Sains Malaysiana 42(3)(2013): 365–371

Association of Fat Mass and Obesity-Associated (FTO) Gene rs9939609 Variant with Obesity Among Multi-Ethnic Malaysians in Kampar, Perak

(Kaitan Gen Jisim Lemak dan Berkaitan Obesiti (FTO) Polimorfisma rs9939609 dengan Obesiti dalam Masyarakat Berbilang Etnik Malaysia di Kampar, Perak)

WEI-WEI CHEY, SOOK-HA FAN & YEE-HOW SAY*

ABSTRACT

Obesity is a multifactorial disease caused by the interaction of genetic, lifestyle and environmental factors. Common single nucleotide polymorphisms in the recently-described Fat Mass and Obesity-Associated (FTO) gene have been related to body weight and fat mass in humans and genome-wide association studies in several populations have indicated that the FTO rs9939609 variant is associated with obesity. Therefore, the objective of this study was to investigate the association of the FTO rs9939609 variant with obesity among 324 multi-ethnic Malaysians (98 Malays, 158 Chinese, 68 Indians) who attended the Kampar Health Clinic, Perak. With the overall minor A allele frequency (MAF) of 0.199, the distribution of genotypes and alleles was significantly different among ethnicities (MAF highest among Malays), but no association was found for obesity, related anthropometric measurements and gender. Subject with allele A had marginally but significantly higher waist circumference (p=0.015), thus the FTO rs9939609 allele was associated with central obesity [p=0.034 by Chi-square analysis; Odds Ratio (OR)=1.680 (CI=1.036, 2.724; p=0.035)]. However, this association was abolished when adjusted for age, gender and ethnicity (OR=1.455, CI=0.874, 2.42; p=0.149). In conclusion, the MAF of the FTO rs9939609 SNP was low as in other Asian populations and there was no evidence for an involvement of this SNP in obesity and obesity-related traits in this multi-ethnic Malaysian study group.

Keywords: Fat mass and obesity-associated gene (FTO); Malaysia; obesity; rs9939609; single nucleotide polymorphism

ABSTRAK

Obesiti adalah satu penyakit yang disebabkan oleh pelbagai faktor, termasuk genetik, cara hidup dan persekitaran. Pelbagai polimorfisme nukleotid tunggal yang biasa dijumpai di dalam satu gen yang baru ditemui, jisim lemak dan berkaitan obesiti (FTO), telah dikaitkan dengan berat badan dan jisim lemak manusia. Kajian kaitan genom-menyeluruh dalam beberapa populasi telah mendapati bahawa FTO polimorfisme gen rs9939609 berkaitan dengan obesiti. Oleh itu, objektif penyelidikan ini adalah untuk mengkaji kaitan gen jisim lemak dan Berkaitan Obesiti (FTO) polimorfisme rs9939609 dengan obesiti dalam kalangan 324 masyarakat majmuk Malaysia (98 Melayu, 158 Cina, 68 India) yang menghadiri Klinik Kesihatan Kampar, Perak. Dengan frekuensi alel A minor (MAF) 0.199, taburan genotip dan alel adalah berbeza secara signifikan antara kaum (MAF paling tinggi dalam kalangan Melayu), tetapi tidak berkaitan dengan obesiti, ukuran antropometri dan jantina. Subjek dengan alel A mempunyai ukuran lilitan pinggang yang marginal tetapi secara signifikannya lebih tinggi (p=0.015), oleh itu FTO rs9939609 berkaitan dengan obesiti pusat [p=0.034 daripada analisis Chi-square; Odds Ratio (OR) 1.680 (CI=1.036, 2.724; p=0.035)] untuk mempunyai obesiti pusat. Namun, kaitan ini dimansuhkan apabila dilaras dengan umur, jantina dan etnik (OR=1.455, CI=0.874, 2.42; p=0.149). Secara keseluruhannya, MAF FTO rs9939609 SNP adalah rendah seperti dalam populasi Asia lain, dan tidak terdapat bukti bahawa polimorfisme ini terlibat dalam obesiti dan ciri-ciri obesiti dalam kalangan masyarakat majmuk Malaysia dalam kajian ini.

