

# Uncoupling protein gene UCP1-3826A/G, UCP2 Ins/Del and UCP3-55C/T polymorphisms in obese Turkish children

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## ABSTRACT

**Background.** Mitochondrial uncoupling proteins (UCP) 1, 2 and 3 are members of the anion carrier protein family located in the inner mitochondrial membrane. There are various controversial reports on UCP genotypes and obesity in adults and children. This study aims to investigate the link between mostly studied UCP polymorphisms (*UCP1-3826A/G*, *UCP2 Insertion/Deletion (Ins/Del) polymorphism of exon 8*, and *UCP3-55C/T* Polymorphisms) and obesity in Turkish children. Furthermore, the relationships of UCP polymorphisms are also analyzed within the scope of metabolic parameters of obese children.

**Methods.** Molecular screening of the UCP1, UCP2, and UCP3 gene polymorphisms was carried out in 189 children aged 6 to 18 years, 102 of who had exogenous obesity (54 girls) and 87 of whom were healthy controls (48 girls). In the obese group, fasting lipids, glucose and insulin levels were measured. In 60 obese children, an oral glucose tolerance test (OGTT) was performed with 0, 30, 60, 90 and 120 minutes of sampling for plasma glucose and insulin levels.

**Results.** The frequency of UCP polymorphisms was similar in obese and non-obese children. In obese children, fasting lipids, glucose and insulin levels were not different among the UCP 1, 2 and 3 genotypes. While no relationship was found between the UCP 1 and 3 genotypes and glucose/insulin levels during OGTT, carriers of the Insertion allele with *UCP2 Ins/Del* polymorphism had significantly higher 30-minute insulin levels ( $p=0.018$ ).

**Conclusions.** Polymorphisms of the *UCP1-3826A/G*, *UCP2 Ins/Del*, and *UCP3-55C/T* are not associated with obesity and related pathologies in Turkish children. However, the presence of the Ins allele of the UCP2 gene has been found to have an unfavorable influence on early insulin excursion after glucose loading.

**Key words:** UCP, polymorphism, obese, children, OGTT.

Obesity is caused by an imbalance between energy intake and output, which is influenced by numerous environmental, biological, and genetic factors. The consumption of high-calorie foods and having a sedentary lifestyle are reported as the main causes of an increasing rate of obesity; only a minority of obese people have a genetic defect. However, controversial

results have been published about single nucleotide polymorphisms in obesity-related genes.<sup>1-5</sup>

Mitochondria are known as the primary organelle regulating metabolic and energy homeostasis and mitochondrial dysfunction is thought to play a key role in the pathogenesis of metabolic disorders in obesity. Mitochondrial uncoupling proteins (UCP) 1, 2 and 3 are the members of the anion carrier protein family located in the inner mitochondrial membrane. They act as a proton transporter, that uncouples oxidative metabolism from ATP synthesis

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and they dissipate energy through heat production.<sup>6</sup> Since the energy in this process is transferred to heat and not stored as fat in the body, polymorphisms in the UCP genes may contribute to variation in energy balance and potentially to the development of obesity.<sup>7</sup> This uncoupling effect subsequently leads to tissue-specific functions, such as thermogenesis and energy expenditure (UCP1), the regulation of free-fatty acids metabolism (UCP2 and UCP3), decrease in reactive oxygen species production by mitochondria (UCP1, UCP2, UCP3) and the regulation of insulin secretion by pancreatic beta-cells (UCP2), which are all associated with obesity and/or type 2 diabetes mellitus (T2DM) pathogenesis.<sup>8</sup>

Some well-known polymorphisms of the UCPs have been reported to be functional variants on gene expression because of their location within the genome. The polymorphisms located on the upstream region of the gene as *UCP1-3826 A/G* and *UCP3-55C/T* may associate with the quantity of the mRNA expression while *UCP2* exon 8 *Ins/Del* polymorphism may associate with mRNA transcript stability.<sup>6-11</sup>

Given the location and proposed biological effects of the UCPs, it might be interesting to further evaluate the association among the *UCP1-3826A/G* (rs1800592), *UCP2 Ins/Del* and *UCP3-55C/T* (rs1800849) genotypes with childhood obesity and metabolic parameters.

