Prevalence of Melanocortin Receptor 4 (MC4R) V103I Gene Variant and Its Association with Obesity among the Kampar Health Clinic Cohort, Perak, Malaysia

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SUMMARY
This study investigated the prevalence of the Melanocortin receptor 4 (MC4R) V103I gene variant and its association with obesity among a cohort of 254 patients (101 males; 118 obese) attending the Kampar Health Clinic. Genotyping revealed the mutated I allele frequency of 0.02, no homozygous mutated (II), and similar distribution of V and I alleles across BMI groups, genders and ethnic groups. No significant difference was found for the means of anthropometric measurements between alleles. Prevalence of this gene variant among the Malaysian cohort was similar with previous populations (2-4% of mutated allele carrier), but was not associated with obesity.

INTRODUCTION
Melanocortin Receptor 4 (MC4R) is a seven trans-membrane G protein-coupled receptor, consisting of 332 amino acids, and is encoded by a single exon located in chromosome 18q22. The secretion of leptin hormone after food intake stimulates pro-opiomelanocortin and cocaine and amphetamine related transcripts (POMC/CART) neurons in the arcuate nucleus. Activated POMC neuron stimulates the post-translational processes and gives rise to α-melanocyte stimulating hormone (α-MSH), which binds to MC4R and triggers the activation of anorexigenic pathway. Mutations of MC4R have been implicated with large number of obesity cases. And among all the variants, V103I is the most common in humans, resulting from a single nucleotide changes from guanine (G) to adenine (A) leading to amino acid change at codon 103 from Valine (GTC) into Isoleucine (ATC). Inconsistent findings and ambiguous association of MC4R V103I with obesity in various populations therefore merits further investigation among multi-ethnic Malaysians in this study.

MATERIALS AND METHODS
Convenience sampling was performed among a cohort of 254 patients (101 males; 118 obese) attending the Kampar Health Clinic from October-December 2010, ranging from 21 to 80 years (M±SD=52.27±14.2 years) from the Kampar Health Clinic (Klinik Kesihatan Kampar), Perak. Almost half of the subjects were Chinese, followed by Malays (29.1%), Indians (20.1%), and others (2.0%). 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 that have participated in this study signed informed consent forms and all samples were taken in accordance with the Declaration of Helsinki (revised in Seoul, 2008). Duplicate measurements of Systolic blood pressure (SBP), diastolic blood pressure (DBP) and pulse rate were taken using the SEM-1® Automatic Blood Pressure Monitor (Omron, Japan) after the subjects have rested for 10 minutes. Waist circumference (WC), hip circumference (HC) and height were obtained in cm using a measuring tape. Waist-hip ratio (WHR) was calculated by dividing WC with HC. Weight, body mass index (BMI), total body fat (TBF), subcutaneous fat (SF), visceral fat level (VFL), resting metabolism (RM), and skeletal muscle (SM) were determined using the Karada Scan HBF-362® bio-impedance scale (Omron, Japan). Subjects with BMI ≥ 27.0 kg/m² were considered obese. Five millilitres of blood was collected into EDTA tube, genomic DNA was extracted from the nucleated leukocytes using the Wizard® Genomic DNA Purification Kit (Promega Inc., Madison, WI). Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) analysis was performed according to previous protocol. Statistical analysis was performed using SPSS Version 17.0 software and p< 0.05 was considered as statistically significant.

RESULTS
In this study, no homozygous mutated genotype (II) was found. The mutated I allele frequency was 0.02, and there was no significant difference in the MC4R allele distributions between BMI groups, genders and ethnicities, as shown in Table I. Besides, no significant difference was found for the means of 13 anthropometric measurements related with obesity.

DISCUSSION
To the best of our knowledge, there was no similar study carried out in the Malaysian population for the V103I polymorphism of MC4R and its association with obesity and related anthropometric measurements. In the present study, absence of association was found between genotypes and alleles for V103I polymorphism with BMI groups, genders and ethnic groups. The high V allele frequency and the frequency for I allele among world populations ranging from

KEY WORDS:
Melanocortin Receptor 4 (MC4R); single nucleotide polymorphism; obesity; Malaysia
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Variables | MC4R Alleles | V | 1 
| --- | --- | --- | --- 
| BMI status | | | 
| Non-obese | 267 (98.2) | 5 (1.8) | 0.820; 0.051; 1.406, 1.494 
| Obese | 231 (97.9) | 5 (2.1) | 
| Gender | | | 
| Male | 200 (98.5) | 3 (1.5) | 0.516; 0.422; 1.540, 1.627 
| Female | 298 (97.7) | 7 (2.3) | 
| Ethnicity | | | 
| Malay | 143 (97.9) | 3 (2.1) | 0.830; 0.882; 1.867, 1.999 
| Chinese | 246 (98.4) | 4 (1.6) | 
| Indian | 99 (97.1) | 3 (2.9) | 
| Others | 10 (100) | 0 (0.0) | 
| Anthropometric Measurements | | | 
| SBP (mmHg) | 139.08 ± 22.54 | 142.40 ± 17.23 | 0.643 
| DBP (mmHg) | 81.69 ± 10.92 | 82.10 ± 6.82 | 0.906 
| Pulse Rate (mmHg) | 72.90 ± 12.96 | 68.20 ± 12.06 | 0.256 
| WC (cm) | 90.78 ± 11.20 | 88.35 ± 10.73 | 0.497 
| WHR | 0.89 ± 0.08 | 0.89 ± 0.09 | 0.985 
| Height (cm) | 159.82 ± 9.25 | 160.65 ± 10.12 | 0.779 
| Weight (kg) | 69.64 ± 14.39 | 67.76 ± 15.22 | 0.683 
| BMI (kg/m²) | 27.21 ± 5.09 | 26.22 ± 5.83 | 0.545 
| TBF (%) | 33.34 ± 7.09 | 35.79 ± 5.46 | 0.288 
| SF (%) | 27.33 ± 8.45 | 28.78 ± 7.68 | 0.392 
| VFL (%) | 12.63 ± 7.41 | 11.90 ± 7.30 | 0.758 
| RM (%) | 1450.30 ± 270.21 | 1401.90 ± 270.52 | 0.575 
| SM (%) | 24.91 ± 4.12 | 23.46 ± 3.42 | 0.269 

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. 
# p, χ² and 95% CI values by Chi-square Test, significant at p< 0.05.

0.5% to 3.4%, as reviewed in a meta-analysis by Young et al. (2007), are consistent with the current study. The lack of association of this gene variant with obesity and lack of significant difference between allele distribution and anthropometric measurements are supported by previous studies where I allele was not associated with obesity and obesity-related phenotype³⁴. Functional studies revealed that there was no difference between the mutated receptor and the wild-type receptor; therefore, V103I polymorphism was believed to have no effect on the receptor's stimulatory ability and activity⁴. Taken together, although the MC4R V103I gene variant has been associated with obesity in animal and human studies, the very low prevalence of the mutated I allele across BMI groups, genders and ethnicities rules out this association in this Malaysian cohort. Although this could be due to the small sample size in our study which limits statistical power, other genes or environmental factors such as dietary habits and lifestyle factors could be the other contributors to obesity in the sampled cohort.

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