher in obese compared to the non-obese subjects (5.9% vs. 1.1%, *P* = 0.007) (Table 2).

3.3. Comparison of anthropometric parameters and lipid profiles in relation to the PPAR γ 2 gene Pro12Ala polymorphism

The differences between anthropometric parameters and lipid profiles in relation to the PPAR γ 2 gene Pro12Ala polymorphism in all subjects, obese and non-obese, respectively are presented in Table 3 and Table 4. In all subjects, the Pro12Ala genotype were older (42.43 ± 12.14 vs. 35.57 ± 10.42; *P* = 0.019) and had higher BMI and body fat percentage than the Pro12Pro (29.26 ± 3.43 vs. 26.59 ± 6.04; *P* = 0.016) and (33.29 ± 8.25 vs. 27.95 ± 8.18; *P* = 0.019), respectively. Moreover, weight and body adiposity index were marginally higher in subjects with Pro12Ala genotype than the Pro12Pro but the differences did not reach statistical significance (71.24 ± 11.49 vs. 67.77 ± 17.86; *P* = 0.095) and (34.73 ± 4.34 vs. 31.49 ± 6.17; *P* = 0.055), respectively (Table 3). Subgroup analyses based on obesity status (obese and non-obese) in relation to PPAR γ 2 genotypes revealed that obese subjects with Pro12Ala genotype were significantly older (45.55 ± 1.18 vs.

Table 2
Genotypic distributions and allelic frequencies of PPAR γ 2 gene Pro12Ala polymorphism with obesity.

	Obesity (n = 217)	
	Obese (n = 94)	Non-obese (n = 123)
Genotype		
Pro12Pro	83 (88.3%)	120 (97.6%)
Pro12Ala	11 (11.7%)	3 (2.4%)
Ala12Ala	0	0
Chi-square value (<i>P</i> -value)	7.575 (0.006 ^a)	
Allele frequency		
Pro12	177 (0.941)	243 (0.989)
Ala12	11 (0.059)	3 (0.011)
Chi-square value (<i>P</i> -value)	7.322 (0.007 ^a)	
OR (95% CI)	0.132 (0.029–0.602)	

Data are n (%) for genotypes and n (frequency) for alleles.

OR: odds ratio, CI: confidence interval.

^a Chi-squared χ^2 -test; *P* value < 0.05 represented in bold is considered significant.

Table 3
Comparison of the anthropometric parameters and lipid profiles in relation to the PPAR γ 2 gene Pro12Ala polymorphism in all subjects.

	Pro12Pro (n = 203)	Pro12Ala (n = 14)	P-value
Age (year)	35.57 ± 10.42	42.43 ± 12.14	0.019 ^a
Weight (kg)	67.77 ± 17.86	71.24 ± 11.49	0.095
Height (m)	1.59 ± 0.08	1.56 ± 0.07	0.309
Body mass index (kg/m ²)	26.59 ± 6.04	29.26 ± 3.43	0.016 ^a
Waist circumference (cm)	81.75 ± 15.06	86.07 ± 12.08	0.295
Hip circumference (cm)	99.21 ± 12.01	102.14 ± 6.80	0.368
Waist-hip ratio	0.82 ± 0.09	0.84 ± 0.09	0.483
Waist-height ratio	0.51 ± 0.09	0.55 ± 0.07	0.106
Conicity index	1.15 ± 0.10	1.17 ± 0.10	0.643
Abdominal volume index	14.10 ± 5.14	15.32 ± 3.70	0.382
Body adiposity index	31.49 ± 6.17	34.73 ± 4.34	0.055
Body fat (%)	27.95 ± 8.18	33.29 ± 8.25	0.019 ^a
Triglycerides (mmol/L) ^b	1.00 (0.30–14.30)	1.05 (0.40–6.10)	0.967
Total cholesterol (mmol/L)	5.52 ± 1.40	5.92 ± 1.16	0.293
HDL-C (mmol/L)	1.30 ± 0.33	1.35 ± 0.32	0.581
LDL-C (mmol/L)	3.89 ± 1.21	4.27 ± 0.96	0.254

Independent *t*-test was used to compare the anthropometric and blood lipid profiles between the genotypes. Data are presented as mean ± standard deviations for the indicated number of subjects (n) in each group unless indicated otherwise.

HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.

