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Peroxisome Proliferator Activated Receptor Gamma (PPARγ) Gene Variant in Relation to Physical Activity and Obesity Among Malay Children

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Abstract

Introduction: The Pro12Ala variant of the Peroxisome Proliferator Activated Receptor Gamma (PPARγ) is one of the critical genetic factor predispose to positive energy balance leading to obesity. **Objective**: This study aimed to determine the association between Pro12Ala variant in the PPARγ gene with body mass index (BMI) status, physical activity and fat intake among Malay children. **Methods**: A total of 119 participants aged between 9-11 years old from primary schools in Kota Bharu, Kelantan were recruited. Anthropometric measurements were taken and activity counts of the participants were recorded using accelerometer (Actigraph GT3X+). A food diary was distributed to all participants to collect the data of their fat intake. Genotyping was performed using High Resolution Melting (HRM) analysis. Data obtained were analysed using SPSS version 19. **Results**: There was a significant association between Pro12Ala variant in the PPARγ gene with BMI status. The allelic frequency of wildtype (Ala/ala) and heterozygous (Pro/ala) among overweight group were 0.83 and 0.17 respectively and 0.92 and 0.08 in the normal weight group (p=0.03). There was a significant difference in BMI, waist circumference and hip circumference between heterozygous and wildtype groups. **Conclusion**: The study found that there was a significant role of Pro12Ala variant in the PPARγ gene in overweight Malay children.

Keywords: PPARγ gene, physical activity, fat intake, children, Malaysia

Introduction

Overweight is one of the most health related problems affecting children in developed countries. The Third Malaysian National Health and Morbidity Survey showed that the prevalence of overweight children was 5.4% (NHMS III, 2006). This number is increasing every year and the overweight and obesity trends are also observed worldwide and have been viewed as a global epidemic of obesity.

Generally, overweight is a multi-factorial syndrome influenced by both environmental and genetic factors (Barbieri et al., 2005 and Duran-Gonzalez et al., 2011). Environmental factors such as reduced physical activity, intake of energy-dense food, cultural and socioeconomic factors significantly contributed to the development of obesity. A study stated that genetic factors account for 40-90% of the body mass index (BMI)

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variations among populations (Duran-Gonzalez et al., 2011).

There is growing evidence suggesting that Peroxisome Proliferator Activated Receptor Gamma (PPARγ) may be one of the most critical genetic factors predisposing to positive energy balance and, ultimately obesity (Mela, 2005). Located on chromosome three and specifically expressed in adipose tissue, PPARγ is induced during adipocytes differentiation (Yen et al., 1997).

In this study the PPARγ2 was selected. The human PPARγ2 has 28 additional amino acids and expressed exclusively in adipose tissue. A missense mutation Pro12Ala was identified in this gene. Located on codon 12 at 34 nucleotide position of PPARγ2 a missense mutation CCA (Pro) ->GCA (Ala) was detected (Yen et al., 1997). It induces the fibroblasts to differentiate into adipocytes hence leading to adipocyte differentiation and thus fat cell accumulation (Cecil et al., 2006). The Pro12Ala variant in PPARγ also found to be associated with insulin resistance and type 2 diabetes among Europeans, in interaction with a high-fat diet (Stryjecki et al., 2016).The more active the PPARγ, the higher the BMI and the risk for insulin resistance. A cross-sectional study conducted among Malay adults suggested that the Pro12Ala of the PPARγ gene is predispose to obesity (Zahri et al., 2016).

Besides genetic factor, lack of physical activity and excess caloric consumptions are some of the reasons for the development of obesity (Anderson, 2003). For instance, a cohort study in the USA revealed a higher increase in BMI for preadolescents who reported higher caloric intakes and less physical activity (Kiess, 2004). Indeed, a study reported that overweight children had significantly lower physical activity especially during weekend compared to normal weight boys (Lin, 2012). Moreover, engaging in sedentary behaviour such as television viewing is also one of primary factors of the current worldwide obesity epidemic.

Evidence also suggests that diet during childhood may have an important implication for the development of obesity and chronic diseases in later life. It has been reported that obese children consumed significantly larger amounts of total calories, protein and fat compared to the normal weight children (Soo et al., 2011). The causes of increased energy intakes include larger portion sizes, eating in restaurants, eating late at night, instant availability of energy dense food and fast food and frequent snacking (Kiess et al., 2004).

