

**Kertas Asli/Original Article**

**Association between Polymorphisms of Insulin and Insulin Receptor Gene with Childhood Obesity in Malay Population**  
(Hubungan Antara Polimorfisme Gen Insulin dan Gen Reseptor Insulin dengan Obesiti Kanak-kanak dalam Populasi Melayu)

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ABSTRACT

*Childhood obesity is a global epidemic, which leads to the increasing number of studies on genetic locations associated with obesity-related traits. Polymorphisms of insulin (INS) gene have been shown to be associated with obesity-related phenotypes in Europeans; while insulin receptor (INSR) gene has been associated with energy regulation. Therefore, this study was conducted to investigate the association between the INS (rs689) and INSR (rs3745551) gene polymorphisms with childhood obesity risk in a Malay childhood population. Normal weight (538) and overweight or obese (557) children aged 6-12 years old were genotyped using semi-automated Sequenom iPLEX® Gold. Body mass index (BMI) was calculated from measured body weight and height. The rs689 (T/T: 0.006, A/T: 0.159 and A/A: 0.835) and rs3745551 (G/G: 0.054, A/G: 0.378 and A/A: 0.568) genotype distributions were consistent with Hardy Weinberg equilibrium. The T-minor allele frequency for rs689 was 8.6% and G-minor allele frequency for rs3745551 was 24.3%. Minor allele of INS gene polymorphisms significantly increased risk of obesity among Malay children (sex- and age-adjusted OR=1.580; 95%CI: 1.134-2.201). However, INSR gene polymorphisms were not significantly associated with childhood obesity. In conclusion, the polymorphisms of INS gene, rather than INSR gene, were associated with childhood obesity in the Malay population.*

*Keywords: Insulin gene; insulin receptor gene; polymorphisms; obesity; Malay children*

ABSTRAK

*Obesiti kanak-kanak merupakan epidemik di seluruh dunia dan telah menyebabkan pertambahan kajian mengenai lokasi gen berkaitan dengan ciri-ciri obesiti. Polimorfisme gen insulin (INS) telah dikaitkan dengan fenotip obesiti dalam kalangan orang Eropah manakala gen reseptor insulin (INSR) berhubung kait dengan regulasi tenaga. Oleh itu, kajian ini dijalankan untuk mengkaji hubungan antara polimorfisme gen INS (rs689) dan gen INSR (rs3745551) dengan risiko obesiti kanak-kanak dalam populasi Melayu. Kanak-kanak yang berumur 6-12 tahun dan mempunyai berat badan normal (528 orang) dan kanak-kanak yang mempunyai berat badan berlebihan atau obes (557 orang) digenotip dengan menggunakan Sequenom iPLEX® Gold yang bersemi-automasi. Indeks jisim badan dikira daripada ukuran berat badan dan ketinggian. Distribusi genotip rs689 (T/T: 0.006, A/T: 0.159 and A/A: 0.835) dan rs3745551 (G/G: 0.054, A/G: 0.378 and A/A: 0.568) adalah dalam keseimbangan Hardy Weinberg. Kekerapan T-alel minor untuk rs689 adalah 8.6% dan G-alel minor untuk rs3745551 adalah 24.3%. Alel minor untuk gen INS meningkatkan risiko obesiti secara signifikan dalam kalangan kanak-kanak Melayu (OR diselaraskan seks dan umur=1.580; 95%CI: 1.134-2.201). Walau bagaimanapun, polimorfisme gen INSR tiada hubungan yang signifikan dengan obesiti kanak-kanak. Kesimpulannya, polimorfisme gen INS, tetapi bukan gen INSR, adalah berkaitan dengan obesiti kanak-kanak dalam populasi Melayu.*

*Kata kunci: Gen insulin; gen reseptor insulin, polimorfisme; obesiti; kanak-kanak Melayu*

INTRODUCTION

Obesity is a multifactorial disease and determined by interactions between gene, environment and behavioural factors (Knecht et al. 2008; Farooqi 2010). Many gene loci have been investigated to be associated with obesity or obesity-related traits (Bradfield et al. 2012; Thorleifsson et al. 2009). The insulin gene (INS) has been proposed as one of the candidate genes because insulin resistance

appears to be linked with obesity (Gallagher et al. 2010). Polymorphisms of the INS gene have been found to be associated with obesity-related traits in children (Huede et al. 2004; Ong et al. 2004). However, there are also controversial results showing insignificant association of these polymorphisms with obesity in children or adults (Bouatia-Naji et al. 2008; Sandhu et al. 2005).

Human insulin receptor (INSR) is a glycoprotein on the cell surface membrane and categorized as tyrosine kinase

receptors (Sesti et al. 2001). It has been demonstrated that adipose tissue selective *INSR* gene knockout has protective effects on obesity in mice (Blüher et al. 2002). Besides that, polymorphisms of *INSR* gene (rs3745551 and rs3756668) showed to be associated with BMI in diabetic patients (Malodobra et al. 2011). Insulin receptors expressed in the brain were found to reduce food intake (Stockhorst et al. 2004; Plum et al. 2006). On the other hand, increased in lipid synthesis and storage in adipose tissues due to high energy intake appeared to be associated with dysfunction of *INSR* (Alzahrani et al. 2012; Wong dan Sul 2010). Therefore, disruption of *INSR* gene may cause body energy homeostasis imbalance that lead to obesity.