^a *P* value < 0.05 is considered significant.

^b Median (minimum–maximum) for the skewed data.

38.34 ± 9.47; *P* = 0.023), had lower body weight (76.04 ± 5.89 vs. 85.59 ± 1.28; *P* < 0.001), BMI (30.93 ± 0.80 vs. 33.13 ± 3.46; *P* < 0.001), waist circumference (90.82 ± 6.57 vs. 95.81 ± 1.14; *P* = 0.046) and abdominal volume index (16.74 ± 2.31 vs. 18.84 ± 4.38; *P* = 0.021) and a higher trend of body fat percentage (36.73 ± 5.26 vs. 33.31 ± 6.40; *P* = 0.093) compared with the Pro12Pro subjects from the same group. A higher body fat percentage was also found in non-obese subjects with Pro12Pro genotype than the Pro12Ala (24.25 ± 7.18 vs. 20.67 ± 0.58; *P* < 0.001) (Table 4).

3.4. Association of PPAR γ 2 gene Pro12Ala polymorphism and other predictor variables with obesity

The odds ratio (OR) analysis from univariate logistic regression showed the risk for the PPAR γ 2 gene variants and other predictor variables with obesity (Table 5). Female gender, age, a high level of triglyceride and LDL-C, and low level of HDL-C and PPAR γ 2 gene Ala12 allele (OR = 5.30; 95% CI = 1.44–19.59; *P* = 0.012) were the risk factors of obesity. Subsequent analysis in multivariate logistic regression showed that age (adjusted OR = 1.04; 95% CI = 1.01–1.08; *P* = 0.007), a high level of triglycerides (adjusted OR = 1.90; 95% CI = 1.34–2.70; *P* < 0.001) and LDL-C (adjusted OR = 13.83; 95% CI = 3.91–48.85; *P* < 0.001) and PPAR γ 2 gene Ala12 allele (adjusted OR = 5.46; 95% CI = 1.27–23.40; *P* = 0.022) were independent risk factors for obesity (Table 6).

4. Discussion

The main aspect of this study was to identify the PPAR γ 2 gene variants for its association with anthropometric parameters, lipid profiles and the risk for obesity in Malay subjects. It is known that obesity results from a combination of causes and contributing factors including unhealthy diet, physical inactivity, lifestyle choices and genetics (Yao et al., 2015). Among those genes that have been found to be associated with obesity are *ACE*, *MC4R*, *BDNF*, *LEP*, *LEPR* and *PPARG* (Rankinen et al., 2006). PPAR γ plays a crucial role in the molecular control of adipogenesis by enhancing the expression of specific genes in adipose tissue (Auwerx, 1999). The Pro12Ala polymorphism is the primarily studied variant in PPAR γ 2 gene in relation with obesity (Hsiao and Lin, 2015). In this study, the Ala12 allele frequency of 5.9% in our obese subjects was similar to that reported in other Asians, ranging from 3.6–10.2%

Table 4
Comparison of the anthropometric parameters and lipid profiles in relation to the PPAR γ 2 gene Pro12Ala polymorphism in obese and non-obese subjects.

