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No Association of Peptide Tyrosine-Tyrosine (*PYY*) Gene R72T Variant with Obesity in the Kampar Health Clinic Cohort, Malaysia

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ABSTRACT

Introduction: Peptide Tyrosine-Tyrosine (PYY) is a 36-amino acid peptide hormone released post-prandially from the endocrine cells in the intestinal tract to suppress pancreatic secretions and eventually reduce appetite. The R72T variant in the *PYY* gene (rs1058046) has been associated with increased susceptibility to obesity. Therefore, the objective of this study was to investigate the association of this variant with obesity and its related anthropometric measurements among the Kampar Health Clinic cohort, Malaysia. **Methodology:** A total of 197 (78 males, 119 females; 98 non-obese, 99 obese) subjects were recruited by convenience sampling and anthropometric measurements were taken. Genotyping was performed using *Stu*I Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP), revealing 61 RR, 94 RT and 42 TT subjects. **Results:** Most of the obese subjects had the RT genotype (50.5%), while only 18.2% were TT. *PYY* R72T genotypes and alleles had no association with obesity (p=0.535; 0.074, respectively), gender (p=0.767; p=0.100, respectively) but were associated with ethnicity (p=0.003; p=0.002, respectively). Among the 13 anthropometric measurements taken, significant difference was only found in Waist Circumference (WC) and Visceral Fat Level (VFL) among the alleles, suggesting that subjects with T allele will have an increment of 1.82 cm in WC and 1.32% in VFL. **Conclusion:** The R72T variant in *PYY* gene was not associated with obesity and most of its related anthropometric measurements. This suggests that other genes and / or environmental factors like dietary habits and lifestyle factors may be the contributors of obesity.

Keywords: Anthropometric measurements, Malaysia, obesity, Peptide Tyrosine-Tyrosine, single nucleotide polymorphism

INTRODUCTION

Obesity is the excessive accumulation of body fat in the body. It is a multifactorial disease which has great socio-economic and personal health impacts for the nation. The World Health Organisation (WHO) stated that globally in 2005, approximately 1.6 billion adults aged 15 and above were overweight and at least 400 million adults were obese (WHO, 2011). Besides, WHO have also projected that by 2015,

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approximately 2.3 billion adults will be overweight and more than 700 million adults will be obese (WHO, 2011). According to the Ministry of Health Malaysia's Third National Health and Morbidity Survey (2006), the combined prevalence of overweight and obesity in Malaysia was alarmingly high at 43.1%. Therefore, obesity is a major public health threat in Malaysia.

Peptide Tyrosine-Tyrosine (PYY) is a 36-amino acid polypeptide which is secreted from the L cells of the ileum, colon and rectum in response to food intake. It contains several tyrosine residues and requires C-terminal amidation for biologic activity (Austin & Marks, 2008). There are 2 endogenous forms of PYY in the human body, which are PYY_{1-36} and PYY_{3-36}. PYY_{3-36} is the peripheral active anorectic signal and the most abundant form of PYY found in the human colon (Grandnt *et al*., 1994). It is created by the cleavage of N-terminal Tyr-Pro residues by dipeptidyl peptidase IV (DPP-IV). Furthermore, PYY_{3-36} binds to the neuropeptide Y2 receptor, which inhibits the NPY neurons and stimulates proopiomelanocortin (POMC) neurons (Austin & Marks, 2008). Studies have reported that PYY plays an important role in reducing food intake in rodents and suppressing appetite in humans post-prandium (Hung *et al*., 2004). In humans, PYY mediates post-prandial satiety through the appetite-regulating centres in the hypothalamus (Hung *et al*., 2004; Torekov *et al*., 2005).