Kata kunci: Jisim lemak dan berkaitan obesiti (FTO); Malaysia; Obesiti; rs9939609; polimorfisme nukleotid tunggal

INTRODUCTION

Obesity is now recognized as an alarming global epidemic throughout the developed and developing world. According to the Malaysian Third National Health and Morbidity Survey in 2006 the combined prevalence of overweight and obesity in Malaysia was alarmingly high at 43.1% (Institute of Public Health Malaysia 2008). The susceptibility to obesity of an individual is determined by combined effects of genetic and environmental factors and one of the candidate genes for obesity that have been identified is the Fat Mass and Obesity-associated gene (FTO), encoding for the Fat mass and obesity-associated protein (FTO) or also known as alpha-ketoglutarate-dependent dioxygenase (Frayling et al. 2007). Single nucleotide polymorphisms (SNPs) in the Fat Mass and Obesity-associated (FTO) gene had previously been identified in genome-wide association

study (GWAS) for type 2 diabetes (T2D) (Frayling et al. 2007). However, the association of the gene variants and T2D was abolished by adjustment for body mass index (BMI). This implies that the relationship between FTO variants and T2D is actually mediated by an association of BMI. The function of the FTO protein is still largely elusive, but it is expressed in tissues like hypothalamus, muscles, pituitary and adrenal glands (Frayling et al. 2007). Studies have suggested that the FTO protein regulates body weight through fat cell lipolysis (Wahlen et al. 2008) and protects against excessive food intake by increasing body sensitivity to satiety (Wardle et al. 2009). FTO variant risk allele, on the other hand, mediates weight gain by increasing appetite and energy intake but not for energy expenditure (Cecil et al. 2008).

SNP rs9939609, identified with the T to A missense mutation in the first intron of the FTO gene on chromosome 16q12.2, is a common variant that is widely studied in different ethnic populations. It is a representative SNP widely studied in different populations worldwide because it was the SNP in the original European adult study and had one of the strongest associations with BMI, with the Odds Ratio (OR) of 1.67 (Confidence Interval (CI) 1.47-1.89), $p = 1 \times 10^{-14}$ for the homozygous A allele condition (Frayling et al. 2007). Association of FTO SNPs with obesity has also been consistently observed in other population of European origin, such as the French (Legry et al. 2009) and Belgians (Peeters 2008). However, the impact of the FTO SNPs on obesity in other ethnic populations seems contradictory. For example, a study in Chinese Han population of 3210 individuals from Beijing and Shanghai (Li et al. 2008) showed none of the variants including rs9939609 were significantly associated with BMI, as well as waist circumference (WC) and total body fat (TBF). The discrepancy could be explained by the different genetic makeup among ethnicities. The researchers also suggested that SNPs in FTO would only give remarkable effect in population with higher minor allele frequencies (MAF) and stronger Linkage Disequilibrium (LD) strength. The MAF of FTO rs9939609 SNP is comparatively rare in Asians (~0.12) (Xi & Mi 2009) compared with Europeans (0.45) and West Africans (0.52) (Frayling et al. 2007).

Therefore, the objectives of this study were to perform genotyping of the FTO rs9939609 SNP among Malaysian subjects from Kampar Health Clinic, Perak, to determine the prevalence of the variant genotypes and alleles and to investigate if there is any association between this SNP with obesity and the related anthropometric measurements.