## Material and Methods

This study was approved by Baskent University Institutional Review Board (Project No: KA15/344), and supported by Başkent University Research Fund.

Written informed consent was obtained from the parents of the subjects. One hundred and two exogenously obese and 87 healthy age-matched controls were included in this study. In the obese group, fasting glucose, lipids and insulin levels were measured. All patients were clinically free of symptoms except for obesity and they were not taking any medication.

The heights of all subjects were measured by using a Harpenden wall-mounted stadiometer. Weight was measured with a calibrated electronic scale. Body mass index (BMI) was calculated by using the weight/height<sup>2</sup> (kg/m<sup>2</sup>) formula. BMI -Z scores were calculated and children with a BMI-Z score greater than or equal to 2 were also accepted as obese.<sup>12,13</sup>

Serum low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglyceride levels were studied by using Roche diagnostics methods (GmbH, Germany).

A standard oral glucose tolerance test (OGTT) (1.75 g/kg or a maximum of 75 g of glucose) following a 3-day, high-carbohydrate diet (300 g/day) and a 12-hour overnight fast was performed in 60 obese children who accepted to be tested. For glucose and insulin assessments, their blood samples were taken at 0, 30, 60, 90 and 120 minutes, after glucose administration. The plasma glucose levels were measured with the glucose oxidase method and a modified Trinder color reaction was catalyzed by the peroxidase enzyme and their insulin levels were measured with an immunoradiometric assay kit.

Genomic DNA was isolated from leukocyte pellets by phenol/chloroform extraction, and ethanol precipitation.<sup>14</sup>

*UCP1-3826A/G* (rs1800592) and *UCP3-55C/T* (rs1800849) SNPs were determined by real-time PCR, using a panel of LightSNiP from TIB MolBiol (assays based on SimpleProbe®). The SimpleProbe® included in the LightSNP assay can detect single base mismatches, thus enabling the analysis of polymorphisms. At the end of the amplification, a melting curve analysis was performed according to the protocol (LightCycler 480, Roche). Polymorphic alleles were identified by the specific melting temperature (T<sub>m</sub>) of the resulting amplicons.

For *UCP1-3826A/G* (rs1800592) individuals with -3826 A allele show a single melting peak at 57.94°C, while individuals with -3826 G allele also show a single melting peak but at 65.16°C

and individuals with both alleles (A/G) show two melting peaks at 57.94 and 65.16°C in this analysis (Fig. 1).

For *UCP3-55C/T* (rs1800849); individuals with two copies of the C allele show a single melting peak at 62.01°C, individuals with two copies of the T allele also show a single melting peak but at 67.31°C and individuals with both alleles (C/T) show two melting peaks at 62.01 and 67.31°C in this analysis (Fig. 2).

Ins/Del polymorphism of the *UCP2* gene was also detected by electrophoretic separation of PCR products.<sup>15</sup> The presence of a 450-bp product indicated the Ins allele, whereas the presence of a 412-bp product indicated the Del allele. Each DNA sample revealed 1 of the 3 possible patterns after electrophoresis: a 412-bp band (genotype Del/Del), a 450-bp band (genotype Ins/Ins), or both bands (genotype Ins/Del).

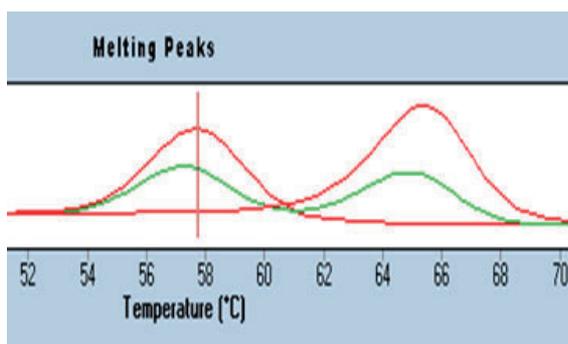


Fig. 1. Melting Curve Analysis *UCP1* -3826A/G (rs1800592).