According to several researchers (Hung *et al*., 2004; Ma *et al*., 2005; Torekov *et al*., 2005), the variants in the gene encoding *PYY* may be associated with obesity. Moreover, the studies have also examined the variants in *PYY* for association with metabolic disorders. They have found the plasma PYY concentration to be lower in the obese individuals compared to the lean individuals. Therefore, the genetic variation in *PYY* gene has shown a major correlation with obesity and metabolic syndrome. Several studies reported the presence of genetic variants at the human *PYY* locus which influences the PYY biosynthesis (Hung *et al*., 2004; Ma *et al*., 2005; Shih *et al*., 2009; Torekov *et al*., 2005).

One of the common polymorphisms in the PYY gene is the R72T variant (rs1058046), located at exon 3 with a base change in nucleotide +778 from G to C, resulting in a non-synonymous amino acid residue change at position 72 from Arg to Thr. This variant is located at 5 amino acids downstream from the $Lys_{66}Arg_{67}$ dibasic cleavage of pro-PYY (Shih *et al*., 2009). The alteration of the helical structure in the proximity of this site may impair the efficiency of the post-translational proteolytic liberation of PYY from its flanking peptide (Torekov *et al*., 2005). The association studies of R72T polymorphism in *PYY* gene with obesity were carried out among the British (Torekov *et al*., 2005) and Swedish (Lavebratt *et al*., 2005) populations. Currently, there is limited data and evidence on this association in the Malaysian population. As different populations show different associations in the existing research, the data on the association of R72T variant with obesity in other populations cannot be used to extrapolate for the Malaysian population. Therefore, in this pilot study among the Kampar Health Clinic cohort in Perak, genotyping of *PYY* R72T gene variant was performed to determine the prevalence of the mutated genotypes and alleles, and to investigate if there was any association with obesity.

METHODOLOGY

Subjects

A convenience sampling method was adopted for this study. The blood sample collection was carried out at Kampar Health Clinic (*Klinik Kesihatan*), Perak**,** from October to December, 2010. A short introduction was given to the subjects and blood drawing was conducted with the aid of a qualified

phlebotomist. As the plasma will be used for biochemical tests in future studies, the exclusion criteria of the subjects include hyperthyroidism, pituitary diseases, chronic liver disease, chronic renal disease, acute infection, haematologic diseases and patients under medications that affect the glucose metabolism.

This study was registered under the National Medical Research Registry NMRR-09-826-4266 and the protocol was approved by the Medical Research and Ethics Committee, Ministry of Health, Malaysia. All individuals who participated in this study signed informed consent forms and all samples were taken in accordance with the Declaration of Helsinki (revised in Seoul, 2008).

Questionnaire and anthropometric measurements

The questionnaire-interview session was conducted by the field team to evaluate the socio-demographic data, which provided information on age, gender, race, marital status, occupation, monthly household income, educational status and family history of obesity. The systolic and diastolic blood pressures (SBP; DBP) and pulse rates of the subjects were measured using the HEM-712C Omron blood pressure monitor and duplicate readings were obtained after the subjects were in resting condition for at least 10 minutes. Besides, the height, waist and hip circumferences were determined using a measuring tape. Waist-Hip Ratio (WHR) was calculated by dividing the waist circumference (WC) by hip circumference. The HBF-362 Omron Karada scan bio-impedance scale was used to analyse body compositions namely weight, body mass index (BMI), total body fat (TBF), subcutaneous fat (SF), visceral fat (VFL), resting metabolism (RM) and subcutaneous muscle (SM). The BMI cut-off point for obesity for this study was 27 kg/m^2 (Deurenberg-Yap *et al*., 2000).

DNA extraction and genotyping

Five millilitres of blood sample was collected and genomic DNA was then extracted from the nucleated leukocytes using the Wizard® Genomic DNA Purification Kit (Promega Inc., Madison, WI) according to the manufacturer's protocol. Basically, there were 4 crucial steps in this purification procedure, which included cell lysis, nuclei lysis, protein precipitation and DNA rehydration.

