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ORIGINAL ARTICLE

Effects of Leptin Gene Variants on Obesity and Its Attributes in Malay Population

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ABSTRAK

Leptin adalah hormon yang mengawal pengambilan dan pengeluaran tenaga yang dikodkan oleh gen leptin. Varian gen leptin telah dikaji secara komprehensif berhubung dengan status berat badan, tetapi bukti-bukti itu tidak muktamad. Kajian ini adalah untuk menentukan hubungan antara varian gen leptin, G2548A, H1328080 dan A19G dengan obesiti serta sifatnya dalam kalangan penduduk di Terengganu, Malaysia. Kajian ini melibatkan seramai 249 subjek Melayu (101 orang dewasa yang sihat dengan BMI biasa sebagai kumpulan kawalan dan 148 subjek berat badan berlebihan dan obes). Data antropometrik diperoleh, sampel darah telah diambil untuk analisa bagi penanda genetik dan profil lemak. Teknik PCR-RFLP dilakukan untuk menentukan pembahagian genotip dan alel bagi gen varian leptin. Frekuensi genotip dan allelik varian gen leptin tidak menunjukkan perbezaan yang signifikan antara kumpulan, G2548A (P = 0.93 dan 0.74); H1328080 (P = 0.58 dan 0.56); dan A19G (P = 0.72 dan 0.38). Walau bagaimanapun, terdapat perbezaan secara statistik antara tahap trigliserida dan genotip varian G2548A (P = 0.016); antara tahap kolesterol dan genotip H1328080 (P = 0.027). Disamping itu, regresi logistik multivariat memperlihatkan jantina lelaki (OR = 26.27; CI = 1.06-1.25; P = 0.009), ukur lilit pinggang (OR = 1.15; CI = 1.06-1.25; P = 0.001) dan peratus lemak badan (OR = 1.43; CI = 1.20-1.70; P<0.001) adalah faktor risiko bebas untuk obesiti. Data mencadangkan bahawa varian G2548A, H1328080 dan A19G tidak dikaitkan dengan obesiti. Walau bagaimanapun, ukur lilit pinggang dan peratusan lemak badan menyumbang kepada peningkatan risiko obesiti dalam populasi Melayu.

Kata kunci: leptin, Melayu, obesiti

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ABSTRACT

Leptin is a hormone that regulates the energy intake and expenditure which is encoded by leptin gene. Leptin gene variants were studied comprehensively in relation with body weight status, but the evidences were indecisive. This study was to determine the association between leptin gene variants, G2548A, H1328080 and A19G with obesity and its attributes in Terengganu, Malaysian population. This study involved a total of 249 Malay subjects (101 healthy adults with normal BMI as the control group and 148 overweight and obese subjects). The anthropometrics data were obtained, blood samples were collected for genetic markers and lipid profile analyses. PCR-RFLP technique was performed to determine the genotype and allele distribution of leptin gene variants. The genotypic and allelic frequencies of leptin gene variants presented no significant difference between groups, G2548A (P = 0.93 and 0.74); H1328080 (P = 0.58 and 0.56); and A19G (P = 0.72 and 0.38) correspondingly. However, there was statistical significant difference between triglyceride level and genotypes of G2548A variant (P = 0.016); between total cholesterol level and H1328080 genotypes (P = 0.027). In addition, multivariate logistic regression projected the male gender (adjusted OR= 26.27; CI= 1.06-1.25; P = 0.009), waist circumference (adjusted OR = 1.15; CI = 1.06-1.25; P = 0.001) and body fat percentage (adjusted OR = 1.43; CI = 1.20-1.70; P<0.001) were the independent risk factors for obesity. The data suggest G2548A, H1328080 and A19G variants were not associated with obesity. However, waist circumference and body fat percentage may increase risk for obesity in Malay population.

Keyword: leptin, Malay, obesity

INTRODUCTION

Globesity is a deteriorating phenomenon which reflects the common health problems of being obese and overweight and exacerbating the risks of metabolic dysfunction, heart diseases as well as cancers (Ismail et al. 2002). According to Fifth National Health Malaysia Survey (NHMS V) in 2015, the prevalence of overweight and obesity were 17.7% and 30.0% respectively from the whole population, which showed a rapid increment from previous years (Institute for Public Health 2006, 2011, 2015). Besides, ethnicity and gender have been shown to yield significant impact in the prevalence of obesity in Malaysia (Mohamud et al. 2011).

