Haplotype analysis of leptin gene polymorphisms in obesity among Malays in Terengganu, Malaysia population

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ABSTRACT

Introduction: The prevalence of overweight and obesity has developed the critical global threat which leads to metabolic risks and mortality. A Leptin hormone that regulates the food intake as well as food expenditure is encoded by Leptin gene. The gene has shown a pivotal role in obesity pathogenesis. This study was sought to determine the SNPs and haplotype association of the Leptin gene that were assigned as G2548A, H1328080, and A19G with obesity among Malays in Terengganu, Malaysia.

Methodology: This study comprised of 249 participants (148 overweight/ obese as a case group and 101 lean participants as controls). The PCR-RFLP technique was performed to distinguish the genotype distribution of Leptin gene polymorphisms. The allele and genotype frequencies were assessed for single and haplotype analyses.

Result: Single association analysis of G2548A (P=0.74), A19G (P=0.38), and H1328080 (P=0.56) polymorphisms yielded no statistically significant association. However, haplotype association analysis showed a suggestive indication of AAG haplotype (G2548A, H1328080, and A19G sequence) with susceptibility effect towards obesity predisposition [P=0.002, OR=8.897 (1.59–9.78)].

Conclusion: This data on single and haplotype might disclose the preliminary exposure and pave the way for the obesity development with an evidence of revealed susceptibility to obesity.

KEY WORDS:
A19G, G2548A, H1328080, Haplotype, Leptin, Obesity, Polymorphisms

INTRODUCTION

Obesity has substantially diagnosed as a serious condition and becomes a worldwide public health concern which associated with metabolic diseases, cardiovascular diseases as well as cancer.[1] The prevalence of overweight and obesity in Malaysia had shown rapid increment within 20 years.[2,3]

Elucidating the complex pathogenesis of obesity and its related influences gives a better insight to provide an effective management that indirectly combats the globesity phenomenon. Obesity is a multifactorial disease, arising from genetic, lifestyles, environmental factors as well as the complex interaction among them.[4–6] Furthermore, early researches on the Human Genome Project, International Hap Map Project and Genome-Wide Association Studies (GWAS) had promoted more investigation on human genetic diseases, especially in genetic obesity propensity. Researches on obesity genes have facilitated in giving the clear explanation of the energy homeostasis, body weight, and adiposity regulation.[7] Therefore, screening for susceptible genetic markers in obese persons can assist exposition of underlying mechanisms of genetic obesity development.[5]

However, previous studies reported the genetic predisposition towards obesity might be varied across the different population. Several numbers of genetic factors are believed to have strong associations with obesity and have proven as the candidate genes in body weight regulation which are leptin receptor (LEPR), Leptin, proopiomelanocortin (POMC), proconvertase 1 (PC-1), and melanocortin 4 receptor (MC4R).[8,9] Leptin gene regulates human body weight and energy homeostasis by coding leptin hormone, a satiety signal that is secreted by white adipose tissue.[10,11] The mutation in Leptin gene which encodes the major inhibitory tonic satiety signal of leptin often results in individuals who constantly feeling hungry and demanding for food that consequently leads to the food overconsumption.[12,13]

Identification of Leptin gene as a genetic obesity marker has led to the extensive researches on the characterization of Leptin polymorphisms. However, the results were conflicting across various populations. The causative role of Leptin SNPs, (G2548A), rs2167270 (A19G), and rs12535747 (H1328080) in body weight regulation were intrigued to determine the possible linkage and involvement in obesity pathogenesis. In Malaysia, serious issue on the prevalence of obesity and overweight has shown rapid increment among Malays which documented in NHMS within 20 years. Few of studies were conducted on the effects of SNPs with obesity susceptibility in Malay adults which is not represented as overall Malay population that might be due to population stratification among adults in Malaysia.[14,15] Furthermore, lack of study on Leptin gene polymorphisms in Malaysia especially in Kuala Terengganu, Terengganu had been conducted as a risk factor of obesity. The genotypic information as a preliminary data may be assisted in elucidating the genetic basis of obesity development as well as improving the inconsistency of the research finding. Therefore, this study was sought to determine the single and haplotype association of the Leptin gene polymorphisms (G2548A, H1328080, and A19G) with obesity among Malays in Terengganu, Malaysia Population.

