Association of melanocortin-4 receptor gene polymorphisms with obesity-related parameters in Malaysian Malays

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INTRODUCTION

Obesity is an abnormal accumulation of body fat due to an increase in energy input, a decrease in energy output or both. The World Health Organization (WHO) defines obesity as a Body Mass Index (BMI) of \( \geq 30 \text{ kg/m}^2 \) and overweight as a BMI of \( \geq 25 \text{ kg/m}^2 \). The WHO reported that obesity has reached epidemic rates, with \( \sim 2.6 \) million people dying every year as a consequence of being overweight or obese. Over the past decade there has been a 3-fold rise in prevalence of obesity in Malaysia, from 4.4% to 14.2%. The prevalence of obesity is the highest in Malays, at 15.3%, compared to other ethnic groups in Malaysia.

The Melanocortin-4 receptor (MC4R) gene is located at human chromosome 18q22 and encoded by a single exon gene. As a key regulator of appetite, MC4R plays an important role in homeostasis of long-term energy balance in humans. The effect of MC4R on body weight regulation was first observed in targeted disruption of the MC4R gene resulting in hyperphagia, hyperinsulinemia, mature-onset obesity and elevated linear growth in mice (Huszár et al. 1997). MC4R deficiency is a common form of monogenic obesity and those with severe early-onset obesity exhibit pathogenic mutations in MC4R (Farooqi et al. 2003).

Polymorphisms of MC4R have been found to be linked to obesity (Heid et al. 2005; Stutzmann et al. 2007). Association studies involving MC4R variants have been widely carried out in many populations across the globe. To date, four meta-analyses have been performed by combining data from case-control studies to study the effect of rs2229616 SNP (Loos 2011). Although many studies have shown that polymorphism in the MC4R gene is positively associated with obesity, other studies have shown no association (Clement 2006; Farooqi and O’Rahilly 2007).

In this study, we aimed to genotype genetic variants of the MC4R gene and to assess the genetic association of MC4R SNPs with obesity-related parameters in Malaysian Malays. This is the first study conducted in this population to investigate the effects of MC4R variants on obesity.

METHODS

A total of 652 participants were recruited, the majority of whom were employees from an annual health screening programme of a public university in Kuala Lumpur, while some were from villages of the Bera district of Pahang in which a health screening was carried out. The WHO cut-off for obesity was applied. A total of 483 non-obese
(BMI: 25.05 ± 3.02 kg/m²) and 169 obese (BMI: 33.50 ± 3.10 kg/m²) Malaysian Malays were included in this study, with the obese group making up 25.9% of all study participants. The obese group were made up of 51 men (age: 48.94 ± 7.68) and 118 women (age: 48.11 ± 9.71), while the non-obese group were made up of 242 men (age: 48.34 ± 10.42) and 241 women (age: 48.23 ± 10.08). The Medical Ethics Committee of the university medical centre approved the study protocol (MEC reference number: 672.23) and written informed consent was obtained from all the participants.

Anthropometric measurements were performed using calibrated stadiometers, weighing scales and circumference measurement tape. Blood pressure was measured using a digital automatic blood pressure monitor (Omron HEM-907, Omron Healthcare, Kyoto, Japan). A volume of 10–15 ml blood was taken from overnight fasting participants for routine chemistries. Lipid levels were measured using standard clinical laboratory techniques by the clinical diagnostic laboratory of the university medical centre. Genomic DNA extraction from the buccal swab samples was performed using the i-genomic CTB DNA extraction kit (iNtRON Biotechnology, Inc., Gyeonggi, Korea). The genotyping of MC4R SNPs were performed using the Sequenom MassARRAY® iPLEX platform.

Haploview software (version 4.2) was used to construct Linkage Disequilibrium (LD) block and haplotype analysis. Hardy-Weinberg equilibrium in both cases and controls were checked. The GLM method, which was adjusted for age and gender, was used in assessing effects of MC4R SNPs on obesity parameters. Statistical analysis was performed using SPSS 16.0. Bonferroni correction was performed for the validity of the significance test. Sample size and power of the study was calculated using Quanto version 1.2.4 software.