Variety of health consequences can occur due to pediatric obesity. The consequences and co morbidities of childhood obesity are not only suffered during childhood but also affect their adulthood. The consequences of obesity that commonly affect children and adolescent are increased growth then stunting, increased in fat free mass, early menarche, hyperlipidemia, increased heart rate and cardiac output, abnormal glucose metabolism and others (Schonfeld-Warden and Warden, 1997). These physical health problems then lead to lower physical fitness consequently affecting their psycho and social wellbeing and lowering their quality of life (Lin et al., 2012). For example, obese children are frequently the target of discrimination and stigmatization. As a result, they always suffer significant psychosocial consequences such as being shamed, marginalized and rejected (Eisenmann, 2006).

As mentioned before, genetic influence is one of the major contributing factors in people with severe and early onset of obesity. However, there are also some individuals who develop obesity while others do not although they have obesity-related genes. The contribution of obesity-related genes is influenced by an interaction with environmental factors predisposing to obesity, such as overeating and a sedentary lifestyle (Kiess et al., 2004). Due to the rising prevalence and health consequences of pediatric obesity, the assessment of factors that leads to overweight and obesity at an earlier stage of life is essential. Therefore, the present study was proposed to investigate the genetic factor of PPARγ gene variant which is one of the strong candidate gene influencing physical activity participation and dietary intake among Malay children.

Materials and methods

One hundred and nineteen (119) participants were recruited in this study (aged 9 to 11 years). The study was approved by ethical board of Ministry of Education and Universiti Sains Malaysia. Participants involved were of both genders with Malay ethnicity, healthy and free

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from any illness or physical disabilities. Informed consent was obtained from their parents prior to recruitment of the study participants. Procedure was started with anthropometric measurements which included the height and weight measurements. Height measurement was taken using wall-mounted stadiometer (Seca Bodymeter 208, Germany) with the participant standing straight with feet slightly apart at shoulder width without wearing shoes and looking straight. Body weight measurement was taken using a weighing scale (Tanita THD-306, Japan) with their uniforms on, without shoes or any accessories and pockets emptied.

The height and weight of the participant was measured for Body Mass Index (BMI) calculation. The BMI was calculated and classified based on WHO standard for children.

Then, waist-to-hip girth ratio (WHR) was done using a measuring tape and calculated in centimeter. The waist girth represents the smallest girth around the abdomen (the natural waist) and the hip girth reflects the largest girth measured around the buttock. The measurements were taken with the participant wearing minimal clothes and standing with feet together (Katch et al., 2011). The WHR was calculated using the formula:

$$\text{WHR} = \text{Waist girth (cm)} / \text{Hip girth (cm)}$$

Body fat percentage was also measured using the Omron body fat monitor (Omron HBF-302, Japan). The bioelectrical impedance analysis (BIA) is a simple, quick and non-invasive technique to estimate the fat and fat-free mass especially in children. This method uses a small alternating current flowing between two electrodes passing more rapidly through hydrated fat free body tissues and extracellular water than through fat or bone tissue. Impedance to electrical current flow relates to total body water content, which in turn relates to fat free mass, body density, and percentage body fat (Ritz et al., 2007). Participants were asked to empty their urinary bladder before the start of measurement. During the measurement process, participants were required to stand still with their feet slightly apart and arms straight forward at a 90° angle with straight elbows. The height, weight, age and gender were keyed in the Omron body fat monitor to obtain the body fat percentage and fat mass (Powers and Howley, 2007).