Materials and Methods

Subject Recruitment and Anthropometric Measurements

Three hundred and twenty four subjects were recruited by random convenience sampling from the Kampar Health Clinic between April and December 2010. This study consisted of 178 non-obese subjects and 146 obese subjects, ranged from 21 to 80 years old (Mean ± SD = 52.8 ± 14.2 years), with 126 males and 198 females. Almost half of the subjects (48.8% or 158) were Chinese, followed by Malays (30.2% or 98) and Indians (21.0% or 68), reflective of the Kampar population. This study was registered under the National Medical Research Registry of Malaysia (NMRR-09-826-4266) and the protocol was approved by the Medical Research and Ethics Committee, Ministry of Health, Malaysia. Informed consent form was signed by all the respondents in this study and the blood samples were taken in accordance with the World Medical Association (WMA) Declaration of Helsinki (as revised in Seoul, 2008). Subject's demographic information including age, gender and ethnicity were obtained. The systolic blood pressure (SBP), diastolic blood pressures (DBP) and pulse rate were determined by using Omron SEM-1 model automatic blood pressure monitor after the subjects have rested for 10 min. Height, waist circumference (WC) and hip circumference (HC) of subjects were measured by using a measuring tape to the nearest 0.1 cm and their waist-to-hip ratio (WHR) was calculated by dividing the WC by HC. A bio-impedence body fat weighing scale (Omron model HBF-362 karadaScan body composition monitor with scale) was used to determine the weight as well as the body compositions such as percentage of skeletal muscle (SM), total body fat (TBF), visceral fat level (VFL) and subcutaneous fat (SF). Body mass index (BMI) and resting metabolism (RM) was also obtained by using the weighing scale. Subjects with the BMI cut-off point of ≥27 kg/m^2 were considered as obese (Deurenberg-Yap et al. 2000), while male and female subjects with WC of ≥90 cm and ≥80 cm, respectively, were considered as having central obesity according to the 'Harmonised' criteria for metabolic syndrome (Alberti et al. 2009).

Genomic DNA Extraction and Genotyping

Five millilitre of blood sample was collected from the subject by experienced phlebotomist in the clinic. Genomic DNA was then extracted from the white blood cells using the Wizard® Genomic DNA Purification Kit (Promega Corporation, Madison Wisconsin) according to the manufacturer's protocol. Partial amplification of the FTO gene was conducted using the forward and reverse primers and polymerase chain reaction (PCR) conditions as described by Lopez-Bermejo et al. (2008). Genotypes for FTO rs9939609 were then determined by restriction enzyme length polymorphism (RFLP) with restriction enzyme *Sca*I. The RFLP products were resolved by performing electrophoresis on a 2.5% agarose gel, where the T allele produced a 182 bp band and the A allele produced 154 bp and 28 bp bands (Lopez-Bermejo et al. 2008). Therefore, homozygous wild-type TT genotype has the 182 bp band only, heterozygous TA genotype has the 182, 154 and 28 bp bands, while homozygous mutated AA genotype has the 154 and 28 bp bands.

STATISTICAL ANALYSIS

The statistical analysis of sample data was obtained by using the statistical package for social sciences (SPSS®) for Windows® Version 17.0 (SPSS Inc., Chicago, IL). The descriptive statistics were used to analyze socio demographic characteristics of the subjects. Allele frequencies of FTO rs9939609 with respect to BMI status, gender and ethnicity were assessed for association by Pearson's Chi-square test. Because majority of the subjects had the FTO rs9939609 TT and TA genotype and only a few had the AA genotype (less than 5 in a cell), Pearson's chi-square test was only performed for alleles but not genotypes. Anthropometric measurements between genotypes and alleles were compared using one way analysis of variance (ANOVA) and Student's *t* test, respectively. Associations between the genotype as well as the allele and obesity were examined using logistic regression ('Enter' method; unadjusted and adjusted with age, gender and ethnicity). Allele frequencies were also analysed separately by Pearson's chi-square test based on BMI status, gender, ethnicity and age groups. $p<0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

The genotype frequencies for the rs9939609 in FTO were 0.623, 0.355 and 0.022, for TT, TA, and AA genotype, respectively (Table 1). There was no deviation from the Hardy-Weinberg equilibrium in the distribution of genotypes. The combined minor allele frequency (MAF) of the A allele for the overall subjects was 0.199, while for Malays, Chinese and Indians were 0.270, 0.165 and 0.176, respectively. Pearson's Chi-Square test showed that the allele frequencies were not associated with BMI status and gender. However, there was a significant association between the allele frequencies with ethnicity ($p=0.011$), with Malays having the significantly highest A allele frequency compared with other ethnicities (Table 1). The anthropometric and clinical measurement were not significantly different between genotypes and alleles, except for WC, which was marginally but significantly higher (+ 1.28 cm; $p=0.015$) in subjects with the A allele compared with T allele (Table 1).