Characteristics	Obese (n = 94)			Non-obese (n = 123)		
	Pro12Pro (n = 83)	Pro12Ala (n = 11)	P-value	Pro12Pro (n = 120)	Pro12Ala (n = 3)	P-value
Age (year) ^a	38.34 ± 9.47	45.55 ± 1.18	0.023 ^b	33.66 ± 10.65	31.00 ± 4.00	0.372
Weight (kg) ^a	85.59 ± 1.28	76.04 ± 5.89	<0.001 ^b	55.44 ± 7.31	53.63 ± 9.58	0.675
Height (m) ^a	1.61 ± 0.08	1.57 ± 0.06	0.128	1.58 ± 0.07	1.52 ± 0.08	0.139
Body mass index (kg/m ²) ^a	33.13 ± 3.46	30.93 ± 0.80	<0.001 ^b	22.08 ± 1.86	23.17 ± 1.58	0.318
Waist circumference (cm) ^a	95.81 ± 1.14	90.82 ± 6.57	0.046 ^b	72.03 ± 7.86	68.66 ± 12.42	0.471
Hip circumference (cm) ^a	109.58 ± 11.07	104.82 ± 4.58	0.163	92.04 ± 5.79	92.33 ± 3.51	0.930
Waist-hip ratio ^a	0.88 ± 0.09	0.87 ± 0.06	0.571	0.78 ± 0.07	0.75 ± 0.13	0.397
Waist-height ratio ^a	0.60 ± 0.06	0.58 ± 0.04	0.381	0.46 ± 0.05	0.45 ± 0.06	0.871
Conicity index ^a	1.21 ± 0.10	1.20 ± 0.07	0.780	1.12 ± 0.09	1.06 ± 0.13	0.262
Abdominal volume index ^a	18.84 ± 4.38	16.74 ± 2.31	0.021 ^b	10.81 ± 2.22	10.11 ± 3.24	0.593
Body adiposity index ^a	36.01 ± 5.91	35.57 ± 4.05	0.811	28.36 ± 4.04	31.63 ± 4.69	0.171
Body fat (%) ^a	33.31 ± 6.40	36.73 ± 5.26	0.093	24.25 ± 7.18	20.67 ± 0.58	<0.001 ^b
Triglycerides (mmol/L) ^c	1.30 (0.50–14.30)	1.10 (0.50–2.20)	0.290	0.90 (0.30–7.90)	0.50 (0.40–6.10)	0.566
Total cholesterol (mmol/L) ^a	5.70 ± 1.51	5.88 ± 1.06	0.706	5.39 ± 1.31	6.07 ± 1.78	0.381
HDL-C (mmol/L) ^a	1.19 ± 0.30	1.35 ± 0.32	0.112	1.38 ± 0.33	1.37 ± 0.38	0.966
LDL-C (mmol/L) ^a	4.11 ± 1.26	4.28 ± 0.93	0.659	3.75 ± 1.16	4.23 ± 1.31	0.473

Data are reported as mean ± SD for the indicated number of subjects (n) in each group unless indicated otherwise.

BMI: body mass index, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol.

^a Independent sample *t*-test.

^b Significant difference between the genotype; *P* value = 0.000 was demonstrated as *P* < 0.001. Both *P* < 0.001 and *P* < 0.05 are considered significant.

^c Mann-Whitney *U* test.

among the Koreans (Oh et al., 2000; Kim et al., 2007), Qatari (Bener et al., 2013), Chinese (Wang et al., 2012) and Palestinian (Erekat et al., 2009) subjects, whereas the frequency is much higher in the Asian Indians (Bhatt et al., 2012) and Iranians (Mirzaei et al., 2009) of 12.8%, 10.2% and 16.6%, respectively. Most plausible explanation to these disproportions is due to that multi-ethnic and cultural backgrounds in Asia itself, which may contribute towards diverse gene effect on the phenotypic expression among different populations of Asian ancestry (Phani et al., 2016). Regarding the Caucasians, the reported Ala12 allele frequency in a range of 9.0–15.0% was comparable, confirming their phylogenetic similarities despite being from different geographical locations (Beamer et al., 1998; Ek et al., 1999; Valve et al., 1999; Clement et al., 2000; Evans et al., 2000; Swarbrick et al., 2001; Gonzalez Sanchez et al., 2002; Ghousaini et al., 2005; Carlos et al., 2013).

This study found a significant association between obesity with Ala12 allele of the PPAR γ 2 gene. Besides, we found that the Pro12Ala polymorphism was an independent risk factor of obesity in our Malay

Table 5
Univariate logistic regression analysis for association of PPAR γ 2 gene Pro12Ala polymorphism and other predictor variables with obesity.

Variables	β	SE	Wald (df)	Crude OR	95% CI	P-value
Gender				1.00 (ref)	–	–
Male	–	–	–	–	–	–
Female	–0.834	0.289	8.351 (1)	0.434	0.247–0.765	0.004*
Age	0.052	0.014	14.075 (1)	1.053	1.025–1.082	< 0.001*
TG (mmol/L)	0.595	0.194	9.354 (1)	1.812	1.238–2.653	0.002*
TC (mmol/L)	0.168	0.101	2.774 (1)	1.183	0.971–1.443	0.096
HDL-C (mmol/L)	–1.655	0.460	12.933 (1)	0.191	0.078–0.471	< 0.001*
LDL-C (mmol/L)	0.266	0.119	4.958 (1)	1.305	1.032–1.649	0.026*
PPAR γ 2 gene				1.00 (ref)	–	–
Pro12Pro	–	–	–	–	–	–
Pro12Ala	1.668	0.667	6.257 (1)	5.301	1.435–19.587	0.012*

Gender (male and female) and PPAR γ 2 gene (Pro12Pro and Pro12Ala) are included as categorical variables. Age, TG, TC, HDL-C and LDL-C are given as continuous variables.