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MATERIALS AND METHODS

Study Participants

This case-control study comprised of 249 Malay adult participants who were volunteers from Kuala Terengganu area which is located in East Peninsular of Malaysia. The Malay ethnic participants were verified via Malaysia's Constitution and self-report claimed at least from three generations. They were designated via primary obesity screening, according to Body Mass Index (BMI) cut-off point by International Classification of Obesity.[16] Obesity and overweight are defined as BMI ≥25 kg/m^2 as the case group, which involved a total of 148 participants while the control group was composed of 101 lean participants with BMI, ≤ 24.99 kg/m^2. The other prerequisite for inclusion in this present study were ascertained through availability of both sexes and the age stratification between 18 until 59 years meanwhile, exclusion criteria were the presence of severe diseases (cardiovascular disease, hypertension, and diabetes), active smoker, pregnancy, or lactation.

The sample size was estimated through Synthetic Validation Approach to Testing Differential Prediction Hypotheses.[17] The Minor Allele Frequencies (MAF) of 0.40 (G2548A), 0.34 (A19G), 0.25 (H1328080) and Odd Ratio (OR) value of 1.69 (G2548A), 1.87 (A19G) from prior studies had been used to calculate sample size.[18,19] The number of 90 and 72 of case and control participants for G2548A polymorphism, meanwhile 132 and 98 participants for case and control groups respectively for A19G were required to achieve 80% power of the study. Unfortunately, the sample size of H1328080 polymorphism was unable to calculate due to lack of studies conducted and the previous studies did not provide the OR value. However, H1328080 polymorphism was still considered to be genotyped in this study since this polymorphism might give clear insight on the onset of obesity in Malay population. This study was carried out with ethical approval from Human Ethics Research Committee (UHREC) at University of Sultan Zainal Abidin (UNISZA) in Terengganu, Malaysia (Reference No: UniSZA.C/1/UHREC/628-1 (17)).

SNPs genotyping

Genomic DNA was extracted from peripheral blood using a commercial DNA extraction kit (Vivantis, California, USA). The detection of the genotypes for G2548A (A/G), H1328080 (C/A) and A19G (A/G) polymorphisms of Leptin was performed using Polymerase Chain Reaction-Restriction Fragment Length Polymorphisms (PCR-RFLP) technique. PCR technique was executed in the total of 25µl that consisted of 5X PCR buffer, 50mM $MgCl_2$, 25mM dNTP, 5U/µl of Taq polymerase (Promega, Madison, USA) and specific primers. The conditions used for PCR amplification were initial denaturation at 94˚C for 2 min, followed by 35 cycles of the denaturation at 94˚C for 30-40 sec, the annealing step at 54-60˚C for 30-40 sec, and the extension step at 72˚C for 1 min. Then, the PCR amplification was followed with final extension phase at 72˚C for 5 min.

The PCR primers used for G2548A were 5′-GCTTTCTAAGCCAAGGCAAA-3′ in forward direction while 5′-GCTCTTTTTCAAGGTGCACTG-3′ for reverse direction that results in 500bp of PCR product and cleavage products into 320/180bp fragments using 1.0U of HhaI restriction enzyme; PCR primers for H1328080 were 5′-ACCCATGTGTTCTTGGCACT-3′ in forward direction while 5′-CTTGAACCAGGGAACACACA-3′ for reverse direction that results in 300bp of PCR product and cleavage products into 222/78bp fragments using 1.0U of EcoNI restriction enzyme; PCR primers for A19G were 5′-CTCTGGAGGGACATCAAGGA-3′ in forward direction while 5′-CGGGATCCAGAGTTGTGTG-3′ for reverse direction that results in 386bp of PCR product and cleavage products into 296/90bp fragments using 1.0U of HpyCH4III restriction enzyme (Thermo Scientific, USA).[20] The PCR and PCR-RFLP product were visualized under UV light after running electrophoresis in 3% agarose gel.

Statistical analysis

The SHEsis Online software (http://shesisplus.bio-x.cn/SHEsis.html#) was employed to assess SNPs and haplotype association in which allelic and genotypic distribution were compared between BMI-stratified groups, case (overweight/obesity) and control (normal BMI). The Odd Ratio (OR) value with 95% Confidence Interval (CI) were generated through Pearson Chi-Square test and Hardy-Weinberg Equilibrium (HWE) value was also calculated for cases and controls participants using SHEsis online software.[21] Data were presented as frequencies (%). P-value <0.05 was considered as a significant association.