Since WC was significantly different among FTO rs9939609 alleles, we re-categorised the subjects based on central obesity status, according to their genotype,

TABLE 1. Obesity status, ethnicity and gender frequencies and body measurement values of the Kampar Health Clinic subjects according to their FTO rs9939609 genotypes and alleles

Variable	Genotype			*p*-value	Allele		*p*-value
	TT	TA	AA		T	A	
BMI Status							
Non-obese	117 (65.7)	58 (32.6)	3 (1.7)	NP§	292 (82.0)	64 (18.0)	0.174
Obese	85 (58.2)	57 (39.0)	4 (2.7)		227 (77.7)	65 (22.3)	
Ethnicity							
Malay	48 (49.0)	47 (48.0)	3 (3.1)		143 (73.0)	53 (27.0)	
Chinese	108 (68.4)	48 (30.4)	2 (1.3)	NP§	264 (83.5)	52 (16.5)	0.011*
Indian	46 (67.6)	20 (29.4)	2 (2.9)		112 (82.4)	24 (17.6)	
Gender							
Male	81 (64.3)	45 (35.7)	0	NP§	207 (82.1)	45 (17.9)	0.297
Female	121 (61.1)	70 (35.4)	7 (3.5)		312 (78.8)	84 (21.2)	
Anthropometric and clinical measurements							
SBP (mmHg)	139.0 ± 22.57	139.37 ± 21.49	136.29 ± 14.93	0.933	139.04 ± 22.29	139.04 ± 20.81	0.245
DBP (mmHg)	81.26 ± 10.50	81.15 ± 10.66	80.43 ± 12.34	0.977	81.24 ± 10.52	81.07 ± 10.74	0.980
Pulse Rate (mmHg)	73.94 ± 13.10	72.50 ± 13.35	74.86 ± 15.79	0.621	73.62 ± 13.14	72.75 ± 13.51	0.520
WC (cm)	89.98 ± 12.10	91.74 ± 10.09	90.86 ± 8.03	0.413	90.37 ± 11.69	91.65 ± 9.84	0.015*
WHR	0.89 ± 0.08	0.90 ± 0.07	0.90 ± 0.07	0.360	0.89 ± 0.08	0.90 ± 0.07	0.125
BMI (kg/m^2)	26.75 ± 5.15	27.67 ± 5.04	27.19 ± 3.45	0.301	26.95 ± 5.13	27.62 ± 4.88	0.335
TBF (%)	32.98 ± 6.92	34.46 ± 6.65	34.80 ± 5.88	0.157	33.31 ± 6.87	34.50 ± 6.50	0.954
SF (%)	26.92 ± 8.18	28.36 ± 8.47	30.09 ± 6.54	0.233	27.24 ± 8.25	28.54 ± 8.26	0.811
VFL (%)	12.22 ± 7.97	12.93 ± 6.11	10.71 ± 4.39	0.578	12.38 ± 7.59	12.69 ± 5.96	0.234
RM (kcal)	1427.40 ± 257.99	1447.55 ± 271.10	1404.43 ± 182.12	0.769	1431.86 ± 260.57	1442.87 ± 262.19	0.584
SM (%)	25.10 ± 4.04	24.45 ± 4.06	24.33 ± 3.50	0.359	24.96 ± 4.04	24.43 ± 3.98	0.821

p-values for obesity status, ethnicity and gender are by Pearson's Chi-square Test, significant at **p*-value<0.05; § Pearson's Chi-Square Test was not performed (NP) for genotypes as some AA frequencies had values of less than 5; numbers in parenthesis are percentage within the same obesity status, ethnicity or gender.
Values for measurements are Mean ± SD Values; *p*-values are by Student's *t* test (for alleles) or one way analysis of variance (ANOVA) (for genotypes), significant at **p*-value < 0.05
SBP, systolic blood pressure; DBP, diastolic blood pressure; WC, waist circumference; WHR, waist-to-hip ratio; BMI, body mass index; TBF, total body fat; SF, subcutaneous fat; VFL, visceral fat level; RM, resting metabolism; SM, skeletal muscle.

allele, gender, ethnicity and age groups (Table 2). Unlike categorisation of obesity based on BMI, FTO rs9939609 alleles were associated with central obesity based on WC (p=0.034), while central obesity was associated with ethnicity (p=0.041).