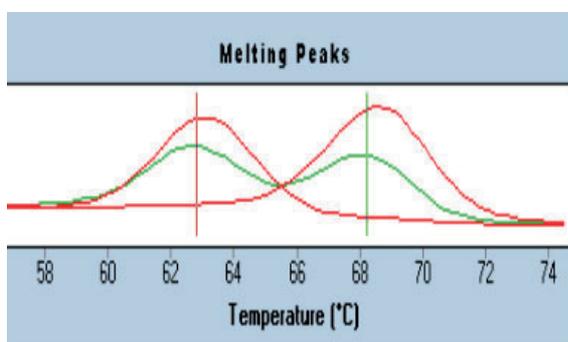


Fig. 2. Melting Curve Analysis *UCP3* -55C/T (rs1800849).

### Statistical analysis

Compliance with the normal distribution of continuous variables was checked with the Shapiro-Wilk test. The homogeneity of group variances was checked by Levene's test. If parametric test requirements were met, two independent group means would be compared by Student's t-test. If requirements were not met, the Mann Whitney U test would be used for the comparisons of groups' medians.

The allele and genotype frequencies were calculated for all investigated polymorphisms by direct counting. The distribution of genotypes for all polymorphisms was tested for conformity with Hardy-Weinberg equilibrium. To determine the relationships between genotypes and study groups, categorical variables were statistically evaluated by Pearson  $\chi^2$  test and the Likelihood ratio test was used for small frequencies in some cells of the contingency tables. Relationships between alleles and study groups were analyzed by the Fisher Exact test. Data analyses were performed using the Statistical Package for the Social Sciences, version 19.0 (SPSS 19, Armonk, NY: IBM Corp). *p* values smaller than 0.05 were considered to be statistically significant.

### Results

The frequencies of *UCP1*, *UCP2*, *UCP3* genotypes were not different in the obese and control groups. The allele frequencies were also similar (Table I).

In obese children, gender, fasting lipids, glucose, and insulin levels were not different among the *UCP 1, 2 and 3* genotypes (Table II). OGTT was performed in 60 obese children who approved of being tested.

Plasma glucose and insulin levels during OGTT were not different between G allele carriers and noncarriers in *UCP1-3826A/G* polymorphism and also between T allele carriers and noncarriers in *UCP3-55C/T* polymorphism genotypes. For *UCP2 Ins/Del* polymorphism, similarly, plasma

**Table I.** Genotype frequencies of the obese and control groups.

	Obese group (n=102) mean $\pm$ SD	Control group (n=87) mean $\pm$ SD	P
	female/male 54/48	female/male 48/39	
Age (year)	12.3 $\pm$ 2.8	11.9 $\pm$ 3.2	0.29
BMI-Z score	2.6 $\pm$ 0.5	-0.7 $\pm$ 0.8	<0.001
UCP1 -3826A/G			
AA	54 (53%)	57 (66%)	0.12
AG	40 (39%)	22 (25%)	
GG	8 (8%)	8 (9%)	
ALLELE FREQUENCY			
A	148 (73%)	136 (78%)	0.20
G	56 (27%)	38 (22%)	
UCP2 Ins/Del			
DD	72 (71%)	53 (61%)	0.11
DI	29 (28%)	29 (33%)	
II	1 (1%)	5 (6%)	
ALLELE FREQUENCY			
D	177 (87%)	150 (86%)	0.87
I	27 (13%)	24 (14%)	
UCP3 55C/T			
CC	75 (74%)	63 (72%)	0.86
CT	27 (26%)	24 (28%)	
TT	0 (0%)	0 (0%)	
ALLELE FREQUENCY			
C	177 (87%)	150 (86%)	0.87
T	27 (13%)	24 (14%)	

SD: standard deviation, BMI: body mass index

glucose and insulin levels during OGTT were not different between Ins allele carriers and noncarriers except for the 30<sup>th</sup>-minute insulin levels. The mean of the serum insulin level at the 30<sup>th</sup>-minute was significantly higher in the Ins allele carrier group (Table III).