Polymerase Chain Reaction (PCR) was carried out under the standard conditions using the MJ Mini™ Personal Thermal Cycler (Biorad) for 35 cycles. The cycle began with a hot start at 95°C for 5 minutes, denaturation at 95°C for 30 seconds, annealing at 68°C for 15 seconds and extension at 72°C for 15 seconds. The *PYY* R72T gene variant was amplified by using a set of forward and reverse primers adapted from Hung *et al.* (2004), with the forward primer sequence 5' CCC GCC GTG TAG GGT CGA GGC TT 3' and reverse sequence 5' GTG CGT ATG CAA ATG ACG TGG GC 3'. Each of the PCR reaction vials contained 20 *µl* of solution, consisting of forward primer (2μM), reverse primer (2μM), *Taq* buffer with ammonium sulphate (1X), *Taq* DNA polymerase recombinant (1U / μl)(Fermentas, Lithuania), dNTP (200μM) and magnesium chloride (1.5mM).

Genotypes were determined by Restriction Fragment Length Polymorphism (RFLP), where restriction enzyme *Stu*I cut the DNA at the restriction site and formed restriction fragments with the size of 176bp and 22bp. Hence, the *PYY* genotypes were RR (undigested, homozygous wild-type), RT (digested, heterozygous) and TT (digested, homozygous mutant) (Figure 1). The fragments were resolved by 2.5% agarose gel electrophoresis at constant 90V for 45 minutes before staining with ethidium bromide and viewed under MultiDoc-It Digital Imaging System with M-20 Transilluminator. The gel images were

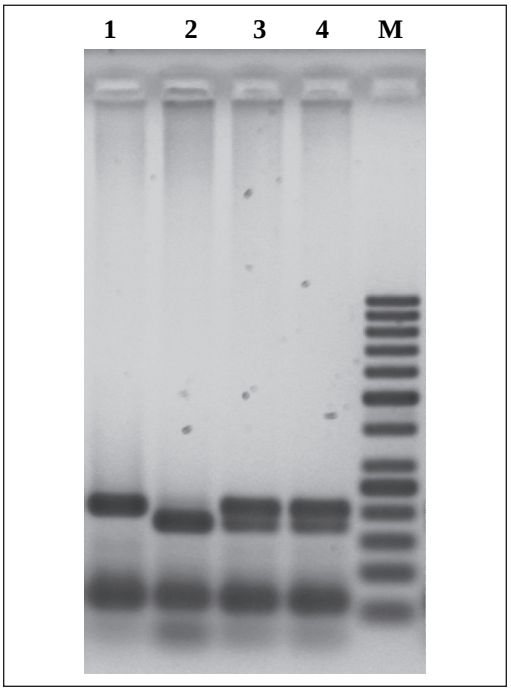

Figure 1. *PYY* R72T Genotyping of the subjects by *Stu*I Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) analysis on 2.5% agarose gel. Lane 1 – Homozygous wild-type RR (198bp), Lane 2 – Homozygous mutated TT (176bp, 22bp), Lane 3 and 4 – Heterozygous mutated RT (198bp, 176bp), M – Fermentas GeneRuler™ 50bp DNA ladder. The unspecific bands at around 50bp indicate primer-dimers.

captured using DigiCam 120 digital camera with 15.1 megapixel resolutions and documented using the UVP's capture software. The three genotypes were validated by sending to an outsourced DNA sequencing service.

Statistical analysis

The compiled data was analysed using Statistical Package for Social Sciences, SPSS® Statistics for Windows® Version 17.0. Descriptive statistics was used to compute frequencies and percentages for socio-demographic data, genotype and allele frequencies. It was also used to calculate the means and standard deviations for anthropometric measurements. Besides, the Pearson's Chi-square analysis was used to compare the genotype and allele frequencies between groups. Also, Student's *t*-test was utilised to determine the differences between means. One-Way Analysis of Variance (ANOVA) was used to test the equality of three or more means at one time. $P<0.05$ was denoted as statistically significant.