To further test for the direct association between FTO rs9939609 alleles with overall and central obesity, logistic regression ('Enter' method) was performed, with and without adjustment for other confounders of obesity such as age, gender and ethnicity (Table 3). Having a variant allele (A) appeared to have an effect on the central obesity status of the subjects (p=0.035), but not on overall obesity. Subjects with an A allele were 1.680 times more likely to be centrally-obese. However, when adjusted for age, gender and ethnicity, this association of FTO rs9939609 with central obesity was abolished.

Due to the high heterogeneity of the sampled subjects, their allele frequencies were also analysed separately by Pearson's chi-square test based on BMI status, gender, ethnicity and age groups (Tables 4 and 5). Within the non-obese group, ethnicity was associated with the distribution of the FTO rs9939609 alleles (Table 4) while within the 41-60 years age group, the distribution of the alleles were significantly different between genders (Table 5). Other than these, the distribution of the alleles was not significantly different among the different stratifications even when analysed separately.

To the best of our knowledge, this is the first study examining the prevalence of the FTO rs9939609 SNP and its association with obesity in the Malaysian population. Homozygous mutated AA genotype was rather uncommon in the sampled cohort, yielding an overall MAF of 0.199.

TABLE 2. FTO rs9939609 genotype and allele, ethnicity, gender and age group frequencies according to central obesity status (based on waist circumference measurement)

Variables	Central obesity (based on waist circumference)		p-value
	Absent	Present	
Genotype			
TT	53 (63.1)	149 (62.1)	
TA	30 (35.7)	85 (35.4)	NP[§]
AA	1 (1.2)	6 (2.5)	
Allele			
T	144 (27.7)	24 (18.6)	0.034*
A	375 (72.3)	105 (81.4)	
Gender			
Male	36 (42.9)	90 (37.5)	0.386
Female	48 (57.1)	150 (62.5)	
Ethnicity			
Malay	23 (27.4)	75 (31.2)	
Chinese	50 (59.5)	108 (45.0)	0.041*
Indian	11 (13.1)	57 (23.8)	
Age group (years)			
21-40	16 (19.0)	42 (17.5)	
41-60	42 (50.0)	118 (49.2)	0.905
61-80	26 (31.0)	80 (33.3)	

p-values for obesity status, ethnicity and gender are by Pearson's Chi-square Test, significant at *p-value<0.05; [§] Pearson's Chi-square Test was not performed (NP) for genotypes as some AA frequencies had values of less than 5

TABLE 3. Association studies of FTO rs9939609 alleles with overall and central obesity among subjects

FTO rs9939609	Unadjusted		Adjusted[§]	
	Odds Ratio (95% CI)	p-value	Odds Ratio (95% CI)	p-value
Overall obesity				
T	1.000			
A	1.306 (0.888, 1.922)	0.175	1.150 (0.768, 1.724)	0.497
Central obesity				
T	1.000			
A	1.680 (1.036, 2.724)	0.035*	1.455 (0.874, 2.423)	0.149

[§]Adjusted for age, gender and ethnicity; Value are by logistic regression enter method, *p-value significant at <0.05

TABLE 4. Separate analysis of FTO rs9939609 allele frequency distribution according to BMI status and gender

Variables	T	A	*p*-value	T	A	*p*-value
			BMI status			
		Non-obese			Obese	
Gender						
Male	128 (41.8)	26 (40.6)	0.865	85 (37.4)	19 (29.2)	0.223
Female	170 (58.2)	38 (59.4)		142 (62.6)	46 (70.8)	
Ethnicity						
Malay	55 (18.8)	21 (32.8)	0.045*	88 (38.8)	32 (49.2)	0.281
Chinese	177 (60.6)	33 (51.6)		87 (38.3)	19 (29.2)	
Indian	60 (20.5)	10 (15.6)		52 (22.9)	14 (21.5)	
			Gender			
		Male			Female	
BMI status						
Non-obese	122 (58.9)	26 (57.8)	0.866	170 (54.5)	38 (45.2)	0.132
Obese	85 (41.1)	19 (42.2)		142 (45.5)	46 (54.8)	
Ethnicity						
Malay	49 (23.7)	17 (37.8)	0.096	94 (30.1)	36 (42.9)	0.062
Chinese	122 (58.9)	24 (53.3)		142 (45.5)	28 (33.3)	
Indian	36 (17.4)	4 (8.9)		76 (24.4)	20 (23.8)	

p-values by Pearson's Chi-Square Test, significant at **p*-value <0.05; numbers in parenthesis are percentage within the same allele