RESULTS

SNPs association of H1328080, G2548A, and A19G

The single association data as presented in Table 1 was found not significantly correlated between G2548A, H1328080, A19G polymorphisms and obesity with the P-value of 0.74, 0.56 and 0.38 respectively. For G2548A polymorphism, G allele is assigned as a wild-type, projected the MAF with 31.8% for case group and 33.2% for the control group and anticipated the OR with 1.066 (0.72-1.56). C wild-type allele of H1328080 polymorphism was observed higher in case (59.5%) and control group (60.4%) while A mutant type with the MAF for case and control groups were 25.0% and 22.0% correspondingly [OR = 1.13 (0.74-1.72)]. Besides, mutant A allele of the A19G polymorphism of control group had higher MAF with 29.0% compared to case group with MAF value of 25.0% and projected the OR value of 1.19 (0.80-1.78).

Haplotype analysis

In a further investigation, haplotype analysis was conducted to evaluate the predisposing effect of haplotypes of Leptin gene polymorphisms with obesity. Haplotype AAG in sequential SNPs of G2548A, H1328080, and A19G polymorphisms was postulated to have a significant difference with P-value of 0.002 with obesity and the trend of association was toward susceptive manner as interpreted by OR with 8.897 (1.59 – 49.78). However, other haplotypes or allele combination of Leptin gene polymorphisms did not show any significant association with obesity (Table II). Odd ratio of GAG and GCA haplotypes could not be calculated due to the frequency was less than 3%, in which was ignored for analysis based on SHEsis online software.

Table I: SNPs association analysis of Leptin gene polymorphisms with obesity

SNPs	Genotype data			Case-control analysis		
	Wild-type	Heterozygous	Mutant type	MAF	P-value	OR (95% CI)
G22548A						
Case	14 (9.5)	66 (44.6)	68 (45.9)	94 (31.8)	0.74	1.066
Control	10 (9.9)	47 (46.5)	44 (43.6)	67 (33.2)		(0.72 – 1.56)
H1328080						
Case	88 (59.5)	46 (31.1)	14 (9.5)	74 (25.0)	0.56	1.13
Control	61 (60.4)	34 (33.7)	6 (5.9)	46 (22.8)		(0.74 – 1.72)
A19G						
Case	87 (58.8)	46 (31.1)	15 (10.1)	76 (25.7)	0.38	1.19
Control	55 (54.5)	33 (32.7)	13 (12.9)	59 (29.2)		(0.80 – 1.78)

*Pearson Chi-Square; data represent as (freq, %); MAF: Minor Allele Frequency

Table II: Haplotype analysis of Leptin gene polymorphisms with obesity

Allele combination	Obesity/ Overweight	Normal BMI	Odd Ratio (CI)	P-value
A*A*G	17.31 (5.80)	1.42 (0.70)	8.897 (1.59 – 49.78)	0.002
A*A*A	8.84 (3.00)	8.25 (4.10)	0.731 (0.27 – 1.92)	0.52
A*C*G	159.06 (53.70)	109.89 (54.40)	0.99 (0.68 – 1.43)	0.97
A*C*A	16.78 (5.70)	15.44 (7.60)	0.73 (0.35 – 1.50)	0.39
G*A*G	5.26 (1.80)	4.02 (2.00)	-	-
G*A*A	42.58 (14.40)	32.32 (16.00)	0.89 (0.54 – 1.46)	0.65
G*C*G	38.36 (13.00)	27.67 (13.70)	0.94 (0.56 – 1.60)	0.84
G*C*A	7.79 (2.60)	2.99 (1.50)	-	-

* A sequential in allele combination represent for G2548A, H1328080 and A19G respectively;

Table III: Comparison of MAF value throughout various populations with current data

Population SNPs	AFR (n=661)	AMR (n=347)	EAS (n=504)	EUR (n=503)	SAS (n=489)	ALL (n=2504)	CURRENT DATA	
							Case	Control
G2548A	0.39	0.41	0.27	0.44	0.49	0.40	0.31	0.33
A19G	0.44	0.41	0.20	0.37	0.28	0.34	0.25	0.29
H1328080	0.12	0.38	0.20	0.37	0.28	0.25	0.25	0.22

MAF: Minor Allele Frequency; AFR: African; AMR: American; EAS: East Asian; SAS: South Asian; ALL: Average of All Populations

DISCUSSION

Preliminary indication on the characterization of Leptin gene polymorphisms and their association with obesity had revealed no single association of G2548A, H1328080 and A19G polymorphisms with obesity. The current data was supported by other studies that no significant association was found between G2548A and obesity in Polish, Spanish, as well as Japanese populations.[22,23] However, the contradicting results were observed in Brazilian, Tunisian, Turkish, Taiwanese and French populations in which G2548A polymorphism was referred as one of the obesity markers.[18,24–27] It is notable that the wild-type allele of G2548A was hypothesized as minor allele in this population, in accordance with other Asian Population such as Japanese and Chinese population.[28]