RESULTS

Socio-demographic characteristics of subjects

Table 1 illustrates the socio-demographic data of the 197 subjects, who ranged from 21 to 80 years, with a mean ±SD of 55.1 ± 11.0 years. Non-obese and obese subjects were equally distributed in age, 56.9 ± 10.4 and 53.4 ± 11.4 years, respectively. The majority of the non-obese subjects were females and Chinese. Most of the obese subjects were Malays and between age 51 to 60. The majority of the subjects were retired or not working, had monthly household incomes below RM1000 and had primary level education. Most of the non-obese and obese subjects had no self-reported family history of obesity.

Anthropometric measurements

Table 2 shows the means of 13 anthropometric measurements within obesity, gender and ethnic groups. As expected, SBP, DBP, weight, WC, BMI, TBF, SF, VFL and RM were significantly higher in obese subjects compared to non-obese subjects, while it was the other way around for SM. In contrast, there was no significant difference between obese groups for pulse rate, height and WHR, indicating that WC may be a better predictor than WHR for obesity. Within genders, pulse rate, BMI, TBF and SF were significantly higher in females

Table 1. Socio-demographic characteristics of the subjects

Variables		*Total*		*BMI Class*			
				Non-obese		*Obese*	
		N	*%*	*N*	*%*	*N*	*%*
N		197	100	98	49.7	99	50.3
Sex	Male	78	39.6	44	44.9	34	34.3
	Female	119	60.4	54	55.1	65	65.7
Race	Malay	75	38.1	25	25.5	50	50.5
	Chinese	77	39.1	51	52.0	26	26.3
	Indian	40	20.3	20	20.4	20	20.2
	Others	5	2.5	2	2.0	3	3.0
Age groups (years)	21-30	4	2.0	2	2.0	2	2.0
	31-40	15	7.6	4	4.1	11	11.1
	41-50	49	24.9	24	24.5	25	25.3
	51-60	65	33.0	30	30.6	35	35.4
	61-70	47	23.9	26	26.5	21	21.2
	71-80	17	8.6	12	12.2	5	5.1
Occupation	Professional	6	3.0	2	2.0	4	4.0
	White-collar	10	5.1	6	6.1	4	4.0
	Blue-collar	36	18.3	17	17.3	19	19.2
	Retired / Not working	124	62.9	63	64.3	61	61.6
	Own / Others	21	10.7	10	10.2	11	11.1
Monthly household income	Below RM1000	112	56.9	60	61.2	52	52.5
	RM1001-3000	71	36.0	31	31.6	40	40.4
	RM3001-5000	11	5.6	4	4.1	7	7.1
	Above RM5000	3	1.5	3	3.1	0	0
Educational status	No Formal Education	25	12.7	12	12.2	13	13.1
	Primary Level	71	36.0	32	32.7	39	39.4
	Lower Secondary Level	45	22.8	25	25.5	20	20.2
	Upper Secondary Level	44	22.3	24	24.5	20	20.2
	Pre-University Level	5	2.5	2	2.0	3	3.0
	University Level	7	3.6	3	3.1	4	4.0
Family history of obesity	Yes	58	29.4	18	18.4	40	40.4
	No	139	70.6	80	81.6	59	59.6

while SBP, DBP, height, weight, WC, WHR, VFL, RM and SM being significantly higher in males. Besides, SBP, pulse rate, WC, WHR, BMI, TBF, SF and VFL were significantly highest among the Malays. However, DBP, height, WHR, RM and SM had no association with ethnicity.