## Discussion

In this study, the genotype and allele frequencies of UCP1-3826A/G, UCP2 Ins/Del, and UCP3-55C/T polymorphisms were found similar in obese and non-obese Turkish children. Our study may indicate that the presence of the Ins allele of the UCP2 gene has an unfavorable influence on early insulin excursion after glucose loading.

UCP1 is expressed in brown adipose tissue and plays an important role in thermogenesis, regulation of energy metabolism, and reduction of reactive oxygen species production by mitochondria, which are important pathways in obesity and T2DM.<sup>16</sup>

UCP1-3826A/G polymorphism is one of the well-described polymorphisms for UCP1. In a study, it was reported that there is a positive correlation between UCP1 GG genotype and lower weight, while in two other studies it was mentioned that there is a causative effect in people carrying G allele and having a higher BMI, especially in women.<sup>17-20</sup> On the other hand, a report which supports an association between the -3826G allele and T2DM in adults<sup>21</sup> and a meta-analysis improves the lack of

**Table II.** Laboratory results of obese children in each polymorphism.

UCP1 -3826 A/G		Female/ Male	Age	HDL (mg/dl)	LDL (mg/dl)	Triglyceride (mg/dl)	Glucose (mg/dl)	Insulin μIU/ml
	Mean		12.9	42.6	98.5	118.9	86.7	20.1
AA	Std. Deviation	30/24	2.2	9.5	26.3	68.6	12.7	14.1
	Minimum		5.0	21.0	45.0	27.0	17.0	2.0
	Maximum		17.8	68.0	167.0	460.0	116.0	85.0
	Mean		12.6	44.8	96.5	117.1	87.2	21.0
AG+GG	Std. Deviation	24/24	2.6	9.8	23.8	60.0	6.6	16.7
	Minimum		5.0	26.0	50.0	40.0	73.0	6.0
	Maximum		17.3	72.0	159.0	304.0	100.0	87.0
p-value		0.57	0.80	0.34	0.70	0.89	0.81	0.76
UCP2 I/D		Female/ Male	Age	HDL (mg/dl)	LDL (mg/dl)	Triglyceride (mg/dl)	Glucose (mg/dl)	Insulin μIU/ml
	Mean		12.5	43.4	98.1	120.4	86.7	87.6
DD	Std. Deviation	39/33	2.8	9.7	26.8	66.1	11.3	7.3
	Minimum		5.0	21.0	45.0	27.0	77.0	74.0
	Maximum		17.8	72.0	167.0	460.0	116.0	101.0
	Mean		12.0	43.8	96.2	112.5	86.9	20.5
II+ID	Std. Deviation	15/15	2.8	9.6	20.1	60.9	10.3	15.4
	Minimum		5.5	26.0	51.0	41.0	17.0	2.0
	Maximum		17.3	64.0	128.0	304.0	116.0	87.0
p-value		0.34	0.78	0.85	0.72	0.57	0.70	0.65
UCP3 -55 C/T		Female/ Male	Age	HDL (mg/dl)	LDL (mg/dl)	Triglyceride (mg/dl)	Glucose (mg/dl)	Insulin μIU/ml
	Mean		11.9	43.3	96.8	114.5	86.1	19.8
CC	Std. Deviation	38/37	3.0	9.5	25.8	68.2	11.3	13.2
	Minimum		5.0	21.0	45.0	27.0	17.0	2.0
	Maximum		17.8	65.0	162.0	460.0	116.0	85.0
	Mean		9.6	43.9	99.8	127.9	89.2	22.4
CT+TT	Std. Deviation	16/11	2.1	10.2	22.8	52.8	6.4	19.9
	Minimum		8.3	30.0	54.0	40.0	73.0	7.0
	Maximum		16.3	72.0	167.0	264.0	100.0	87.0
p-value		0.44	0.67	0.76	0.60	0.36	0.18	0.45