RESULTS

A total of six MC4R SNPs were genotyped in this study. Table I shows the following MC4R SNPs did not deviate from Hardy-Weinberg equilibrium: MC4R rs1295734 SNP (p HWE case: control = 0.907/0.025) and MC4R rs17700144 SNP (p HWE case: control = 0.603/0.033) deviated from Hardy-Weinberg equilibrium. After the 5000 permutation test, there were no significant differences in the allelic and genotype frequencies of each of the MC4R SNPs between the obese and non-obese group.

The results of testing the single marker association of MC4R SNPs with obesity traits are summarized in Figure 1. After Bonferroni adjustment, α = 0.017 (p log 10 = −1.78). For the MC4R rs571312 SNP, there were significant associations with log BMI (p = 0.008) and systolic blood pressure (SBP) (p = 0.005) after Bonferroni correction and adjustment for age and gender. For the MC4R rs2229616 SNP, there was a significant association with TC level (p = 0.016) after Bonferroni correction and adjustment for age and gender. MC4R rs7227255 SNP had no effect on obesity traits in the Malaysian Malays.

Figure 2 shows the LD pattern of the MC4R gene. Low LD was observed at MC4R rs571312 SNP with MC4R rs2229616 (D’ = 0.05) and rs7227255 (D’ = 0.33) SNPs. Low LD was observed at MC4R rs2229616 SNP with MC4R rs7227255 SNP (D’ = 0.67). Table II shows the haplotypes of MC4R gene. After correction for permutation testing with 5000 permutations, none of the haplotypes were associated with obesity.

DISCUSSION

Allelic and genotype frequencies of all the MC4R SNP showed no significant differences between the obese and non-obese group. The MAF for rs2229616 was in the range of 0.01–0.02 in Europeans (Stutzmann et al. 2007). This is in agreement with our results in Malaysian Malays. The MC4R rs1016862 SNP was monomorphic among Malaysian Malays because we could not detect any heterozygous genotype in the study sample. The MC4R rs1016862 SNP was also monomorphic in the Japanese population (Okuda et al. 2002). A similar monomorphic pattern of MC4R rs1016862 SNP was observed in Singaporean Malays, Chinese and Indians (Teo et al. 2009). The homozygotes

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Table I. Allelic distribution of MC4R SNPs among obese and non-obese groups.

<table>
<thead>
<tr>
<th>SNP ID</th>
<th>Position</th>
<th>MAF</th>
<th>p HWE (Obese/non-obese)</th>
<th>Obese frequency (Allele: Genotype)</th>
<th>Non-obese frequency (Allele: Genotype)</th>
<th>p a/p b</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs571312</td>
<td>57839769</td>
<td>0.134</td>
<td>0.145/0.319</td>
<td>0.139: GG = 0.73, GT = 0.26, TT = 0.01</td>
<td>0.133: GG = 0.76, GT = 0.22, TT = 0.02</td>
<td>0.981/0.195</td>
</tr>
<tr>
<td>rs2229616</td>
<td>58039276</td>
<td>0.025</td>
<td>0.753/0.576</td>
<td>0.976: AA = 0, AG = 0.03, GG = 0.97</td>
<td>0.975: AA = 0, AG = 0.02, GG = 0.98</td>
<td>1/0.297</td>
</tr>
<tr>
<td>rs7227255</td>
<td>58055731</td>
<td>0.010</td>
<td>0.845/0.854</td>
<td>0.015: AA = 0, AG = 0.03, GG = 0.97</td>
<td>0.008: AA = 0, AG = 0.03, GG = 0.97</td>
<td>0.654/0.903</td>
</tr>
</tbody>
</table>

p a/p b = p for allelic frequencies/p for genotype frequencies between obese and non-obese groups.
AA were not detected for the MC4R rs2229616 and rs7227255 SNPs in the Malaysian Malays. The homozygotes AA for the rs2229616 were also undetected in the Finnish population (Rutanen et al. 2004).

