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Association of *IRX3* rs3751723 polymorphism with the risk of overweight and obesity: case-control study and meta-analysis

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ABSTRACT

Association of the iroquois homeobox 3 (*IRX3*) rs3751723 polymorphism has been shown to increase the risk of obesity, but the data are underreported in the Malaysian population. Therefore, this study sought to investigate this association in the Malaysian population using both a case-control study and a meta-analysis. Genotyping of the *IRX3* rs3751723 polymorphism was performed for 1030 age-matched Malaysians using hydrolysis probe. Odds ratios were calculated with 95% confidence intervals. A meta-analysis was conducted using Comprehensive Meta Analysis software Ver. 2.2.064. Our results showed that the variant G/G genotype was significantly associated with a higher risk of obesity in the Malaysian population, especially in individuals who did not intake fast-food. The presence of the variant G allele was linked to overweight susceptibility in Malaysian females, but seemed to be protective against being overweight and obese among current smokers. However, a meta-analysis showed no significant associations between this polymorphism and obesity in all genetic comparison models. In summary, this study established a significant relationship between the *IRX3* rs3751723 polymorphism and gender and lifestyle differences with respect to susceptibility of overweight and obesity in the Malaysian population; yet no significant association was found in a meta-analysis. Taken together, these data could be beneficial for informing personal health management decisions and population-based study designs that target overweight susceptibility and obesity.

1. Introduction

Overweight and obesity are described as excessive body fat accumulation that has been linked with increased risks for several chronic diseases, including stroke, heart attack, type 2 diabetes, and cancer (Kopelman, 2007). Body mass index (BMI), calculated by dividing an individual's weight in kilograms by height in meters squared (kg/m^2), is commonly used to characterize normal (BMI $< 25\ kg/m^2$), overweight (BMI = 25.0–29.9 kg/m^2), and obese (BMI $\geq 30\ kg/m^2$) individuals. In 2014, the World Health Organization (WHO) reported that more than 1.9 billion adults were overweight and over 600 million of them were obese (WHO, 2016).

In Malaysia, nearly half of Malaysian adults have been reported as overweight, and about 17.7% of them are obese (MIPH, 2015). Malaysia has been ranked as the top country with the highest frequency of overweight and obese individuals in Southeast Asia (WHO, 2011). In addition, the Malaysian Ministry of Health reveals that Malaysia is at the top of obesity prevalence chart among all Southeast Asian countries. Starting in the early years of the 21st century, the Ministry introduced several efforts for monitoring the overweight/obesity rate in the country, including establishment of a collaboration with the Malaysian Association for the Study of Obesity (MASO) to update population's obesity statistics every five years (MASO, 2005). Unpromising statistics showed that the prevalence of obesity in Malaysia dramatically increased from 4.4% in 1996 to 17.7% in 2015. This condition's seriousness indicates that understanding the factors causing overweight and obesity in the population is absolutely essential.

Overweight and obesity are more likely to be influenced by genetic factors, especially an individual's inherited genetic variations. Single nucleotide polymorphisms (SNPs) within the fat mass- and obesity-associated (*FTO*) gene became the first to be linked with body mass in 2007 (Dina et al., 2007; Frayling et al., 2007; Scuteri et al., 2007), and subsequent studies have successfully associated the *FTO* SNPs with an increased risk for overweight and obesity in different populations (Grant et al., 2008; Hubacek et al., 2008; Okuda et al., 2011; Shahid et al., 2013; Wu et al., 2014). While researchers believe that they have found the key gene for overweight and obesity, a recent study reported a "game changer" that has been termed the iroquois homeobox 3 (*IRX3*) gene and appears to be an important determinant of body mass and composition. Smemo et al. (2014) empirically demonstrated that the

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non-protein coding region of the *FTO* gene that previously has been reported to be associated with obesity can physically interact with the *IRX3* gene promoter sequence. A significant reduction in body weight of 25%–30% in a *IRX3*-knockout mice model through the loss of fat mass has been shown (Smemo et al., 2014); these findings make *IRX3* an attractive gene to be studied for overweight/obesity risks in humans.

Recently, one of the SNPs within the promoter region of *IRX3*, namely rs3751723, has been significantly associated with a higher risk of obesity in the North Indian population (Srivastava et al., 2016), but has yet to be determined in the Malaysian population. Therefore, in this study, our aim was to investigate the association between *IRX3* rs3751723 SNP and the risk of overweight/obesity in the Malaysian population by including gender and lifestyle parameters such as smoking status, alcohol consumption, and fast-food intake. In addition, a meta-analysis that emphasized the genetic perspective of this SNP for obesity risk was also conducted.

2. Materials and methods

2.1. Study subjects

From 2014 to 2016, five mL of peripheral blood was collected from 1030 age-matched volunteers with written consent. Subjects were selected based on the following inclusion criteria: (1) healthy Malaysians who were able to provide a blood sample for this study and (2) subjects who did not take dietary supplements or attend an exercise therapy course. Subjects were excluded when they had existing health problems (such as diabetes and depression) that may influence subjects' BMI. Information about age, gender, height, weight, blood pressure, and lifestyles was obtained from all recruited subjects. The subjects were divided into three groups according to their BMI: (1) normal group with BMI $< 25.0\,kg/m^2$ ($N = 694$); (2) overweight group with BMI between 25.0–29.9 kg/m^2 ($N = 223$); and (3) obese group with BMI $\geq 30.0\,kg/m^2$ ($N = 113$). Lipid profiling ($N = 25$ from each group) was performed by Gribbles Pathology Laboratory, Kota Kinabalu, Sabah. This study conformed to The Code of Ethics of the World Medical Association (Declaration of Helsinki), and ethical approval was obtained from Universiti Malaysia Sabah Medical Research Ethics Committee with reference JKEtika 1/15 (7).