Environmental and genetic factors and the multifaceted interactions among them have been associated with body weight problems. Besides, the psychological effect such as vulnerability towards depression also contributed to development of obesity (Ainsah & Osman 2007). Hereditary factor in personal obesity genetic make-up of human was noted to be involved

in body weight stability by controlling certain processes such as appetite and adipogenesis that may lead to an individual response to energy supply and deficit. Several potential causal genes can contribute to the onset of obesity such as leptin, leptin receptor, proopiomelanocortin, proconvertase 1, and melanocortin 4 receptor (Loos et al. 2006; Ramachandrappa & Farooqi 2011). Leptin hormone encoded by leptin gene which is located at chromosome 7q31.3, responsible in producing leptin for food ingestion and energy expenditure (Isse et al. 1995; Zhang et al. 1994). The leptin production and secretion can be illustrated when the fat mass decreases due to low energy consumption; the leptin level is dropped which reducing energy expenditure and enhancing the response of satiety signal. Conversely, when the fat mass increases due to energy excess or high-calorie intake, more leptin are produced that suppress appetite and stimulate energy expenditure (Mantzoros et al. 2011; Rosenbaum et al. 2002). However, when mutation takes place across the leptin gene sequence, it may disrupt normal function of leptin in maintaining body weight. Current studies revealed leptin gene mutation may lead to early-onset obesity in children (Kohlsdorf et al. 2018; Ozsu et al. 2017). Leptin level and genetic screening could assist clinicians to pinpoint children with monogenic obesity due to functionally mutation in leptin gene.

Since identification of leptin gene as one of the candidate genes for obesity, variations in leptin gene were studied extensively. Furthermore, genetic variations of leptin gene play the significant role in determining the inter-individual variances in susceptibility or resistance to obesegenic environment. Several SNPs were selected and tested which assigned as G2548A (rs7799039), A19G (rs2167270) and H1328080 (rs12535747). Several studies were conducted to correlate polymorphisms of G2548A, A19G and H1328080 of LEP gene with obesity. The polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) is a treasured technique for genotyping of species-species variation that had been exploited for this study (Rasmussen 2012).

The present study was sought to associate the SNPs of leptin gene with obesity phenotypes, such as body weight, BMI, height, waist circumference, body fat percentage and the obesity-related parameter, i.e. lipid profile parameters in the Terengganu among Malaysian Malay population.

MATERIALS AND METHODS

STUDY SUBJECTS

This study was conducted in two groups. Briefly, one group was composed of 148 unrelated obese and overweight subjects, while the second group was consisted of 101 normal BMI subjects as the control group, who were volunteers from Terengganu's residents. Terengganu is located in Eastern Peninsular Malaysia and one of Malaysia's most homogenous states by means of Malay as the main ethnic.

During recruitment, subjects aged 18 and above, who were Malays with self-claimed report at least for three generations, and body mass index (BMI) stratification for case (<24.99 m^2/kg) and control (>25.00 m^2/kg) groups were enlisted in this study. However, we excluded the subjects who were smoking, taking lipid-lowering drugs and all subjects having chronic diseases such as renal failure, hepatic failure and cardiovascular diseases. Structured questionnaires and signed informed consent were obtained from subjects to get information on social demographic characteristic. This study was approved by the University of Human Ethics Research Committee (UHREC) at University of Sultan Zainal Abidin (UNISZA) in Terengganu, Malaysia (Reference No: UniSZA.C/1/UHREC/628-1 (17)).

ANTHROPOMETRIC PARAMETERS

BMI was calculated as body weight (kg) / height^2 (m^2). Obesity and overweight are defined as BMI 25 kg/m^2 while normal BMI is 24.99 kg/m^2 based on the International Classification of Obesity. Waist circumference (WC) was dignified using measuring tape (SECA 201, Hamburg, Germany) while the body fat percentage (BF) was measured using body composition analyzer (Tanita BC106, Amsterdam, Netherlands).