The single association of A19G polymorphism with obesity in this study was consistent with Japanese, Tunisian and Brazilian populations. [20,29] A19G is the substitution of G (guanine) to A (adenine) nucleotide of the Leptin gene sequence and was postulated to exert an effect on gene transcription.[30] In addition, few studies were also conducted to associate H1328080 polymorphism with obesity. This polymorphism is located at the flank region of Leptin sequence and demonstrated the alteration of C (cytosine) to A (adenine) nucleotides. H1328080 had proved as the obesity marker in multiple marker analysis which is in agreement with present study.[31]

The demographic characteristics (age and sex) in a current data (data not shown) that comprised the median age of participants was 14 (18.00) years and ranged from 18 until 52 years old. The former investigation had reported the risks of developing obesity constantly increase with age until a peak in middle ages. Nevertheless the prevalence of overweight and obese declined at the age of 60 years and upwards.[32–34] In addition, the women participants were higher in the current study and also showed the same trends when classified based on BMI. Previous studies also disclosed the female had higher risks to develop obesity within Malaysian population[32] which might be explained due to hormonal changes especially during pregnancy and lactation period.[35]

Noteworthy, prior studies were conducted among Malaysian suggesting G2548A and A19G polymorphisms were not a potential obesity predisposing marker among Malays, but G2548A showed association among Indian male.[14,15] Hence, further study should be considered to clarify the mechanisms of DNA sequence variability of Leptin gene polymorphisms.

The MAF distribution of G2548A, A19G, and H1328080 polymorphisms was found lower in both groups among Malay adults when compared to global MAF obtaining from Ensembl database with 0.40, 0.34 and 0.25 respectively.[36] This data was in accordance with the previous study that had been conducted in Kampar, Perak has demonstrated the MAF of 0.26 and 0.33 for A19G and G2548A correspondingly.[14] Furthermore, Table III shows the differences of MAF of the current study in Kuala Terengganu, Terengganu Malaysian Malays with the global MAF which are reported by 1000 Genome Project and interfaced in Ensembl database website.[36] Meanwhile, in American population conducted by Jiang et al., (2004), the MAF of A19G and G2548A were 0.34 and 0.35 correspondingly.[31] This might explained the varied MAF value could be varied across different ethnic or population as well as sample size.

The haplotype structure would provide the critical information on human evolutionary history and identification of genetic polymorphisms underlying the traits of diseases.[37] This study postulated that individual with AAG haplotype that is represented with the combination of the mutant allele of G2548A, mutant allele of H1328080 and wild-type allele of A19G polymorphisms was prone to develop the body weight problems despite the environmental factors. Any changes of the allele from this haplotype combination lead to insignificant association with obesity. An earlier study of G2548A polymorphism also depicted the synergist effects when combined with LEPR gene polymorphism and was contributed to a 58% increase the obesity risk. In addition, they also demonstrated no significant difference in the genotype frequency of G2548A polymorphism in single association with obesity, between lean and obese in which agreement to the present study.[38] The allele combination of AAG has reflected the interaction of H1328080, G2548A, and A19G with obesity that influenced in the modulation of energy homeostasis.

The treatment and prevention of the obesity and its comorbidities are principally challenging because of the multifactorial to the onset of obesity.[5,39] Therefore, this study might give some information about the role of the Leptin gene polymorphisms that related to obesity risks among Malay in Terengganu as one of Malaysia's most homogenous states by means of Malay as the main ethnic. There are some limitations of this study in which samples size and the sampling method were restricted to the certain region in Malaysia. The post-priori power calculation was implied in the case-control analysis using obtained sample size, MAF, and effect size to determine the power of the study.[17] This study disclosed the power of G2548A, H1328080, and A19G polymorphisms were 5.2%, 9.8% and 14.3% correspondingly. Therefore, future study should be considered on the increase in the sample size to strengthen the power of the study and other ethnic should be deliberated since Malaysian population has multi-ethnicity. Besides, serum leptin level and other adipokines should be deliberated in future studies since these parameters might give an impact in the understanding of obesity propensity.

CONCLUSION

Haplotype AAG of G2548A, H1328080, and A19G conferred the significant association with obesity among Malay in Terengganu, Malaysia population. This suggestive preliminary evidence might give new insight in elucidating the development of obesity with regard to Leptin gene polymorphisms.

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