Genotype and allele frequencies

The genotype and allele frequencies are shown in Table 3. The allele frequency for the mutated T allele was 0.45 and most of the obese subjects had heterozygous mutated RT genotype. Overall, the mutated TT genotype and T allele were both not associated with obesity and the odds ratio for obesity was 0.946 for those with T allele. Besides, the majority of the ethnic groups had mutated RT genotype with most of the Malays and Indians having T alleles. However, R alleles were dominated by the Chinese and other races had the same allele

Table 2. Means of the anthropometric measurements according to BMI status, gender and ethnicity

Anthropometric Measurements	*Mean ± SD BMI Status*		*p#*	*Mean ± SD Gender*		*p#*	*Mean ± SD Ethnicity*				*p#*
	Non-Obese	*Obese*		*Male*	*Female*		*Malay*	*Chinese*	*Indian*	*Others*	
SBP (mmHg)	139.37 ± 21.29	146.52 ± 20.64	0.018	144.26 ± 20.19	142.11 ± 21.90	0.027	148.69 ± 22.71	140.05 ± 17.87	138.13 ± 22.82	140.40 ± 18.39	0.027
DBP (mmHg)	79.97 ± 10.42	84.20 ±10.62	0.005	85.10 ± 10.68	80.13 ± 10.30	0.592	83.25 ± 11.49	81.29 ± 10.07	81.95 ± 9.79	78.40 ± 16.24	0.592
Pulse Rate (bpm)	74.41 ± 13.11	73.29 ± 12.81	0.547	72.83 ± 13.33	74.51 ± 12.69	0.046	76.79 ± 13.79	71.38 ± 12.24	73.90 ± 11.98	67.40 ± 10.46	0.046
Height (cm)	159.09 ± 8.84	157.53 ± 8.83	0.216	165.73 ± 7.15	153.44 ± 6.02	0.074	156.74 ± 7.90	159.98 ± 9.01	157.45 ± 8.89	163.00 ± 15.17	0.074
Weight (kg)	60.91 ± 8.61	76.68 ± 11.22	<0.001	73.27 ± 11.55	65.92 ± 12.67	0.038	71.58 ± 14.76	66.15 ± 10.82	68.04 ± 10.73	75.26 ± 15.06	0.038
WC (cm)	86.19 ± 7.66	97.27 ± 7.45	<0.001	94.48 ± 8.41	89.97 ± 9.55	0.001	94.40 ± 10.02	88.53 ± 8.96	92.79 ± 6.77	93.60 ± 10.84	0.001
WHR	0.91 ± 0.07	0.91 ± 0.07	0.671	0.95 ± 0.05	0.88 ± 0.07	0.093	0.92 ± 0.08	0.90 ± 0.07	0.91 ± 0.07	0.87 ± 0.10	0.093
BMI (kg/m^2)	23.97 ± 1.93	30.94 ± 3.63	<0.001	26.66 ± 3.76	28.01 ± 4.94	<0.001	29.08 ± 4.91	25.86 ± 3.86	27.48 ± 4.09	28.26 ± 4.55	<0.001
TBF (%)	31.62 ± 5.89	36.78 ± 5.30	<0.001	28.73 ± 4.45	37.80 ± 4.16	0.004	35.68 ± 5.59	32.24 ± 6.08	35.23 ± 6.48	34.32 ± 6.65	0.004
SF (%)	24.52 ± 6.10	31.70 ± 7.85	<0.001	20.37 ± 4.27	33.21 ± 5.05	0.009	30.01 ± 7.81	25.80 ± 7.43	28.98 ± 7.82	28.82 ± 9.64	0.009
VFL (%)	8.82 ± 3.17	16.46 ± 4.77	<0.001	14.22 ± 4.95	11.64 ± 5.74	0.009	14.28 ± 6.12	11.29 ± 5.00	12.20 ± 5.12	13.20 ± 3.35	0.009
RM (kcal)	1333.50 ± 194.18	1536.60 ±215.913	<0.001	1607.06 ± 182.09	1323.15 ± 181.95	0.365	1462.55 ± 251.50	1413.56 ± 213.46	1414.03 ± 201.98	1542.00 ± 298.18	0.365
SM (%)	25.19 ± 3.5474	23.65 ± 3.3857	<0.001	27.99 ± 2.22	22.08 ± 1.92	0.057	23.76 ± 3.28	25.24 ± 3.52	23.99 ± 3.70	25.02 ± 4.96	0.057

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.