TABLE 5. Separate analysis of FTO rs9939609 allele frequency distribution according to ethnicity and age group

Variables	T	A	*p*-value	T	A	*p*-value	T	A	*p*-value
					Ethnicity				
		Malay			Chinese			Indian	
BMI status									
Non-obese	55 (38.5)	21 (39.6)	0.882	177 (67.0)	33 (63.5)	0.617	60 (53.6)	10 (41.7)	0.290
Obese	88 (61.5)	32 (60.4)		87 (33.0)	19 (36.5)		52 (46.4)	14 (58.3)	
Gender									
Male	49 (34.3)	17 (32.1)	0.773	122 (46.2)	24 (46.2)	0.994	36 (32.1)	4 (16.7)	0.131
Female	94 (65.7)	36 (67.9)		142 (53.8)	28 (53.8)		76 (67.9)	20 (83.3)	
					Age group (years)				
		21-40			41-60			61-80	
BMI status									
Non-obese	56 (57.1)	8 (44.4)	0.319	125 (49.8)	29 (42.0)	0.252	111 (65.3)	27 (64.3)	0.902
Obese	42 (42.9)	10 (55.6)		126 (50.2)	40 (58.0)		59 (34.7)	15 (35.7)	
Gender									
Male	37 (37.8)	9 (50.0)	0.329	93 (37.1)	15 (21.7)	0.017*	77 (45.3)	21 (50.0)	0.584
Female	61 (62.2)	9 (50.0)		158 (62.9)	54 (78.3)		93 (54.7)	21 (50.0)	
Ethnicity									
Malay	27 (27.6)	7 (38.9)	0.593	74 (29.5)	30 (43.5)	0.082	42 (24.7)	16 (38.1)	0.201
Chinese	62 (63.3)	10 (55.6)		93 (37.1)	19 (27.5)		109 (64.1)	23 (54.8)	
Indian	9 (9.2)	1 (5.6)		84 (33.5)	20 (29.0)		19 (11.2)	3 (7.1)	

p-values by Pearson's Chi-square Test, significant at **p*-value <0.05; numbers in parenthesis are percentage within the same allele

The results showed a high number of wild type TT genotype followed by heterozygous mutated TA genotype. This FTO SNP was not associated with overall obesity (based on BMI) and central obesity (based on WC, after adjusting for gender, age and ethnicity). Previous studies in Asian populations with larger sample size for the FTO rs9939609 SNP yielded similar MAF but with inconsistent association findings. Population studies in mainland and greater China showed the MAFs were around 0.12 - 0.13, which Chang et al. (2008), Fang et al. (2010) and Xi et al. (2010) showed that there was a strong association of the SNP with BMI in Beijing children and adolescents (n=3503, p=1.39 ×

10^{-6}), Beijing children (n=670, p=0.004) and Taiwanese adults (n=2248, $p=7 \times 10^{-4}$), respectively. However, Li et al. (2008) reported a lack of association of the SNP with risk of obesity in adults from Beijing and Shanghai (n=3210, p=0.96). Other East Asian studies like Hotta et al. (2008) reported an association of the SNP with severe obesity in the Japanese (MAF=0.24, n=2454, $p=2 \times 10^{-5}$), while a study among Korean children and adults reported significant association of rs9939609 with BMI and obesity (MAF=~0.13, n=711; 8842, p=0.023; 0.001 for adults and children, respectively) (Lee et al. 2010).

A similar Singaporean multi-ethnic study which has the closest resemblance to the Malaysian study also reported significant association of rs9939609 with BMI and obesity among Malays and Chinese (MAF=0.118 – 0.333, n=2919, p<0.0001), but not among Indians (Tan et al. 2008). They claimed that the non-association in Indians may due to the small sample size of that ethnic group. On the contrary, the present results showed no association between FTO variant rs9939609 and obesity within respective ethnicities among the Kampar Health Clinic cohort. Furthermore, although differing in sample size, the MAFs of Malays and Chinese in their study were similar with the present study (0.282 *vs*. 0.270 and 0.118 *vs*. 0.165, respectively), while the MAF of Singaporean ethnic Indians were higher than Malaysian ethnic Indians (0.333 *vs*. 0.176) (Tan et al. 2008).