Differences in ethnicity and geographical area may contribute to genetic architecture variances and therefore influence the gene-gene or gene-environment interactions in different populations (Greene et al. 2009). The polymorphisms of *INS* gene and *INSR* gene have not been studied in the Malays, especially in children. Furthermore, the relationship between *INS* gene polymorphisms with obesity are inconclusive and the study on association of *INSR* gene polymorphisms with obesity risk are still lacking. Therefore, the aim of the study was to determine the association between polymorphisms of *INS* gene and *INSR* gene with childhood obesity among Malay children.

## MATERIALS AND METHODS

A total of 1095 male and female children (aged 6-12 years old) were recruited from primary schools around Klang Valley. All subjects reported that they belonged to the Malay ethnic group for at least three generations. Approval was obtained from the Medical Research and Ethics Committee of the Universiti Kebangsaan Malaysia (UKM 1.5.3.5/244/NN-040-2011), the Ministry of Education and school authorities. Prior written informed consent was obtained from children, parents or guardians.

Standing height was measured using SECA Bodymeter 213 (SECA, Germany), and body weight was assessed with SECA digital weighing scale 880 (SECA, Germany). Body mass index (BMI) was calculated, and subjects were categorised into normal weight and overweight or obese groups based on WHO (2007) BMI-for-age growth reference. Blood samples were collected from all individuals using EDTA vacutainers and genomic DNA was isolated using salt precipitation method. Genotyping of the two single nucleotide polymorphisms (SNPs) (rs689 and rs3745551) were performed using semi-automated Sequenom Mass Array iPLEX® Gold platform (MALDI-TOF) (Sequenom, USA). The average call rate for genotyping was >95% and results of duplicate samples had > 97% concordance.

Statistical analysis was performed using SPSS software version 19.0 (IBM, USA). Genotype and allele

frequencies of all SNPs were calculated and Hardy-Weinberg equilibrium was checked using chi-square test. Continuous quantitative traits between the two BMI groups were compared with independent sample *t*-test. Sex- and age-adjusted odds ratios (OR) for association of genotypes with risk of childhood obesity were obtained from logistic regression analysis. Data were expressed as mean  $\pm$  SD and  $p < 0.05$  were considered statistically significant.

## RESULTS

### CHARACTERISTICS OF THE STUDY POPULATION

Characteristics of the 538 normal weight and 557 overweight or obese subjects were summarized in Table 1. Significant differences were found between the two BMI groups for all the phenotypes ( $p < 0.001$ ). The *INS* rs689 (Figure 1) and *INSR* rs3745551 (Figure 2) genotype distributions in the overweight children and normal children were in Hardy Weinberg equilibrium ( $p > 0.05$ ). The minor allele frequency of the T-allele for

TABLE 1. Characteristics of subjects

Characteristic	Normal weight N=538	Overweight/Obese N=557
Male (n, %)	195, 36.2%	291, 52.2%
Female (n, %)	343, 63.8%	266, 47.8%
Age (years)	10.4 $\pm$ 1.5	10.7 $\pm$ 1.2***
Weight (kg)	30.4 $\pm$ 7.8	51.2 $\pm$ 12.7***
Height (cm)	136.7 $\pm$ 11.0	143.4 $\pm$ 8.9***
BMI (BAZ score)	-0.67 $\pm$ 1.00	2.36 $\pm$ 0.84***
BMI (kg/m <sup>2</sup> )	16.0 $\pm$ 2.3	24.6 $\pm$ 4.7 ***
<i>INS</i> rs689 Genotype		
T/T (n, %)	3, 0.6%	4, 0.7%
A/T (n, %)	72, 13.4%	102, 18.4%
A/A (n, %)	462, 86.0%	448, 80.9%
<i>INSR</i> rs3745551 Genotype		
G/G (n, %)	25, 4.7%	34, 6.1%
A/G (n, %)	198, 36.9%	214, 38.6%
A/A (n, %)	314, 58.5%	306, 55.2%