TG, triglycerides; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; β , beta logistic regression coefficient; SE, standard error; Wald, Wald statistics; df, degrees of freedom; OR, odds ratio; CI, confidence interval of the odds ratio.

* *P*-value = 0.000 is demonstrated as *P* < 0.001. Both *P* < 0.001 and *P* < 0.05 represented in bold are considered significant.

subjects. Odds ratio analysis in our univariate and multivariate logistic regression model (adjusted for known confounders) showed that the odds of obesity were increased from 5.301 folds to 5.456 in those carriers for Ala12 allele as opposed to the non-carriers. Furthermore, the Ala12 allele was associated with higher BMI, body fat percentage, age and a trend of higher body adiposity index in all subjects suggesting the impact of this genetic polymorphism on the regulation of body mass (Lei et al., 2000; Mirzaei et al., 2009), adiposity (Bhatt et al., 2012; Prakash et al., 2012) and obesity (Mirzaei et al., 2009; Bhatt et al., 2012; Prakash et al., 2012). However, subgroup analyses in obese group showed that the Ala12 allele carriers was associated with lower body weight, BMI, waist circumference and abdominal volume index and a marginally higher body fat percentage than the non-carriers without significant differences. As for the non-obese, the Ala12 allele was associated with lower body fat percentage in this group. Different indices have been used for obesity assessment (Yao et al., 2015). The BMI is mostly applied in clinical practice despite being known for its limited accuracy (Bergman et al., 2011). Other than that waist (WC) and hip (HC) circumferences, waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR), abdominal volume index (AVI), conicity index (CI) and body adiposity index (BAI) were also used in this study due to their high accuracy, more sensitive and cost-efficient (Bergman et al., 2011; Motamed et al., 2015).

Table 6
Multivariate logistic regression analysis for association of PPAR γ 2 gene Pro12Ala polymorphism and other predictor variables with obesity.

Variables	β	SE	Wald (df)	Adjusted OR	95% CI	P-value
Age	0.043	0.016	7.394 (1)	1.044	1.012–1.077	0.007*
TG (mmol/L)	0.643	0.178	13.023 (1)	1.902	1.341–2.696	< 0.001*
LDL-C (mmol/L)	2.627	0.644	16.636 (1)	13.287	3.914–48.853	< 0.001*
PPAR γ 2 gene				1.00 (ref)	–	–
Pro12Pro	–	–	–	–	–	–
Pro12Ala	1.697	0.743	5.217 (1)	5.456	1.272–23.401	0.022*

PPAR γ 2 gene (Pro12Pro and Pro12Ala) is included as categorical variables while age, TG and LDL-C are given as continuous variables.

TG, triglycerides; LDL-C, low density lipoprotein cholesterol; β , beta logistic regression coefficient; SE, standard error; Wald, Wald statistics; df, degrees of freedom; OR, odds ratio; CI, confidence interval of the odds ratio.

* *P*-value = 0.000 is demonstrated as *P* < 0.001. Both *P* < 0.001 and *P* < 0.05 represented in bold are considered significant.