Assessment of physical activity by Accelerometer GT3X+

Accelerometer GT3X+ was used to measure the physical activity of the participants. The GT3X+ measures acceleration in three individual orthogonal planes (Ventrical (VT), Anterior-posterior (AP), and medio-lateral (ML)) and provides activity counts as a composite vector magnitude of these axes (VM3). The participants used the accelerometer at their waist for five days which consisted of three weekdays and two weekends. Prior to giving the GT3X+ to the participants, the accelerometer was initialized. During the process of initialization, setting parameters were set. The sample rate was set at 30 Hz, flash LED during data collection mode was selected whereas start date and time was set at the day the GT3X+ was given to the participant at 12.00pm. Stop time button was also selected by setting the stop date five days after the GT3X+ was given and ended at 12.00pm the next day. The epoch length of data collection was set at 60 seconds. The data recorded by GT3X+ was then analysed using ActiLife 6 Data Analysis Software by Actigraph. As for data screening criteria, a non-wear period was set at default setting which define a non-wear period after 60 minutes of consecutive zeroes. The physical activity level of the children was then classified using cut off points by Fredson et al., 2005.

Calculation of Activity Counts (CPM) is the composite vector magnitude of Axis 1 (vertical axis), Axis 2 (mediolateral axis) and Axis 3 (anterior-posterior axis) was also calculated through accelerometer GT3X+.

Record of food intake

A food diary was given to each participant. The parents were briefed on how to estimate the portion sizes of foods and beverages consumed by giving examples and household measurements like bowls, cups, plates, glass and spoon. They were requested to record in detail the type of food intake, eating frequencies, and amount of food consumed of their child for four days. The dietary data was then analysed by using Diet Analysis Plus® Version 5.1 software to calculate the average percentage of fat intake in daily diet of the participant.

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Genotyping

Blood sample from each participant was obtained through venepuncture. Genomic DNA was extracted from leukocyte of the blood following the protocols from manufacturer using the DNA blood mini kit GeneAll®Exgene™ (GeneAll Biotechnology Co. Ltd., Seoul, Korea). Then, Polymerase Chain Reaction (PCR) was done followed by High Resolution Melting analysis to identify the genetic variation of PPARγ. PCR was prepared consisted of 0.06 μl of 5U Amplitaq Gold DNA Polymerase., 2 mM $MgCl_2$, 2 μl of DNA and 0.3 μl each primer at 10 pmol, 1.0 μl of 10X PCR buffer and deionised water was added to a final volume of 10 μl. The PCR conditions were 94◦C for 7 minutes followed by 40 cycles in three steps: 94◦C for 5 s, 57.7◦C for 5 s and 72◦C for 5 s followed by 72◦C for 10 s in the final extension. High resolution melting (HRM) analysis was performed using the PikoReal 96 real-time polymerase chain reaction (PCR) system (Thermo Fisher Scientific Inc., United States). The HRM assay for the targeted SNP was carried out in a 96-well Piko PCR white plate (Thermo Fisher Scientific Inc., United States). A total of 10μL of final volume was prepared. It contained 5μL of 2× Precision Melt Supermix (Bio-Rad Laboratories, Inc., CA), 1.0μM of each forward and reverse primer (Sigma-Aldrich, Malaysia), 3μL of DNA template and 7μL of assay master mix volume. The following forward and reverse primers were used in the HRM analysis: Forward 5′ ATA TCA GTG TGA ATT ACA GCA 3′ and Reverse 5′ GAA GGA ACT TTA CCT TGT GA 3′. The real-time PCR condition consisted of an initial denaturation step at 95 °C for 2 min that was followed by 45 cycles of denaturation (95 °C for 10 s), annealing (58 °C for 30 s) and extension (72 °C for 30 s). The subsequent HRM program for heteroduplex formation was under the following conditions: denaturation at 95 °C for 30 s and renaturation at 60 °C for 30 s and high resolution melt with continuous fluorescence readings from 65 °C to 95 °C in 0.2 °C increments. The PikoReal Software version 2.1(Thermo Fisher Scientific Inc., United States) was used to analyze the HRM data. The different melting patterns were produced and suggested the different genotypes of the SNP. In order to confirm the results, sequencing analysis was performed.

Results

Characteristics of study participants

There were 119 children participated in this study. All participants were Malay children from seven schools in Kota Bharu district. The characteristics of the participants were shown in (Table 1). From 119, 28 were males and the rest 91 were females. Age of the participants ranged from 9-11 years old. As for the BMI status, 72 were categorized in normal weight and 47 were under overweight group.