HDL: high-density lipoprotein cholesterol, LDL: low-density lipoprotein cholesterol

the association of the relevant genotype and T2DM.<sup>22</sup> The result of our study shows that *UCP1-326A/G* genotype was not associated with obesity, metabolic disorders, gender and also glucose-insulin responses during OGTT.

UCP2 is widely expressed, especially in white adipose tissue, skeletal muscle, and pancreatic islets. The *UCP2 Ins/Del* polymorphism is a 45-bp insertion in exon 8 in the 3'-untranslated

region of the UCP2 gene. It may affect UCP2 mRNA stability, post-transcriptional modification, or translation.<sup>23</sup> Although no significant association has been reported between UCP2 Ins/Del genotype and obese/non-obese adults and children in many reports, Yanovski et al.<sup>24</sup> have reported that children with Ins allele had a greater BMI. However, the metabolic parameters related to obesity were not included.<sup>25,26</sup>

**Table III.** Glucose and insulin levels during OGTT in UCP1. 2. 3 polymorphism genotypes.

	UCP1 3826A/G	Mean	Std. Deviation	Std. Error Mean	P	UCP2 Ins/Del of exon 8	Mean	Std. Deviation	Std. Error Mean	P	UCP3 55C/T	Mean	Std. Deviation	Std. Error Mean	P
	AA	86.7	12.7	1.7	0.81	DD	86.7	11.3	1.3	0.70	CC	86.1	11.3	1.3	0.18
Glucose 0	AG+GG	87.2	6.6	0.9		ID+II	87.6	7.3	1.3		CT+TT	89.2	6.4	1.2	
	AA	20.1	14.2	2.0	0.77	DD	20.9	15.3	1.8	0.66	CC	19.8	13.3	1.6	0.46
Insulin 0	AG+GG	21.0	16.7	2.5		ID+II	19.4	15.5	2.8		CT+TT	22.4	19.9	3.8	
	AA	142.2	25.8	4.4	0.78	DD	140.9	24.4	3.7	0.81	CC	139.0	27.7	4.2	0.46
Glucose 30	AG+GG	140.3	25.7	5.1		ID+II	142.7	28.6	6.7		CT+TT	145.5	18.3	4.6	
	AA	138.6	84.8	14.3	0.78	DD	120.6	74.1	11.4	0.01	CC	130.5	84.5	12.7	0.36
Insulin 30	AG+GG	132.8	72.2	14.4		ID+II	172.7	80.8	19.1		CT+TT	151.8	62.3	15.6	
	AA	140.9	27.9	4.7	0.44	DD	141.3	26.3	4.1	0.25	CC	138.3	26.5	3.9	0.84
Glucose 60	AG+GG	135.6	23.3	4.7		ID+II	132.7	24.9	5.9		CT+TT	139.9	25.4	6.4	
	AA	138.7	88.5	14.9	0.67	DD	136.3	78.2	12.1	0.39	CC	139.0	96.9	14.6	0.58
Insulin 60	AG+GG	149.5	106.4	21.3		ID+II	159.3	128.8	30.7		CT+TT	154.6	93.9	23.5	
	AA	132.9	36.2	6.1	0.45	DD	132.4	32.7	5.0	0.46	CC	129.4	32.2	4.9	0.62
Glucose 90	AG+GG	127.1	15.8	3.2		ID+II	126.2	19.9	4.7		CT+TT	133.7	20.2	5.1	
	AA	145.1	108.0	18.3	0.88	DD	138.6	105.1	16.2	0.63	CC	142.7	122.0	18.4	0.96
Insulin 90	AG+GG	140.5	119.9	23.9		ID+II	153.9	129.7	30.6		CT+TT	144.3	82.2	20.6	
	AA	122.3	25.6	4.3	0.11	DD	116.9	23.2	3.5	0.48	CC	116.9	24.1	3.6	0.43
Glucose 120	AG+GG	113.0	17.4	3.4		ID+II	121.6	21.8	5.1		CT+TT	122.2	18.5	4.6	
	AA	137.1	103.3	17.5	0.71	DD	131.6	109.3	16.7	0.28	CC	141.5	123.7	18.4	0.96
Insulin 120	AG+GG	148.4	129.3	25.4		ID+II	166.6	125.0	29.5		CT+TT	143.2	85.5	21.4	