BIOCHEMICAL MEASUREMENTS

Venous blood samples were collected after a minimum of 8 hours fast. Blood samples were analyzed for triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and total cholesterol (TC) using automated chemistry analyzer (Olympus AU400, Tokyo, Japan). Low-density lipoprotein cholesterol (LDL-C) was calculated based on Friedewald formula, LDL-C: TC-HDL-C-(TG/5) (Knopfholz et al. 2014).

DNA ANALYSIS

Genomic DNA was isolated from peripheral blood using commercial DNA extraction kit (Vivantis, California, USA). G2358A, A19G and H1328080 polymorphisms of leptin gene were genotyped by the PCR-RFLP assay. DNA was amplified

Table 1: Primers and restriction enzymes used for genotyping

Variants	Primers (5′ to 3′)	Restriction enzyme	PCR product (bp)	PCR-RFLP product (bp)
G2548A	For : GCTTTCTAAGCCAAGGCA AA Rev: GCTCTTTTTCAAGGTGCACTG	HhaI	500	A = 500 G = 320/180
H1328080	For: ACCCATGTGTTCTTGGCACT Rev: CTTGAACCAGGGAACACACA	EcoNI	300	A = 300 C = 222/78
A19G	For: CTCTGGAGGGACATCAAGGA Rev: CGGGATCCAGAGTTGTGTG	HpyCH4III	386	G = 386 A = 296/90

For: forward direction; Rev: reverse direction

using flanking primers were briefly described in Table 1 (Fourati et al. 2013). PCR technique was performed in a total of 25 µl that consisted of 5X PCR buffer, 50mM $MgCl^2$, 25mM dNTP, forward and reverse primer and 5U/µl of Taq polymerase (Promega, Madison, USA). The PCR product was digested at restriction cut site with enzyme (refer Table 1). The PCR and PCR-RFLP product was preceded with electrophoresis, stained with ethidium bromide and viewed with the Alpha imager (FluorChem Q system, California, USA).

STATISTICAL ANALYSIS

Sample size calculation and power of the study were estimated for each variant based on synthetic validation approach (Johnson et al. 2001). Data normality was assessed by Kolmogorov-Smirnov test. The results were considered statistically significant when the *P*-value was less than 0.05. The alleles and genotypes frequencies were determined by Pearson Chi-Square and assessed by Hardy-Weinberg Equilibrium (HWE) by using SHEsis online software (Shi & L 2005). Comparison of the anthropometric and lipid profile between control and case groups were executed by t-test and Mann Whitney U-test for the skewed data whilst comparison between the variables and the genotypes of each variable were evaluated by ANOVA with post hoc Scheffe's procedure. Meanwhile, Kruskal-Walis test was performed for the skewed variable and proceed with Mann Whitney U-test repeatedly due to unavailability of post hoc test in Kruskal-Walis in order to determine the difference of each pair. Multiple logistic regression was implemented to determine the predictor that could be associated with obesity. In univariate analysis, genotypes of each variant and gender variable were analyzed as categorical variables, while age, lipid profile and anthropometrics variables were considered as continuous variables. The adjusted odd ratio (OD) and 95% confidence interval (CI) were evaluated to determine the association of the predictor variables with obesity risk. Statistical tests were executed by Statistical Package for Social Sciences (SPSS 21 for Windows; SPSS Inc. Chicago, IL, USA).

RESULTS

SUBJECTS CHARACTERISTICS

The demographics, anthropometrics and lipid profile characteristics of all subjects are summarized in Table 2. For this study, the number of women was higher compared to men (171 vs 78). The study involved adult Malaysian Malays in Terengganu with mean age of 40.00 (18.00) years. All anthropometric and lipid profile variables were shown significantly different between overweight/obesity and normal BMI groups.

GENOTYPE AND ALLELE FREQUENCIES OF LEPTIN GENE VARIANTS

The allelic and genotypic distribution

Table 2: Demographic, anthropometric and biochemical characteristics of the study group