Table 1. Characteristics of the participants

Variables	***Frequency (%)***	***Mean (SD)***
Gender		
Male	28 (23.5)	
Female	91 (76.5)	
Age (years)		10.3(0.8)
Weight (kg)		39.2(12.9)
Height (cm)		139.9(9.2)
Body Mass Index, BMI (kg/m2)		19.7(4.8)
% Body fat (%)		23.9(8.0)
Waist hip ratio		0.8(0.1)
BMI group		
Normal	72 (60.5)	
Overweight	47 (39.5)	
Genotype		
Wildtype	91 (76.5)	
Heterozygous	28 (23.5)	

*SD=Standard Deviation

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Genotype distribution and allele frequency of Pro12Ala PPARγ gene among overweight and normal weight group

Result in Table 2 from the 119 participants showed, 28 of them were heterozygous which (n=16) were from overweight group and (n=12) from normal weight group whereas, 91 children were wildtype with (n= 31) from overweight group and (n=60) from normal weight group. None was recorded for homozygous. The genotype distribution and allele frequency of wildtype, heterozygous and homozygous of Pro12Ala was shown in Table 2. There was a significant association (p=0.03) between Pro12Ala PPARγ gene variant and BMI group with allelic frequency among overweight children Pro12 and Ala12 were 0.83 and 0.17 respectively and in normal weight group the allelic frequency of Pro12 and Ala12 were 0.92 and 0.08 respectively.

Table 2. Genotype distribution and allele frequency of Pro12Ala PPARγ gene variant among overweight and normal weight groups

Group	Wildtype Pro/pro (CC) n (%)	Heterozygous Pro/ala (CG) n (%)	Homozygous Ala/ala (GG) n (%)	P-value	[b]Allele frequency	
					Pro/pro	*Pro/ala*
Overweight (n=47)	31 (0.66)	16 (0.34)	0	0.03[a]	0.83	0.17
Normal weight (n= 72)	60 (0.83)	12 (0.17)	0		0.92	0.08

[a] Pearson Chi-Square test
Significant level set at p< 0.05
[b] Allele frequency is calculated using Hardy-Weinberg equilibrium equation

Comparison of phenotype data between participants with and without genotypic variant of *Pro12Ala PPARγ* gene

Table 3. Comparison of phenotype data between heterozygous and wildtype group

Variables	Heterozygous Pro/ala (CG) (n=28) Mean (SD)	Wildtype Pro/pro (CC) (n=91) Mean(SD)	Mean diff (95% CI)	*T* stats[a] (df)	*P* value[b]
Body Mass Index	21.3 (4.7)	19.2 (4.8)	2.1 (0.06,4.14)	2.039	0.04*
Body fat %	26.3 (7.4)	23.2 (8.1)	3.14 (-.28, 6.56)	1.816	0.07
Waist circumference	65.9 (10.3)	61.0 (11.3)	4.98 (0.23,9.73)	2.07	0.04*
Hip circumference	81.7 (10.4)	76.4 (10.7)	5.35 (0.77,9.94)	2.314	0.02*
Fat intake (%)	84.1 (42.6)	75.9 (38.5)	8.25 (8.6,25.1)	0.96	0.33
Activity counts (CPM)	2503 (615)	2793 (1013)	290 (691, 110)	-1.435	0.15

[a] Independent t test
[b] Significant level at 0.05
*there is significance difference between variable means

Data in Table 3 showed that there were significant different *P*<0.05 on mean of body mass index, waist circumference and hip circumference between participants with wildtype and heterozygous of Pro12Ala PPARγ gene variant group. Participants with Pro/ala carriers have higher BMI, waist and hip circumference compared to Pro/pro group whereas, there were no significant difference in body fat percentage, percentage of fat intake and activity counts between the two groups.

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Discussion

Result showed the overall heterozygous Pro12Ala PPARγ gene variant among Malay children in Kota Bharu, Kelantan is 23.5% which comprised of 13.0 % from overweight children and 10.5% from normal weight children. This finding is supported by many studies from Caucasians in different countries which concluded that Pro12Ala variant was greater than 10%. A study in Malaysia found that 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 Body Adiposity Index, BAI (P = 0.055) than the non-carriers (Zahri et al., 2016). However, this finding is contradicts a statement in one research study that stated Asian people have less than 10% of prevalence of Pro12Ala variant (Danawati et al., 2005).