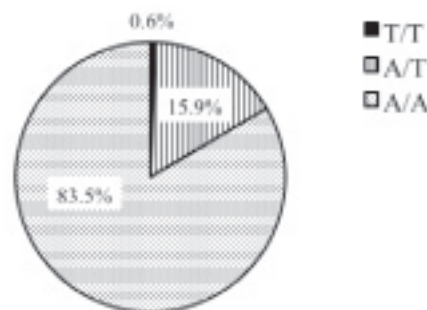


FIGURE 1. Genotypes distribution of *INS* gene rs689 among Malay children

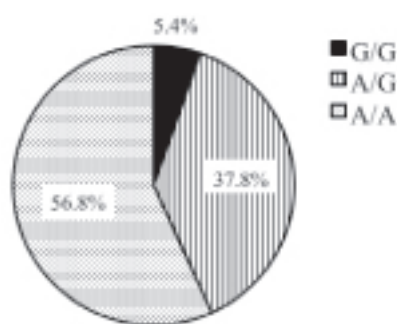


FIGURE 2. Genotypes distribution of INSR gene rs3745551 among Malay children

rs689 and G-allele for rs3745551 were 8.6% and 24.3%, respectively. Data are expressed as proportions per percentages of total for categorical variables and mean  $\pm$  SD for continuous variables. Significantly different by *t*-test, \*\*\**p* < 0.001.

#### ASSOCIATIONS BETWEEN THE INS AND INSR POLYMORPHISMS WITH RISK OF CHILDHOOD OBESITY

Logistic regression analysis revealed that the rs689 A/T variant genotype instead of T/T heterozygote was associated with increased risk of childhood obesity when compared to A/A wild-type homozygote with OR value of 1.578 after adjusting for age and sex (Table 2). Children carrying risk allele of rs689 had significantly increased 1.580 odds of obesity when compared to the wild type carriers. In our study, the rs3745551 variants were not significantly associated with obesity compared to the wild-type A/A genotype.

#### DISCUSSION

We found that INS gene polymorphisms were significantly associated with increased risk of obesity among the Malay children in Malaysia. Our study showed similar results as previous findings from other European studies. Ong et al. (2004) observed that children from the United Kingdom that carried T/T genotype had higher body weight, BMI, and waist circumference when compared to A/A genotype. Significant association between INS polymorphisms with childhood obesity particularly with early onset of severe obesity was also reported (Le Stunff et al. 2001). Besides that, a study from France showed that INS polymorphisms contributed to various levels of adiposity among children and adolescents (Huede et al. 2004). However, they showed that the A/A genotype rather than the T/T genotype was associated with higher mean BMI and waist circumference (Huede et al. 2004; Le Stunff et al. 2001). In contrast, no significant associations between the INS polymorphisms with childhood and adult obesity among the Europeans (Bouatio-Naji et al. 2008; Cimponeriu et al. 2010), and with body composition in early childhood (Maas et al. 2010) were reported.

In our study, there was also lack of association between the INSR rs3745551 and childhood obesity in the Malay population. It has been reported that polymorphisms of INSR gene were associated with insulin resistance and subjects carrying homozygous G/G genotype displayed higher BMI value (Malodobra et al. 2011). To the best of our knowledge, our study is the first to assess the association of INSR polymorphisms with childhood obesity. Previously, studies on association of INSR gene polymorphisms with hypertension (Kuo et al.

TABLE 2. Association analysis of INS and INSR gene polymorphisms and minor allele carriers with respect to the risk of obesity among normal weight and overweight or obese subjects

SNP	Genotype	OR (95%CI)	<i>p</i> value	Adjusted OR (95%CI)	Adjusted <i>p</i> value
INS rs689	T/T	1.375 (0.306, 6.178)	0.678	1.650 (0.354, 7.687)	0.523
	A/T	1.461 (1.052, 2.029)	0.024	1.578 (1.127, 2.211)	0.008
	A/A	1.000	-	1.000	-
	T-allele carrier	1.457 (1.055, 2.013)	0.022	1.580 (1.134, 2.201)	0.007
	Non T-allele carrier	1.000	-	1.000	-
INSR rs3745551	G/G	1.396 (0.813, 2.394)	0.226	1.383 (0.796, 2.403)	0.250
	A/G	1.109 (0.864, 1.423)	0.416	1.124 (0.871, 1.450)	0.368
	A/A	1.000	-	1.000	-
	G-allele carrier	1.141 (0.898, 1.450)	0.280	1.153 (0.903, 1.473)	0.254
	Non G-allele carrier	1.000	-	1.000	-

OR was derived from binomial logistic regression and was adjusted for sex and age

2012), different types of cancer (LeRoy et al. 2011; Cox et al. 2009; Wang et al. 2007) and type 2 diabetes (Bodhini et al. 2012) have been reported but not with obesity.

Insulin signalling plays a crucial role in lipid storage and regulation of glucose homeostasis in adipose tissue (Blüher et al. 2002). Recently, it also has been proposed to control lipogenic gene transcription by activation of the transcription factors (Wong & Sul 2010). Hence, dysregulation of insulin or its receptors potentially influence fatty acid and triacylglycerol metabolism which can lead to obesity. However, the underlying mechanisms of the association between *INS* gene and *INSR* gene with obesity are still unknown. Therefore, functional studies are required to provide an insight into the mechanisms of obesity associated gene polymorphisms.

### CONCLUSION

In conclusion, our study indicated that polymorphisms of the insulin gene (rs689) were associated with obesity-related traits but not for insulin receptor gene (rs3745551) in Malay children.

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