- obesity status and gender values by Student's *t*-test; ethnicity values by one-way ANOVA

Table 3. Obesity status, race, gender and anthropometric measurements values according to genotypes and alleles

Variables	*Genotype*			*Alleles*	
	RR	*RT*	*TT*	*R*	*T*
Obesity Status					
Non-obese	30 (30.6)	44 (44.9)	24 (24.5)	104 (53.1)	92 (46.9)
Obese	31 (31.3)	50 (50.5)	18 (18.2)	112 (56.6)	86 (43.4)
χ^2	1.251	0.786			
df	2	1			
$p^{\S}$	0.535	0.074			
Ethnicity					
Malay	19 (25.3)	38 (50.7)	18 (24.0)	76 (50.7)	74 (49.3)
Chinese	35 (45.5)	34 (44.2)	8 (10.4)	104 (67.5)	50 (32.5)
Indian	6 (15.0)	19 (47.5)	15 (37.5)	31 (38.8)	49 (61.3)
Others	1 (20.0)	3 (60.0)	1 (20.0)	5 (50.0)	5 (50.0)
χ^2	19.459	15.072			
df	6	3			
$p^{\S}$	0.003	0.002			
Gender					
Male	22 (28.2)	38 (48.7)	18 (23.1)	82 (52.6)	74 (47.4)
Female	39 (32.8)	56 (47.1)	24 (20.2)	134 (56.3)	104 (43.7)
χ^2	0.532	2.713			
df	2	1			
$p^{\S}$	0.767	0.100			
Anthropometric Measurements[w]					
SBP (mmHg)	137.62 ± 19.43	145.37± 21.50 $p = 0.060$	145.31± 22.12	141.24 ± 21.14	145.09 ± 21.12 $p = 0.073$
DBP (mmHg)	81.82± 11.17 $p = 0.441$	81.45± 10.32 $p = 0.233$	83.95 ± 10.93	81.52 ± 10.66	82.81 ± 10.73
Pulse Rate (bpm)	74.05 ± 13.01 $p = 0.075$	72.07 ± 12.10 $p = 0.274$	77.52 ± 14.14	73.21 ± 11.95	74.64 ± 14.03

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Variables	*Genotype*			*Alleles*	
	RR	*RT*	*TT*	*R*	*T*
Weight (kg)	68.29 ± 14.34	68.36 ± 11.97	70.67 ± 12.03	68.07 ± 13.19	69.79 ± 12.09
	$p = 0.575$	$p = 0.183$			
WC (cm)	89.91 ± 10.17	92.22 ± 0.34	93.40 ± 7.85	90.92 ± 9.56	92.79 ± 9.00
	$p = 0.143$	$p = 0.048$			
WHR	0.90 ± 0.07	0.92 ± 0.07	0.91 ± 0.07	0.91 ± 0.07	0.91 ± 0.07
	$p = 0.256$	$p = 0.273$			
BMI	26.98 ± 4.60	27.46 ± 4.35	28.22 ± 4.90	28.26 ± 16.72	29. 19 ± 18.45
	$p = 0.398$	$p = 0.600$			
TBF (%)	33.64 ± 5.32	34.38 ± 6.23	34.65 ± 7.15	34.03 ± 5.81	34.42 ± 6.55
	$p = 0.667$	$p = 0.533$			
SF (%)	27.45 ± 6.90	28.20 ± 7.99	28.95 ± 9.01	28.03 ± 7.43	28.24 ± 8.41
	$p = 0.636$	$p = 0.796$			
VFL (%)	11.82 ± 5.85	12.87 ± 5.38	13.40 ± 5.57	12.07 ± 5.53	13.39 ± 5.55
	$p = 0.323$	$p = 0.019$			
RM (kcal)	1428.46 ± 258.58	1428.78 ± 217.11	1461.07 ± 211.10	1420.82 ± 236.09	1453.83 ± 217.95
	$p = 0.720$	$p = 0.154$			
SM (%)	24.74 ± 3.39	24.22 ± 3.38	24.41 ± 4.12	24.44 ± 3.41	25.22 ± 11.37
	$p = 0.677$	$p = 0.337$			

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.