2.2. DNA extraction and measurement

DNA was extracted from all subjects using the alkaline phenol-chloroform method. The Standard Sensitivity Genomic DNA Analysis Kit was used to measure the extracted DNA quality using the Fragment Analyzer™ (Advanced Analytical Technologies Inc., IA, USA). DNA quality was represented with a genomic quality number (GQN), and only high intact DNA with GQN > 7.0 was selected for subsequent analysis.

2.3. IRX3 rs3751723 genotyping analysis

StepOnePlus™ Real-time PCR System (ABI, USA) was utilized for *IRX3* rs3751723 genotyping analysis in this study. In brief, a PCR mixture containing 100 ng of genomic DNA, 5 μL of 2× TaqMan® GTXpress™ Master Mix (ABI, USA), and 0.5 μL of 20x TaqMan® genotyping assay (assay ID: C_27476879_10) (ABI, USA) was prepared. The mixture was topped up with sterile distilled H_2O until a final volume of 10 μL was achieved. The PCR conditions included an enzyme activation step at 95 °C for 20 s followed by 40 cycles of 95 °C for 3 s and 60 °C for 30 s.

2.4. Literature search for meta-analysis

A systematic literature search was performed in the PubMed database for studies reporting the association of the *IRX3* rs3751723 polymorphism to overweight/obesity risk until 31 December 2017 using several keywords including: “Iroquois homeobox 3”, “*IRX3*”, “rs3751723 polymorphism”, “rs3751723 variant”, “obesity”, “overweight”, “fat” and “lipid”. The following inclusion criteria were specified: (1) case-control study assessing the association of *IRX3* rs3751723 polymorphism with overweight/obesity risk and (2) sufficient data on genotype distribution of *IRX3* rs3751723 polymorphism was provided. To avoid duplication, only studies with the most recent data were included when the study populations overlapped. Data including first author’s name, year of publication, studied population, total sample size for both cases and controls, and genotype distribution in both cases and controls were extracted from all the included studies. Data extraction from included studies were individually conducted by two investigators, and disagreements were resolved through discussion.

2.5. Statistical analyses

An independent t-test was applied to compare the mean difference of health characteristics between overweight or obese or overweight/obese to normal group and considered statistically significant when $p < 0.05$. By taking T/T as the reference group, odds ratio (OR) with 95% confidence intervals (95% CI) were calculated in the overall and sub-groups (such as gender, smoking status, alcohol consumption, and fast-food intake) with a significant value of $p < 0.05$.

For meta-analysis, OR with 95% CI was calculated for five genetic comparison models, including the allelic (G versus T), heterozygous (G/T versus T/T), homozygous (G/G versus T/T), dominant (G/T + G/G versus T/T), and recessive (T/T + G/T versus G/G) using the Comprehensive Meta Analysis software Ver. 2.2.064 (Biostat, Inc., USA). Each genetic comparison study's heterogeneity was determined by the I^2 (a value in %) and Q (a p-value) statistical tests. A fixed effect model was used to calculate the pooled OR for I^2 value < 50% and $p \geq 0.10$ in both tests (Mantel and Haenszel, 1959) while the random effect model was used to calculate the pooled OR for I^2 value > 50% and $p < 0.10$ (DerSimonian and Laird, 1986). All p-values in the meta-analysis were two-sided.

3. Results

3.1. Health characteristic of the subjects

The mean age of overweight (mean age ± S.D. = 26.91 ± 12.98), obese (mean age ± S.D. = 26.45 ± 11.97), and overweight/obese (mean age ± S.D. = 26.76 ± 12.63) groups were similar to normal (mean age ± S.D. = 25.91 ± 9.75) group with no significant differences. The mean BMI values for overweight (26.95 ± 1.26 kg/m^2), obese (32.66 ± 2.23 kg/m^2), and overweight/obese (28.80 ± 3.14 kg/m^2) individuals were significantly different when compared with normal (21.71 ± 2.34 kg/m^2) individuals (Table 1). Besides, obese individuals also have significantly higher weight and systolic blood pressure when compared with normal individuals.

3.2. Genotyping and risk association analyses

Genotyping of *IRX3* rs3751723 SNP using real-time PCR showed four distinct clusters of genotyped samples (Fig. 1). Overall, variant G/G genotype was significantly associated with increased risk of obesity when compared to wild-type T/T genotype (OR = 1.72; 95% CI = 1.02–2.91) in the Malaysian population (Table 2). When stratified to different parameters, the presence of the variant G allele in Malaysian females was significantly linked to overweight. Interestingly, smokers who inherited the variant G allele seemed to be protected against overweight and obesity. Another unexpected finding in this study was that individuals who did not consume fast-food but inherited the variant G/G genotype had a 4-fold higher risk of obesity.