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Original Article

UCP3 gene polymorphism and insulin resistance in obese female adolescents

Dian Eurike[1], Harry Freitag Luglio Muhammad[2], Rina Susilowati[1], Madarina Julia[4]

Abstract

Background A previous study on obese female adolescents in Yogyakarta done in the year 2007 suggests that genetic factors might influence insulin resistance. One genetic factor that has been associated with insulin resistance in other populations is the -55C/T polymorphism in the uncoupling protein 3 (UCP3) gene.

Objective To investigate an association between the -55C/T polymorphism in the UCP3 gene and insulin resistance in obese female adolescents in Yogyakarta.

Methods A total of 79 obese female adolescents were enrolled in this cross-sectional study. Genotyping of the -55C/T polymorphism in the UCP3 gene was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results The T/T homozygous individuals had a higher risk of insulin resistance (OR 2.3; 95% CI 0.4 to 14.1), as well as higher fasting insulin concentration and homeostatic model assessment for insulin resistance (HOMA-IR) compared to individuals with other genotypes. The T allele carriers also had a higher risk of insulin resistance (OR 1.3; 95% CI 0.7 to 2.5), as well as higher fasting insulin concentration and HOMA-IR compared to C allele carriers. However, none of these results were statistically significant ($P > 0.05$).

Conclusion The T/T genotype and T allele of the UCP3 gene -55C/T polymorphism was not significantly associated increased risk of insulin resistance in obese female adolescents in Yogyakarta. **[Paediatr Indones. 2012;52:152-6]**.

Keywords: *UCP3, polymorphism, insulin resistance, obesity, female adolescents*

Obesity has become one of the world's most challenging health problems. The number of obese children and adolescents has increased over the last 2 decades.[1-3] Moreover, obesity has been linked to many comorbidities, one of which is insulin resistance.[4] A previous study by Julia *et al* revealed that 6.4% of 2120 female adolescents in Yogyakarta were obese.[5] Of 79 of these obese female adolescents who agreed to further study, 55.7% were insulin resistant. Insulin-resistant and non-insulin-resistant subjects had no significant differences in dietary habits and physical activity levels. Sulistyoningrum suggested that insulin resistance in children was correlated to insulin resistance in parents (OR >2). Furthermore, she found that children whose mothers were insulin resistant had higher fasting blood glucose concentrations.[6] This data suggests that environmental and genetic factors contribute to insulin resistance.

From the genetic factors that may influence insulin resistance, we focused our work on the

From the Department of Histology and Cell Biology,[1] and Department of Health Nutrition,[2] and Department of Child Health,[3] Medical School, Gadjah Mada University Yogyakarta, Indonesia.

Reprint requests to: Dian Eurike, Department of Histology and Cell Biology, Medical School, Gadjah Mada University, Jalan Farmako Sekip Utara, Yogyakarta 55281, Indonesia. Tel. +62-81578147866, Fax. +62-274-546486. E-mail: *dian.eurike@gmail.com*

-55C/T polymorphism in the UCP3 gene. UCP3 gene is located on chromosome 11q13 and is exclusively expressed in skeletal muscle.[7] UCP3 induces glucose transporter type 4 (GLUT4) translocation by inhibiting lipid peroxidation and reactive oxygen species formation.[8] The -55C/T polymorphism in UCP3 gene has been reported to influence UCP3 expression.[9] Several studies have reported an association between the UCP3 -55T/T genotype and insulin resistance,[10-12] but another study reported contrasting results.[13] Given the inconsistency in reported results on this association, we aimed to further analyze it in obese female adolescents in Yogyakarta.

Methods

A total of 79 obese female adolescents who took part in the previous study by Julia *et al* were enrolled in our study.[5] All subjects were junior high school students in Yogyakarta, had menarche, and were obese (according to WHO-CDC 2000 criteria). One individual was excluded due to incomplete data. This study was approved by the Health and Medical Research Ethics Committee, Medical School at Gadjah Mada University. Informed consent was obtained from all subjects.