§ - Values are by Chi-Square test, significant at $p < 0.05$.

ψ – Values are mean ± SD Values, p values are by Student's t-test, significant at $p < 0.05$

( ) - % within the obesity status, ethnicity or gender of respondents

frequencies for both R and T alleles. Therefore, it can be stated that the *PYY* R72T genotypes and alleles were associated with ethnicity. On the other hand, they were not associated with gender as the majority of the males and females had RT genotype and R allele. All the subjects with TT genotype had higher means of anthropometric measurements compared to RR and RT genotypes, except for SBP, WHR and SM - which were higher among the subjects with RT genotype. Besides, subjects with T allele had higher means for all the anthropometric measurements except for WHR. However, significant difference was only found in WC and VFL among the alleles, indicating that subjects with T allele will cause an increment of 1.82 cm in WC and 1.32% in VFL.

DISCUSSION

Based on the socio-demographic data, there were more female obese subjects compared to males. According to Kee *et al*. (2008), the prevalence of obesity in Malaysian women is greater than in men. A national study on prevalence of obesity among Malaysians has also reported a significantly higher prevalence of obesity in females (13.8%) compared to males (9.6%) (Rampal *et al*., 2007). Hence, obesity appears to be more likely to occur in women compared to men in this study. This may be due to the majority of the females being housewives, married and with a lower educational level. Hence, these factors increased the susceptibility of a sedentary lifestyle and reduced health literacy (Kee *et al*., 2008). Among the ethnic groups, obesity was more prevalent among Malays, as supported by the nation-wide study by Rampal *et al*. (2007) and among clinical students in a Malaysian medical school (Boo *et al*., 2010). There was an increase in prevalence of obesity in the age group from 21-30 to 51-60 years and a decrease in the age group of 61-70 and 71-80 years, consistent with the study of Kee *et al*. (2008) which showed that prevalence of obesity increased steadily with age until the age of 50-59 years and decreased after 59 years. This may be due to a change in lifestyle towards a sedentary pattern as age increases. Besides, physiological changes such as an increase in body fat and a reduction in lean body mass, which are associated with older age, may lead to a high risk association of age with obesity (Kee *et al*., 2008). Meanwhile, there was high prevalence of obesity in subjects that were retired or not working. This may be due to the sedentary lifestyle and greater accessibility to food for those who were not working. Among the household income categories, prevalence of obesity was the highest in incomes of less than RM1000. Kampar, a semi-urban town in the state of Perak and which is still under-developed consists of many low-income residents. However, according to Sidik & Rampal (2009), there was no significant association between obesity and total family income. Finally, there was high prevalence of obesity among subjects with a primary level education. According to Kee *et al*. (2008), the relation between prevalence of obesity with level of education is an inverse relationship. This may be due to the observation that high educational attainment has an effect on the attitude of a person towards body weight control, dietary pattern and a healthier lifestyle.

The mutated T allele frequency was 0.45, similar to the European Caucasians (0.45) and Hispanics (0.43), but lower than the sub-Saharan Africans (0.52) (Shih *et al*., 2009). Both the mutated TT genotype and T allele were ethnically-different among Malaysians, but were not associated with obesity, as in the studies of Hung *et al*. (2004) and Lavebratt *et al*. (2006) among the British and Swedish men, respectively. However, according to Torekov *et al*. (2005), there was a modest association between type 2 diabetes and overweight with *PYY* genetic variation among the Danish Caucasians. There was also a strong association of R72T carriers with obesity among normal-weight-range American twins (Shih *et al*., 2009). Among

genders, there was no significant association with the *PYY* R72T genotypes and alleles. According to the study of Lavebratt *et al.* (2006), there was less prevalence of the TT genotypes among obese men compared to lean Swedish men, while there were more TT genotypes in female obese subjects than male obese subjects. But contrary results were obtained by Siddiq *et al.* (2007) who found more evident association of obesity with the R72T genotypes in French Caucasian male children compared to females.