Variables	Total (n=249)	Normal BMI (n=101)	Overweight/ Obesity (n=148)	P-value
Gender (male/female)[a]	78/171	27/74	51/97	
Age (years)[c]	40.0 (18.0)	39.0 (21.0)	41.5 (17.0)	0.003
BMI (kg/m^2)[b]	27.67±5.26	22.40±1.91	31.26±3.48	<0.001
WC (cm)[b]	88.53±13.86	76.76±8.41	96.54±0.78	<0.001
BF (%)[b]	32.32±8.49	26.59±7.15	36.23±6.98	<0.001
TC (mmol/L)[b]	5.40±1.32	5.00±1.21	5.68±1.33	<0.001
HDL-C (mmol/L)[b]	1.28±0.33	1.36±0.36	1.22±0.28	0.002
LDL-C (mmol/L)[b]	3.69±1.13	3.26±1.00	3.99±1.12	<0.001
TG (mmol/L)[c]	1.06 (0.84)	0.81 (0.55)	1.57 (0.94)	<0.001

[a]categorical variable: data are frequencies values; [b]data are mean±SD values: Independent t test; [c]data are median (IQR) values: Mann-Whitney test for skewed data; n indicates number of subjects in each group; P-value = 0.000 demonstrates as P<0.001; both P<0.001 and P<0.05 are considered significant; BMI: body mass index; WC: waist circumference; BF: body fat; TC: Total Cholesterol; HDL-C: HDL-cholesterol; LDL-C: LDL-cholesterol; TG: Triglyceride; SD: standard deviation; IQR: Interquartile range.

for G2548A, H1328080, and A19G variants are presented in Table 3. For G2548A, G allele is assigned as a wild-type with minor allele frequencies (MAF) with 0.31 for case group (HWE; *P*-value = 0.72) and 0.33 for control group (HWE; *P*-value = 0.61). Genotype distribution of G2548A revealed that homogenous mutant AA yielded the highest frequencies in the obese/ overweight group while heterozygous AG was highest in normal BMI group. For H1328080 variant, C wild-type allele frequency was higher in obese and overweight/obese group and normal BMI group while A mutant type with the MAF for case and control groups were 0.25 (HWE; *P*-value = 0.03) and 0.22 (HWE; *P*-value = 0.66) correspondingly. CC homozygous wild-type genotype demonstrated as the dominant genotype for both groups.

Mutant A allele of A19G variant of normal BMI group has higher MAF with 0.29 (HWE; *P-value* = 0.02) compared to overweight/obesity group with MAF value of 0.25 (HWE; *P*-value = 0.03). Based on statistical analysis, no significant difference was observed for allelic frequencies between groups with *P*-value 0.74, 0.56 and 0.38 whereas *P*-value for genotypic distributions were 0.93, 0.58, 0.72 respectively for G2548A, H1328080 and A19G.

CHARACTERISTICS OF ANTHROPOMETRIC AND LIPID PROFILE VARIABLES ACCORDING

Table 3: Allelic and genotypic frequencies of G2548A, H1328080 and A19G and association with obesity

Polymorphisms		All groups, n= 249		Odd ratio (CI)	P-value	MAF	
		Case, (n=148)	Control, (n=101)			Case	Control
G2548A							
Allele	A	202 (68.2)	135 (66.8)	1.066	0.74	0.31	0.33
	G	94 (31.8)	67 (33.2)	(0.74 – 1.56)		HWE (P=0.72)	HWE (P=0.61)
Genotype	AA	68 (45.9)	44 (43.6)	-	0.93	-	
	AG	66 (44.6)	47 (46.5)				
	GG	14 (9.5)	10 (9.9)				
H1328080							
Allele	A	74 (25.0)	46 (22.8)	1.13	0.56	0.25	0.22
	C	222 (75.0)	156 (77.2)	(0.74 – 1.72)		HWE (P=0.03)	HWE (P=0.66)
Genotype	AA	14 (9.5)	6 (5.9)	-	0.58	-	
	AC	46 (31.1)	34 (33.7)				
	CC	88 (59.5)	61 (60.4)				
A19G							
Allele	G	220 (74.3)	143 (70.8)	1.19	0.38	0.25	0.29
	A	76 (25.7)	59 (29.2)	(0.80 – 1.78)		HWE (P=0.03)	HWE (P=0.02)
Genotype	GG	87 (58.8)	55 (54.5)	-	0.72	-	
	GA	46 (31.1)	33 (32.7)				
	AA	15 (10.1)	13 (12.9)				

Data represented as freq (%); Pearson Chi-Square test; n indicates number of subjects in each group; CI, confidence interval.