We found that the MC4R SNPs rs571312, rs2229616 and rs7227255 SNPs were not associated with BMI in the Malaysian Malays after correction for 5000 permutation. The MC4R rs571312 SNP was significantly associated with BMI in the Singaporeans (Dorajoo et al. 2012). Recently, a genome-wide association study (GWAS) reported that MC4R rs7227255 SNP was significantly associated with BMI in Europeans (Speliotes et al. 2010). Previous studies have shown that MC4R rs2229616 SNP (V103I) protects against obesity, by negative association with obesity in Europeans (Stutzmann et al. 2007), UK populations (Young et al. 2007) and in the East Asians (Wang et al. 2010).

Previous studies provided evidence that MC4R gene variants can lead to intracellular cellular retention of receptor molecules, thereby preventing expression of binding sites on the cell surface. This results in reduction in binding affinity for melanocortins. The variants of MC4R can have an effect on the protein structure of the MC4 receptor, thereby preventing its normal inhibitory function on the hypothalamus; hence resulting in dysregulation of energy homeostasis that can consequently lead to obesity (Carroll et al. 2005). The MC4R gene is therefore considered a candidate gene for obesity and melanocortins and their receptors have become the target for drug-based treatment for controlling body weight. This study provides further evidence for the association of MC4R SNPs with BMI in different populations.

Figure 1. Log10 of p-value for single marker association of MC4R SNPs with obesity traits after adjustment for age and gender.

![Figure 1](image1.png)

Figure 2. Linkage disequilibrium (LD) pattern of MC4R gene.

![Figure 2](image2.png)
evidence that MC4R variants are linked to obesity. There were significant differences between rs571312 with logBMI and SBP (p = 0.008 and p = 0.005, respectively) in the Malaysian Malays. The MC4R rs571312 SNP was found to be significantly associated with BMI in Singaporeans (Dorajoo et al. 2012). This finding is consistent with our results that polymorphism has an effect on logBMI in the Malaysian Malays. There were no significant differences between MC4R rs7227255 SNP with obesity traits.

There is a significant association between MC4R rs2229616 polymorphism and total cholesterol level (p = 0.016). This shows the possible involvement of MC4R rs2229616 SNP in cholesterol metabolism in the Malaysian Malay population, but further verification in functional studies is needed. There were no significant differences in height, weight, log BMI, waist circumference (WC), hip circumference (HC), waist hip ratio (WHR), systolic blood pressure (SBP), diastolic blood pressure (DBP), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels in the Malaysian Malays. Our findings were consistent with results reported in the Finnish population (Rutanen et al. 2004).

The MC4R rs7227255 SNP was in low LD ($D^\prime = 0.67$) with the relatively rare MC4R missense variant (rs2229616). In contrast, the MC4R rs7227255 SNP was in perfect LD with MC4R rs2229616 SNP in a European ancestry as reported by Genetic Investigation of Anthropometric Traits (GIANT) Consortium genome-wide association meta-analysis (Speliotes et al. 2010). The difference in genetic pattern may explain the differences in strength of LD in these two different populations. Haplotype analysis showed that none of the three haplotypes of MC4R were associated with obesity in this study of Malaysian Malays. This may be due to the low frequency of these rare MC4R variants in this study population.

To our knowledge, this is the first Malaysian study which provides data on the association between MC4R genetic variants and obesity-related traits. Our study had sufficient power (75%) to detect signals of associations of the MC4R variants. This study was conducted in Malaysian Malays. Therefore, the findings cannot be generalized to other ethnic groups in Malaysia. Results from this study may be applicable to Malays in other parts of South East Asia but further studies will be needed. The subjects are middle-aged and elderly individuals, therefore these findings cannot be generally extrapolated to children and adolescents. It is noted that MC4R polymorphisms may have a potential effect on energy expenditure and control of food intake and therefore the lack of data on the basal metabolic rate and caloric intake in this study may be considered a limitation of our study.

In summary, our data suggest that MC4R SNPs rs571312 and rs2229616 are associated with obesity-related parameters in Malaysian Malays. However, MC4R rs7227255 SNP showed a lack of association with obesity traits. Strength of the LD pattern in the MC4R gene is low in this population. The haplotypes of MC4R gene do not confer risk to obesity in Malaysian Malays.

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REFERENCES