There was significant association between SBP and DBP with obesity. According to several studies, hypertension is highly associated with obesity (Narkiewicz, 2005; Rohrer, Anderson & Furst, 2007). Individuals with elevated blood pressure and high BMI values tend to have higher pulse rate (Martin *et al*., 2004). Based on the results tabulated, the majority of the obese subjects in this study were Malays (50.5%). Therefore, Malays tend to have higher SBP, DBP and pulse rate, which may be due to high BMI. Besides, a higher pulse rate was found in female subjects in this study. This is consistent with research done by Martin *et al.* (2004) who demonstrated in their analysis that females tend to have an elevated heart rate. Non-obese individuals, males and Chinese tend to be taller than other subjects. The studies of Sichieri, Siqueira & Moura, (2000) and Velásquez-Melendez *et al.* (1999) have indicated that weight gain is associated with a short stature. Velásquez-Melendez *et al.* (1999) have also stated that short stature in females is associated with obesity but not in males.

A high WC was found among the obese subjects, males and Malays. The study done by Vazquez *et al.* (2007) states that WC is strongly correlated with BMI. Besides, a study has found that WC is significantly associated with gender with 4 of the 5 obesity-associated risk factors detected (Zhu *et al*., 2002). Kee *et al.* (2008) used WC as the parameter to determine abdominal obesity among the different races. They found that Indians and Malays are at higher risk of abdominal obesity, in parallel with the current study. However, obesity was more prevalent in women compared to men as WC was higher in females (Kee *et al*., 2008). There was an association between WHR with gender, but not for obesity and ethnicity. This is consistent with the research done by Myint *et al.* (2006) who reported a higher WHR in men compared to women in terms of self-reported poor physical functional health. Based on the study done by Deurenberg-Yap *et al.* (2000), Indian men in Singapore had the largest WHR, while the opposite was true for Chinese Singaporean men, thus contradicting the results obtained in this study.

This study found significant associations between TBF, SF, and VFL with obesity, gender and ethnicity. TBF, SF and VFL tend to be higher in obese and Malay subjects. Moreover, TBF and SF were found to be higher in females and VFL higher in males. According to Deurenberg-Yap *et al.* (2000),Chinese females have a lower percentage of body fat compared to Malay and Indian females. However, their study only found body fat percentage difference between Chinese and Indian males in Singapore. Besides, the percentage of body fat in males is lower compared to females. A higher RM was found in obese subjects, males and other races. Based on a study done by Huang *et al.* (2004), RM was found to be higher in severely obese patients. Moreover, males had a higher RM compared to females. Also, Albu *et al.* (1997) reported a lower resting metabolic rate among obese females.

This study has some limitations. Study participants may not represent the general Kampar Health Clinic population as only 197 subjects were studied and their results analysed. Besides, the inconsistency of the results obtained from this study may be due to a small sample size thus limiting statistical analysis and extrapolation. A larger sample size may prevent sampling bias. Future studies need to be done to assess the main contributing factors besides the association of the *PYY* gene to obesity. Besides, insulin

level and lipid profile need to be evaluated to determine the association with obesity and the *PYY* genotypes and alleles.

CONCLUSIONS

Taken together, although the *PYY* R72T gene variant has been associated with obesity in different populations, a similar distribution of its genotypes and alleles across BMI groups and genders has been ruled out in the sampled multi-ethnic Malaysian cohort. Nevertheless, the genotype and allele distribution was ethnically-different, and the mutated T allele could predict a higher WC and VFL. Although the non-association with obesity could be due to the small sample size in our study, other genes or environmental factors such as dietary habits and lifestyle factors could also be other contributors to obesity in the sampled cohort.

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