TO LEPTIN G2548A, A19G, H1328080 GENOTYPES

The anthropometric parameters and lipid profile in relation to the genotypic distribution of G2548A, H1328080 and A19G of leptin gene are shown in Table 4. For G2548A, the mutant homozygous GG genotype revealed a higher mean when compared with GA and AA genotypes for all variables. However, only TG variable was significantly difference between groups. Then, further analysis was performed to confirm the interaction between the genotypes. Based on Mann-Whitney U-test, there were significant differences in TG value between AA and AG genotypes; and between AA and GG genotypes (data not shown). Though, no significant difference was detected in median TG level difference between AG and GG genotypes.

TC mean value for AA, AC and CC genotypes of H1328080 variant showed significant difference (*P*-value = 0.027) (data not shown). Post hoc Scheffe's test revealed statistically difference between AC and CC genotypes. However, there were no significant differences in mean TC between AA and CC genotypes as well as between AA and AC genotypes. Besides, the LDL-C variables showed fair association between H1328080 genotypes (*P*-value = 0.050).

For A19G variant, there was no

Table 4: Anthropometrics and lipid profile variables according to Leptin SNPs genotypes

Variables	G2548A				H1328080				A19G			
	AA	AG	GG	P-value	AA	AC	CC	P-value	GG	GA	AA	P-value
BMI (kg/m2)[a]	27.80 ± 5.24	27.36 ± 5.26	28.52 ± 5.46	0.584	29.71 ± 5.23	27.13 ± 4.62	27.68 ± 5.54	0.147	28.03 ± 5.23	26.96 ± 4.61	27.83 ± 5.40	0.345
WC (cm)[a]	88.32 ± 14.27	88.24 ± 13.76	90.77 ± 12.71	0.706	93.85 ± 14.36	87.53 ± 11.90	88.33 ± 14.69	0.184	89.19 ± 14.68	86.76 ± 12.60	90.07 ± 12.95	0.379
BF (%)[a]	31.82 ± 8.55	32.46 ± 8.17	34.01 ± 9.73	0.505	35.42 ± 9.36	32.84 ± 7.84	31.63 ± 8.64	0.139	31.81 ± 8.44	32.44 ± 8.21	34.56 ± 9.36	0.290
TC (mmol/L)[a]	5.18 ± 1.40	5.58 ± 1.28	5.61 ± 0.94	0.059	5.63 ± 0.80	5.69 ± 1.39	5.22 ± 1.31	0.027	5.34 ± 1.38	5.51 ± 1.33	5.39 ± 0.94	0.652
HDL-C (mmol/L)[a]	1.24 ± 0.32	1.30 ± 0.35	1.32 ± 0.22	0.297	1.29 ± 0.27	1.31 ± 0.32	1.25 ± 0.33	0.415	1.24 ± 0.33	1.32 ± 0.31	1.35 ± 0.32	0.089
LDL-C (mmol/L)[a]	3.55 ± 1.19	3.80 ± 1.13	3.85 ± 0.77	0.184	3.99 ± 0.63	3.88 ± 1.21	3.55 ± 1.12	0.050	3.68 ± 1.19	3.75 ± 1.14	3.58 ± 0.80	0.789
TG (mmol/L)[b]	0.96 (0.77)	1.08 (0.76)	1.30 (0.86)	0.016	1.10 (0.75)	1.15 (0.81)	0.97 (0.86)	0.090	1.00 (0.90)	1.10 (0.80)	1.07 (0.61)	0.491

[a] data presented as mean ± SD: One-Way ANOVA (General Linear Model); [b] data presented as median (IQR); BMI: body mass index; WC: waist circumference; BF: body fat; TC: Total Cholesterol; HDL-C: HDL-cholesterol; LDL-C: LDL-cholesterol; TG: Triglyceride; SD: standard deviation; IQR: Interquartile range

significant difference for all variables. Heterozygous GA genotypes had the lowest mean values when compared with homozygous GG and AA genotypes for BMI, and WC, but yielded the highest value of TC, LDL-C, and TG.

ASSOCIATION OF LEPTIN GENE VARIANTS AND OTHER PREDICTORS VARIABLES WITH OBESITY

The univariate logistic regression exposed the associated risk factors with obesity (Table 5). The odd ratio analysis associated the risk for age, WC, BF, TC, HDL-C, LDL-C and TG with overweight/obesity. In multivariate logistic regression model revealed that men, higher WC and BF were independent risk factors for obesity (Table 6).