The role for the genetic variations in PPAR γ 2 gene, particularly the Pro12Ala polymorphism in the genetic determinants of obesity is supported in many studies, including the Spanish (Gonzalez Sanchez et al., 2002), Brazilian of European descent (Mattevi et al., 2007), native Javanese (Danawati et al., 2005), French (Meirhaeghe et al., 2000), Iranian (Mirzaei et al., 2009), Asian Indian (Bhatt et al., 2012), obese Finnish women (Valve et al., 1999), obese Danish-Caucasian (Ek et al., 1999) and obese American-Caucasian (Beamer et al., 1998), while other studies failed to detect similar associations, as being reported in the Caucasian Portuguese women (Carlos et al., 2013), obese aboriginal Qatari (Bener et al., 2013) and Australian-Caucasians (Swarbrick et al., 2001). These inconsistencies reflect the complex pathogenesis of obesity, as the patterns vary across ethnicities (He, 2009). Other contributing factors include the differences in the study designs (Kim et al., 2007; Hsiao and Lin, 2015), effects of gender (Valve et al., 1999; Gonzalez Sanchez et al., 2002), racial differences in Ala12 allele frequency (Kim et al., 2007) and the use of different BMI criteria to define obesity (Kim et al., 2007; Prakash et al., 2012; Wang et al., 2012; Hsiao and Lin, 2015). Furthermore, Hsiao and Lin (2015) speculated that these disagreements may partly be due to the differences across ethnic groups in the haplotypes of PPAR γ 2 gene Pro12Ala polymorphism with other SNPs in the same gene, thus other neighbouring variants may influence the prevalence of overweight or obesity. In addition to the genetic effect, environmental factors also appear to be very important. It is speculated that low ratio of dietary polyunsaturated fatty acid to saturated fatty acid cause higher BMI in Ala12 carriers relative to the non-carriers, but when the dietary ratio is high, the opposite is seen (Luan et al., 2001).

This study also found no significant interaction effect between Pro12Ala polymorphism and lipid profiles and in the entire subjects, obese and non-obese groups. However, carriers for Ala12 allele exhibits consistently higher total cholesterol and LDL-cholesterol without significant differences. Multiple studies have been conducted previously but the results were controversial. Beamer et al. (1998) reported that Ala12 carriers were associated with considerably higher triglycerides and lower HDL-cholesterol levels are gender dependent. Consistent association, except for the gender-interaction effect (Beamer et al., 1998), was also reported by Swarbrick et al. (2001). Besides, Meirhaeghe et al. (2000) found that Ala12 carriers were associated with increased levels of total cholesterol, LDL-cholesterol and apolipoprotein B than the non-carriers. Other studies found no association at all (Kim et al., 2007; Mirzaei et al., 2009). Differences in results could be attributed to different ethnic backgrounds and nutrition profile (Bhatt et al., 2012; Li et al., 2015).

From the molecular perspective, the effect of Ala12 allele towards metabolic heterogeneity can be explained by the influence of this genetic polymorphism on PPAR γ transcriptional activity (Zarebska et al., 2014). In vitro, the ability for PPAR γ 2 Ala12 allele to bind for specific response elements (PPREs) and activating target genes including the lipoprotein lipase was decreased, resulting in a less active form of PPAR γ 2 protein (Schoonjans et al., 1996; Deeb et al., 1998; Auwerx, 1999). Besides, the specific localisation of the Pro12Ala amino acid substitution within the PPAR γ molecule has been implicated in the transcriptional changes of PPAR γ activity and its associated different physiological effects (Zarebska et al., 2014). This amino acid substitution was positioned within the ligand independent AF-1 activation domain that contain a consensus mitogen activated protein (MAP) kinase site which was shown to play an important role in controlling PPAR γ transcriptional activity (Adams et al., 1997). Phosphorylation of this site reduces PPAR γ 2 binding affinity by its interaction with specific co-repressor proteins (Adams et al., 1997) or by inter-domain communication between the AF-1 and the ligand binding domain (LBD) (Auwerx, 1999), resulting in low transcriptional activation activity of the target genes. This study also found that age, higher levels of triglycerides and LDL-cholesterol independently increases the odds for obesity by 1.90 and 13.83 folds respectively, further indicates that cardiovascular disease risk factor tend to cluster with obesity (Grundy et al., 2004).

Data regarding the association of this genetic polymorphism with obesity among Malay subjects are scarce. Although environmental factors are well established as the significant contributors (Jan Mohamed et al., 2014), having a better understanding of the genetic susceptibility for obesity are also important to decipher the complex genetic architecture of obesity. Therefore, we believe that our research findings are a promising start for extensive research efforts in unravelling the genetic determinants for obesity in Malaysia.

5. Conclusion

In summary, we found that the PPAR γ 2 gene Pro12Ala polymorphism was significantly associated with increased risk for obesity in our subjects and Ala12 allele could predict alterations in adipocyte and lipid metabolism among them. Ala12 allele as well as age and a higher level of triglycerides and LDL-C were regarded as independent risk factors for obesity in this ethnicity.

Disclosure statement

The authors declare that they have no conflict of interest.

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