Insulin-resistance status was established based on the homeostatic model assessment of insulin resistance (HOMA-IR), which is fasting blood glucose level (mg/dL) x fasting insulin level (mU/mL)/405.[14] Subjects whose HOMA-IR was ≥ 3.16 were classified as insulin-resistant. For fasting blood glucose and fasting insulin measurements, peripheral blood specimens were taken in the morning after subjects had fasted for 8 hours, i.e., last evening meal before 10:00pm the previous evening. Fasting insulin was measured by immunoassay method and fasting blood glucose was measured by hexokinase chemical method.[6]

Genomic DNA was extracted from 5 mL of peripheral blood by salting out method.[15] PCR-RFLP was used to determine the genotype the -55C/T polymorphism in the UCP3 gene. Previously described primers were used in this study (forward: 5'- GAG CTA TAT TAA AGC ACC CCG GGT CAA GAG GAC -3' and reverse: 5'- TCT GCT GCT TCT GGC TTG GCA CTG GTC TTA TAC ACC C -3').[16] The PCR conditions were 94ºC for 3 min; followed by 35 cycles of 94ºC for 30 sec, 65ºC for 30 sec, 72ºC for 30 sec; and a final extension at 72ºC for 5 min. After amplification, the PCR product was incubated with restriction enzyme SmaI (New England Biolabs, Beverly, MA) at 25ºC for 4 hours. Restriction fragments were resolved on a 3% agarose gel.

Subjects' genotypic and allelic proportions, as well as odds ratios (OR) were analyzed using Chi square test. Differences in continuous variables between genotypes and alleles were compared using Kruskal-Wallis test or Mann Whitney U test. For all analyses, $P < 0.05$ was considered to be statistically significant. All analyses were performed using SPSS version 16.0 for Windows (SPSS, Inc., Chicago, IL).

Results

Genotyping by PCR-RFLP revealed all possible genotypes of the UCP3 polymorphism. We expected a 189 bp fragment for the T/T genotype, 152 bp and 37 bp fragments for the C/C genotype, and 189 bp, 152bp, and 37 bp fragments for the C/T genotype (**Figure 1**).

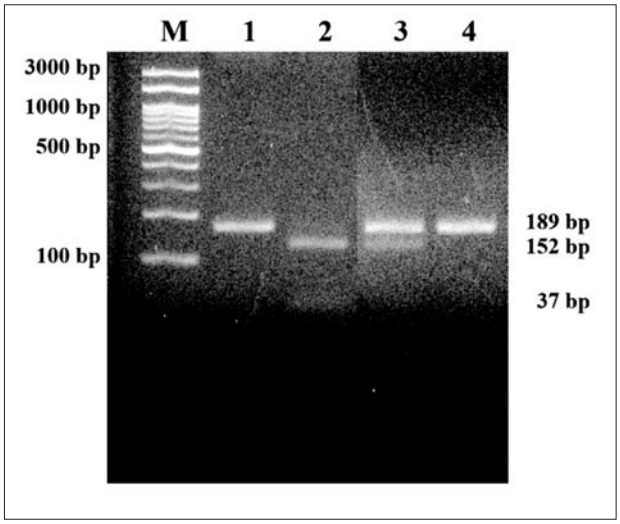

Figure 1. Electrophoresis of the UCP3 gene PCR-RFLP product digested with SmaI. Lane M: 100 bp DNA standard ladder; lane 1: T/T genotype (189 bp fragment); lane 2: C/C genotype (152 and 37 bp fragments); lane 3: C/T genotype (189 bp, 152 bp, and 37 bp fragments); lane 4: uncut UCP3 gene PCR product

Table 1. Genotypic and allelic proportions of the UCP3 -55C/T polymorphism in the insulin-resistant and non-insulin-resistant groups

	n	%	IR, n (%)	NIR, n (%)	OR (95% CI)	P
Genotype						
T/T	7	8.97	5 (71.43)	2 (28.57)	2.32 (0.38 to 14.12)	0.36
C/T	44	56.41	25 (56.82)	19 (43.18)	1.22 (0.47 to 3.20)	0.68
C/C	27	34.62	14 (51.86)	13 (48.14)	1.00 (reference)	
Allele						
T	58	37.18	35 (60.34)	23 (39.66)	1.30 (0.67 to 2.50)	0.45
C	98	62.82	53 (54.08)	45 (45.92)	1.00 (reference)	

IR = insulin-resistant group; NIR = non-insulin-resistant group; OR = odds ratio; CI = confidence interval; P values were calculated by Chi square (χ^2) analysis.