DISCUSSION

This study exposes the preliminary evidence of the role of genetic variants (G2548A, H1328080 and A19G) in obesity risk among Malaysian Malay population. However, the leptin gene variants did not show any association with obesity. Previous studies also have been unable to detect the association

Table 5: Associated factors of obesity and overweight by Simple Logistic Regression Model

Variables	Regression coefficient	Crude odd ratio (CI)	Wald statistics	P-value
Age	0.03	1.03 (1.01 – 1.06)	8.93	0.003
Gender				
Women	0	1.00 (ref)	1.05	0.198
Men	0.36	1.44 (0.82 – 2.51)		
WC (cm)	0.23	1.26 (1.19 – 1.34)	58.37	<0.001
BF (%)	0.19	1.21 (1.15 – 1.28)	55.64	<0.001
TC (mmol/L)	0.41	1.51 (1.22 – 1.87)	14.56	<0.001
HDL-C (mmol/L)	-1.34	0.26 (0.11 – 0.59)	10.29	0.001
LDL-C (mmol/L)	0.63	1.89 (1.45 – 2.46)	22.72	<0.001
TG (mmol/L)	1.18	3.26 (1.95 – 5.44)	20.53	<0.001
G2548A variant				
GG (wild-type)	0	1.0 (ref)		
AG	0.003	1.00 (0.41 – 2.45)	0.00	0.99
AA	0.099	1.10 (0.45 – 2.70)	0.04	0.82
H1328080 variant				
CC (wild-type)	0	1.0 (ref)		
AC	-0.64	0.93 (0.54 – 1.62)	0.05	0.81
AA	0.48	0.61 (0.58 – 4.44)	0.87	0.35
A19G variant				
GG (wild-type)	0	1.0 (ref)		
GA	-0.12	0.88 (0.50 – 1.54)	0.19	0.65
AA	-0.31	0.72 (0.32 – 1.64)	0.57	0.44

Simple Logistic Regression was applied; WC: waist circumference; BF: body fat; TC: Total Cholesterol; HDL-C: HDL-cholesterol; LDL-C: LDL-cholesterol; TG: Triglyceride; CI: confident interval of odd ratio

between G2548A and obesity in Polish, Spanish, and Japanese population in which consistent with our data (Cieslak et al. 2012; Mizuta et al. 2008; Portolés et al. 2006). However, the contradicting results were observed in Brazilian, Tunisian, Turkish, Taiwanese and French which G2548A variant was referred as one of the obesity markers (Boumaiza et al. 2012; Hinuy et al. 2008; Mammès et al. 2000; Sahin et al. 2013; Wang et al. 2006; Zayani et al. 2017). Besides, previous researchers suggested G allele was susceptible to the obesity in particular population (Boumaiza et al. 2012) whereas, in some populations, they claimed that A allele was related to obesity risks (Hinuy et al. 2008; Wang et al. 2006). At molecular level, G2548A region may comprehend the transcription inhibitory element in adipocytes. However, the effect of this SNPs on leptin expression in energy homoeostasis was requisite to be further investigated.

It is noteworthy, the finding from this study was supported by the findings in Japanese, Tunisian and Brazilian population (Angeli et al. 2011; Fourati et al. 2013; Mizuta et al. 2008) in regards to A19G variant but showed strong association between obesity in American population (Hart

Table 6: Associated factors of obesity and overweight by Multiple Logistic Regression Model

Variables	Regression coefficient	Adjusted odd ratio (CI)	Wald statistics	P-value
Gender				
Women	0	1.00 (ref)	6.83	0.009
Men	3.28	26.27 (2.27 – 311.67)		
WC (cm)	0.14	1.15 (1.06 – 1.25)	11.15	0.001
BF (%)	0.35	1.43 (1.20 – 1.70)	16.21	<0.001

Multiple Logistic regression; Forward and backward LR variable selection were applied; Multicollinearity and interaction were not found; WC: waist circumference; BF: body fat; CI: confident interval of odd ratio

Sailors et al. 2007). Leptin A19G has been discovered as one of the obesity markers (Hager et al. 1998). The leptin A19G is located within the first UTR (untranslated region) in the end of promoter region and has been reported that the unknown alteration process might take part in modifying the protein function. This underlying mechanism intrigued researchers to postulate the A19G variant and its effect on gene transcription. Therefore, further investigation is still required to elucidate the mechanism of DNA sequence variability especially A19G leptin.