In Asian adult populations, replication studies have yielded inconsistent results (Chang et al. 2008; Fang et al. 2010; Hotta et al. 2008; Lee et al. 2010; Tan et al. 2008; Xi et al. 2010). All these probably reflect genetic or ethnic heterogeneity between populations; therefore, the data on the association of the FTO rs9939609 SNP with obesity in other populations cannot be used to extrapolate for the Malaysian population. This discrepancy might also partly be due to the low MAF of the FTO rs9939609 A allele, and therefore there is not enough power to dissect the genetic contribution to obesity. The MAF of rs9939609 was substantially lower in the present study (0.199) compared with the European population (0.450) (Frayling et al. 2007). Different environmental exposures might also interact with the gene effect of FTO rs9939609 SNP (gene-environmental interaction). For example, physical activity might interact with the effects of the FTO rs9939609 SNP on BMI (Andreasen et al. 2008; Xi et al. 2010).

A meta-analysis combining all studies in East Asian populations showed that the FTO rs9939609 polymorphism is associated with obesity (Xi & Mi 2009). The lack of association result in this study might probably due to the limitations of study designs and small sample size. Small sample size may not adequately represent the overall population in Malaysia. Thus, there might not be enough statistical strength to examine the genetic contribution to obesity. Furthermore, the loss of association can be resulted from the heterogenic composition of the sample population in the study. Jacobsson et al. (2009) has noted a reduced association between FTO SNPs and BMI at older age. They also reported that FTO SNP rs9939609 was associated with BMI and obesity among female but not male children, as a statistically significant number of obese girls were found to be AA homozygous carrier as compared with the non-obese girls; while the obese and non-obese boys showed similar number of AA homozygous carrier (Jacobsson et al. 2008). However, in this study, when analysed separately according to gender, age group and even BMI status and ethnicity, it seemed that all these stratifications were not factors in affecting the association of FTO rs9939609 with obesity.

This study had some limitations whereby the respondents may not represent the whole Malaysian population as only 324 subjects were studied. In addition, small sample size leads to the inconsistency of the results therefore limiting the power for statistical analysis and extrapolation. The low MAF of rs9939609 in the Malaysian subjects in this study may also affect sufficient power for subgroup analysis. The case-control design in this study does not allow for a causality conclusion to be made. Also as only one SNP of FTO was evaluated in this study, it is unclear whether other FTO SNPs in tight linkage disequilibrium with rs9939609 (Hakanen et al. 2009) instead might have association with obesity. Therefore, population-based studies with large sample sizes and an investigation on the effects of environmental and lifestyle factors (like physical activity and dietary habits) is necessary in order to clarify the possible gene-environment interaction that causes inconsistent findings in different populations.

Conclusion

In conclusion, the MAF of the FTO rs9939609 SNP was low - as in other Asian populations and there was no evidence for an involvement of this SNP in obesity and obesity-related traits in this multi-ethnic Malaysian study group. The distribution of the genotype and allele frequencies of this gene variant was also significantly different among ethnicities, with Malays having the significantly highest MAF. On the basis of the results available so far, the role of FTO specifically its rs9939609 SNP in obesity remains inconclusive among Malaysian subjects.

Acknowledgements

This project was funded by the Universiti Tunku Abdul Rahman Research Fund (IPSR/RMC/UTARRF/C109/S1). We would like to extend our deepest gratitude to the Kinta District Health Office for granting us permission to carry out this study at the Kampar Health Clinic, the nurses who assisted with the blood sampling and all the respondents who have volunteered to participate in this study.

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Department of Biomedical Science
Faculty of Science
Universiti Tunku Abdul Rahman (UTAR) Perak Campus
31900 Kampar, Perak
Malaysia

*Corresponding author; email: sayyh@utar.edu.my

Received: 26 May 2011
Accepted: 15 October 2012