In this study, the allelic frequency of Pro/ala was 0.17 in overweight and 0.08 in normal weight of Malay children (Table 2). Ochoa et al (2008) reported almost similar allelic frequencies in obese Spanish children (allelic frequencies 0.13) than in normal weight Spanish children 0.08 suggested that this variant was associated with obesity.

In contrast, previous reports from study on Asian population from Singapore consisting of 2730 Chinese, 740 Malays and 568 Indians reported that a very low allele frequency for the Ala12 was found in Malays (0.032). However, the study reported that there was a significant association between Pro12Ala variant and BMI in normal group with Pro/ala carriers have higher mean BMI similar with the finding in this study (Table 3) (Tai et al., 2004).

The result in Table 2 showed that there was a significant association between the presence of Pro12Ala PPARγ gene variant with normal weight and overweight groups. This result is supported by a study conducted among children in Asian population (Tai et al. 2004) and Egypt population (Shawky and Sadik, 2012). A study in a large cohort (n=1170) of white British observed that subjects for the homozygous Ala/Ala had significantly higher mean BMI than both Pro/ala and Pro/pro groups, indicating a recessive model for the Pro/ala allele. In addition, from meta-analyses of 30 independent studies found that the common Pro12Ala variant of the PPARγ gene may be a genetic modifier of obesity. These findings also provide a strong genetic evidence of an important roles of PPARγ in the regulation of body weight, insulin sensitivity, glucose homeostasis, and blood pressure (Masud and Ye, 2003).

The mechanism of the development of obesity through this gene can be explained by the ability of PPARγ gene variant to induce fibroblasts to differentiate into adipocytes thus leading to fat cell accumulation (Hwang et al., 2006). The more active PPARγ (activating mutations), the higher the Body Mass Index and the higher risk for insulin resistance. Contradicted with this study finding, there was no association between the presence of Pro12Ala PPARγ gene variant between normal weight and overweight groups, however in opposite effect, it may associate with higher insulin sensitivity and higher long chain polyunsaturated fatty acid that might bring to protective effect from metabolic complications (Scaglioni et al., 2006).

Dedoussis et al. (2009) in their study among Dutch preadolescence observed that there was no significant association between Pro12Ala PPARγ gene mutation and BMI. They also reported that boys with the Ala allele had lower skinfold measures (triceps and subscapular) compared to those with the Pro12 allele which suggest a protective effect of the Ala allele against obesity.

There were low number of studies on Pro12Ala in relation to physical activities to support this finding and so far no study had been reported to determine physical activity in Pro12Ala PPARγ gene variant using accelerometer. However, this gene is one of the most studied genes as potentially linked to the development of obesity and especially related to the interactions with lifestyle factor.

Finding in this study showed that there was no significant difference between Pro12Ala PPARγ gene variant and physical activity among normal weight and overweight children. Dedoussis et al. (2009) in their study observed that there were no significant difference that was observed between Pro12Ala PPARγ genotypes group and sedentary activity.

Nelson et al. (2007) in their study could not find an association between Pro12Ala gene variant and increase in BMI, hence they could not conclude that physical activity altered any association between BMI.

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Table 3 shows that, there was no significant different ($p>0.05$) on percentage of fat intake between group with variant and without variant of Pro12Ala PPARγ gene.

The Ala12 allele was associated with higher BMI, waist circumference, fat mass and a greater accumulation of visceral and subcutaneous adipose tissue accumulation. However, total and saturated fat intakes were more closely associated to components of the metabolic syndrome in Pro12/Pro12 homozygotes than in carriers of the Ala12 allele (Robitaille et al., 2003).

Body Mass Index of PPARγ Ala12 variant allele carriers was higher than BMI of non-carriers. A positive trend between increasing intake of total fat and BMI was observed in Pro/Pro homozygotes but not in PPARγ Ala12 carriers. However, intake of saturated fat was directly associated with increased BMI among individuals of both genotypes (Memisoglu et al., 2003).

Conclusion

Pro12Ala of the PPARγ gene is suggested to play a significant role in the development of obesity. The study also found that physical activity and fat intake does not give significant impacts on Malay children with and without obesity.

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