Previous study had been conducted with regards to this variant, H1328080 which is located at the flank region and manifested the substitution of C to A nucleotides (Ma et al. 2009). In accordance with the previous study, H1328080 postulated a strong association in single and multiple marker haplotype analysis (Jiang et al. 2004). Nevertheless, family-based association study that was performed in Tunisian population reported that H1328080 was not linked with obesity in concordance with the results of this study (Fourati et al. 2013).

It is noteworthy the divergence in HWE values were demonstrated in current study which involved the case group (P = 0.03) of H1328080, the case (P = 0.03) and control (P = 0.02) groups of A19G polymorphism in their associations with obesity respectively. The phenomenon of genetic drift and natural selection have become major factors that cause the population to violate from HWE assumptions. Besides, these results might be explained due to limited number of sample size that had been used in current study. Population admixture is also well-known factor that contributed to deviation of HWE (Deng et al. 2001). Previous study mentioned the genetic influx was detected from Indian to Malay especially in *Melayu Kedah* and *Melayu Kelantan* sub-ethnics which is genetically distinct from other sub-ethnics (*Melayu Minang*, *Melayu Jawa* and *Melayu Bugis*) but showed similarity with Thai *Pattani* (Hatin et. al. 2011). They postulated genetic admixture was existed among Malaysian Malay population.

This study included 249 participants with the median age of participants was 40 (18.00) years and ranged from

18 until 52 years old. As indicated by (Lim 2016), the risks of developing obesity was reported two-fold to a peak in middle age, but the prevalence of overweight and obese was projected the lowest among young adults. In addition, the enrollment of women subjects were higher in this study with 68.67% and demonstrated the same trend when stratified into case and control groups. Prior study disclosed Malaysian women had higher risks than men to develop obesity due to hormonal and metabolic changes (Suzana et al. 2012).

From the findings in this study, the WC, BF, TC, LDL-C, and TG of obesity-related traits parameters were significantly higher and HDL-C was significantly lower in obese/ overweight group when compared with normal BMI group. In addition, the lower level of HDL-C and higher TG level parameters also showed strong correlation with metabolic and CVD diseases that proposed to be sensitive markers for detecting the risks of CVD and metabolic syndrome (Nordestgaard & Varbo 2014). The anthropometric and lipid profile measurement evaluated in this study showed significant difference between body weight status and likewise to the prior studies (Chen et al. 2012; Weiss et al. 2004).

Nevertheless, the comparison between genotypes of the variants revealed the unexpected results in which only TG and TC variables were correlated with G2548A and H1328080 variants respectively. This result was consistent with previous studies that revealed A19G and G2548A were not associated with obesity related variables that evaluated, including WC, BF and metabolic parameters (Constantin et al. 2010; Franek et al. 2010). The complex pathogenesis of obesity was hypothesized that might involve the interaction between genetics, hormonal, environmental and lifestyles (Alfredo et al. 2007; Chong et al. 2014; Khor 2012; Paracchini 2005). Therefore, the prevention and management of obesity and its comorbidities are particularly challenging due to multifactorial of obesity development. Previous study suggested the combination of pharmacotherapy, cognitive behavior therapy, and nutritional education should be effective to combat obesity (Ainsah & Osman 2007). Based on the final multiple regression model, the result demonstrated that the women, WC, and BF as the augmented risk factors for obesity and were proved to give better indicators of cardiovascular diseases and metabolic diseases (Recio-Rodriguez et al. 2012; Schneider et al. 2010).

There were few limitations in this study and these include small samples size and sampling method that restricted to certain population. Future studies should be considered with higher sample size and more balance on age group and gender. Besides, other ethnic should be deliberated since Malaysian population has multi-ethnicity. Another restriction of this study was the absence of certain parameters such as other variants of leptin gene, serum leptin level and insulin level that may elucidate the obesity pathogenesis.

CONCLUSION

G2548A, H1328080 and A19G have no single association with obesity. However, the BF percentage, women, and WC may be considered to be at increased risks for obesity in Terengganu, Malay subjects.

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