All glucose levels were expressed as mg/dL and insulin levels were expressed as mU/mL

As for the UCP3, the gene is selectively expressed in human skeletal muscles, a major site of thermogenesis and substrate oxidation, which makes this gene an attractive candidate for studies of energy metabolism and body weight regulation. Recent evidence has suggested that UCP3 plays an important role in modulating the use of lipid and glucose as an energy substrate.<sup>27</sup> *UCP3-55C/T* polymorphism is the most well-documented one. Studies exploring the association of *UCP3-55C/T* genotype and BMI have provided conflicting results. Although no association was reported between *UCP3-55C/T* polymorphism and obesity, in some reports concerning adults, -55TT genotype of UCP3 was mentioned to have both higher BMI and lower BMI.<sup>28-31</sup> No significant association was reported between *UCP3-55C/T* polymorphism and childhood obesity in this study.

Gul et al.<sup>32</sup> studied *UCP1-3826A/G*, *UCP2 Ins/Del*, and *UCP3-55C/T* variants in 268 obese and 185 non-obese Turkish children and reported that the G allele was more frequent in obese subjects with hypertriglyceridemia. Although it has been reported that the polymorphisms of *UCP2 Ins/Del* and *UCP3-55C/T* do not influence the obesity risk, the Ins allele was associated with low HDL levels. In our study, we did not find any relation between UCP polymorphisms and lipid levels in Turkish children.

OGTT provides a dynamic view of glucose and insulin physiology and has been widely used for decades to diagnose diabetes.<sup>33</sup> The 30<sup>th</sup>-minute insulin levels in the test show the first-phase insulin secretion which can be an index of beta-cell function. In a recent study concerning adults,<sup>34</sup> it has been reported that, after glucose ingestion, individuals with insulin-resistance and normal glucose tolerance secreted even more insulin in the early phase (30<sup>th</sup>-minute measurement in OGTT). Therefore, we have investigated the association between UCP genotypes and post-challenge glucose-insulin levels during OGTT. Our results may indicate that the 30-minute post-challenge insulin levels were significantly higher in obese children who

were Ins allele carriers in *UCP2 Ins/Del*. Csernus K. et al.<sup>35</sup> reported that in obese Hungarian children the *UCP2 Ins/Del* polymorphism was associated with a higher degree of obesity, insulin resistance (higher 0 and 120-minute insulin levels during OGTT), dyslipidemia and lower adjusted metabolic rate. The Ins allele of the *UCP2 Ins/Del* polymorphism was associated with worse indices of obesity, insulin resistance, and dyslipidemia. They did not measure insulin levels at 30, 60 and 90 minutes. Although we did not find any relation between the 120<sup>th</sup>-minute insulin levels with Ins allele carriers of the UCP2 gene, 30-minute post-challenge insulin levels were found to be higher.

In conclusion, based on our data from a single-center, we propose that *UCP2 Ins/Del* polymorphism has a significant role in glucose-insulin dynamics. Obese children who are Ins allele carriers of the UCP2 gene have a higher risk for exaggerated early-phase insulin response to glucose. Further studies with other genes are required to be carried out to understand the molecular mechanisms of obesity.

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