Table 2. BMI, fasting glucose concentration, fasting insulin concentration and HOMA-IR of the three UCP3 genotypes

	Genotypes			P
	C/C	C/T	T/T	
Mean BMI, kg/m^2 (SD)	29.05 (2.22)	30.00 (2.81)	30.06 (2.35)	0.30
Mean fasting glucose, mg/dL (SD)	87.44 (9.94)	89.25 (22.11)	85.29 (14.52)	0.64
Mean fasting insulin, µU/mL (SD)	16.98 (7.32)	17.63 (12.90)	21.10 (10.77)	0.50
Mean HOMA-IR, units (SD)	3.60 (1.49)	4.06 (3.97)	4.68 (3.27)	0.66

P values were calculated by Kruskal-Wallis test because the Kolmogorov-Smirnov normality test showed that the data were not normally distributed ($P < 0.05$).

Table 3. BMI, fasting glucose concentration, fasting insulin concentration and HOMA-IR of the C and T alleles of the UCP3 -55C/T polymorphism

	Alleles		P
	C	T	
Mean BMI, kg/m^2 (SD)	29.48 (2.53)	30.01 (2.67)	0.21
Mean fasting glucose, mg/dL (SD)	88.26 (16.45)	88.29 (20.40)	0.42
Mean fasting insulin, µU/mL (SD)	17.28 (10.13)	18.47 (12.34)	0.74
Mean HOMA-IR, units (SD)	3.81 (2.87)	4.21 (3.77)	0.86

Genotypic and allelic frequencies of the UCP3 gene polymorphism are shown in **Table 1**. Most subjects were C/T heterozygous individuals (56.4%). We found that individuals with the T/T genotype had a higher risk for insulin resistance (OR 2.3; 95% CI 0.4 to 14.1). We also observed that the UCP3 T allele increased the risk of insulin resistance (OR 1.3; 95% CI 0.7 to 2.5). Nonetheless, these results were not statistically significant ($P > 0.05$).

We also evaluated the influence of this polymorphism on body mass index (BMI), fasting insulin concentration, fasting glucose concentration, and HOMA-IR measurements. We observed that T/T homozygous individuals and T allele carriers had higher fasting insulin concentrations and HOMA-IR values, but they were not statistically significant (**Tables 2 and 3**).

P values were calculated by Mann-Whitney U test because the Kolmogorov-Smirnov normality test showed that the data were not normally distributed ($P < 0.05$).

Discussion

Although not statistically significant, we observed that subjects with the T/T genotype and T allele were more likely to have insulin resistance. Similar results were reported in other populations, such as in Spanish adults,[17] as well as Spanish children and adolescents.[11] However, conflicting results were reported in other populations. A study of Pima Indians reported that the UCP3 -55T/T genotype was associated with increased skeletal muscle expression of UCP3 and protection

against insulin resistance.[9] In addition, in French adults, the -55T/T genotype was reported to decrease the risk of type 2 diabetes.[13] These contradictory results might be explained by the influence of the UCP3 -55C/T polymorphism on UCP3 expression in skeletal muscle. Further study is required to determine the influence of the -55C/T polymorphism in UCP3 mRNA expression.

Another interesting result of this study was the high frequency of UCP3 T allele of more than 30% (37.2%), similar to that in a Japanese study (30.3%).[18] However, the frequency of the T allele in Pima Indians and Caucasians was reported to be less than 25% (16.0 – 23.2%).[9,11,12,19] Our finding indicates the existence of an ethnic difference in the distribution of the UCP3 -55C/T polymorphism. Since the T/T genotype and T allele were reported to be a high risk genotype and allele for insulin resistance,[11,17] it was suggested that this polymorphism also increased Asians' susceptibility to insulin resistance.

Genotyping can be used to predict risk of insulin resistance. Since insulin resistance is a polygenetic condition, there are many contributing gene to gene interactions and genetic variations. Analysis of more gene to gene interactions and genetic variations may help with prediction of insulin resistance.[20] A Korean study reported a 65% prediction value by combining 14 such polymorphisms.[21] Therefore, to obtain a laboratory prediction test, more genetic variations must be determined.

We suggest that, although not statistically significant, the -55T/T genotype and the -55T allele of the UCP3 -55C/T polymorphism increased the risk for insulin resistance.

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