

Association of the UCP2 –866G/A polymorphism with type 2 diabetes and obesity in Saudi population

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ABSTRACT

Background: Diabetes mellitus is emerging as a major public health problem all over the world particularly Saudi Arabia. Recent studies reported that Uncoupling Protein 2 (UCP2) was associated with obesity and type 2 diabetes (T2D).

Aim of the Study: This study was conducted to clarify the contribution of polymorphism in UCP2 in obesity and T2D in the Saudi population.

Subjects and Methods: The distribution of the –866G/A polymorphism was examined in a case-control study including samples from 110 obese patients, 81 T2D patients, 96 obese-T2D patients and 100 healthy unrelated Saudi subjects. The –866G/A polymorphism were determined by using PCR/RFLP (polymerase chain reaction/restriction fragment length polymorphism) techniques.

Results: The results of this study showed that the frequency of the GG genotype was significantly higher in both obese and T2D patients (p -value= 0.0001, p -value= 0.014, respectively) compared with healthy control. The G allele was significantly associated with increase risk of obesity (odd ratio, OR: 3.3; 95% confidence interval, CI: 1.37-7.98), but not with T2D (OR, 1.97; CI, 0.80-4.87). In obese-T2D patients group, no significant correlation with –866G/A polymorphism (p = 0.067; OR, 1.21; CI, 0.25-2.80). This unrevealed study suggested that the G allele of UCP2 –866G/A polymorphism was related to obesity, which indicated the possible role of this polymorphism in causing metabolic syndrome.

Conclusion: This study concluded that the G allele of UCP2 –866G/A polymorphism might be related to obesity and T2D which might be used as a predictive marker for obesity and T2D.

Key Words:

T2D, Obesity, Uncoupling protein 2 (UCP2) gene, –866G/A polymorphism, Saudi population.

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INTRODUCTION

Type 2 diabetes (T2D) is one of the most common is a health-care problem worldwide affecting both industrial and developing nations. A clear link has

been made between genetic defects and the less common monogenic forms of diabetes, such as maturity onset diabetes of the young (MODY) and neonatal

diabetes^{1,2}. Conversely, the genetics underlying T2D is multifactorial and complex in nature³ with several genes simultaneously involved.⁴

Uncoupling protein 2 (UCP2) belongs to the superfamily of the mitochondrial transporter proteins and is highly expressed in adipose tissue and pancreatic islets⁵. Although UCP2 does not contribute substantially to adaptive thermogenesis in humans, it may play an important role in the regulation of fuel metabolism by direct and indirect actions in the adipose tissue, skeletal muscle and the endocrine pancreas⁶. Moreover, *UCP2* inhibits lipogenesis in the adipose tissue⁷ and insulin secretion in pancreatic islets.⁸

In the Goto-Kazizaki rat, the *UCP2* gene is linked to D1Wox38 marker (at 87.9 cM of the rat chromosome 1)⁹, which is located in a region where a relevant number of linkage peaks for glucose intolerance and adiposity have been identified.

In humans, the 11q13 chromosomal region (which contains the *UCP2* gene) is weakly linked to T2D in Finns¹⁰, but not in other populations¹¹. In Finns, by a genome scan performed at an average resolution of 8 cM, the most relevant logarithm of the odds (LOD) score peak in 11q13, lies about 8 cM from the *UCP2* gene locus¹⁰. Taken together, it is apparent that the *UCP2* gene is an excellent candidate for T2D.

Several *UCP2* gene variants have been reported in human studies: A G/A mutation in the promoter region –866G/A^{12,13}, a valine-alanine substitution at amino acid 55 in exon-4 (Ala55Val)^{14,15} and a 45-base-pair insertion and deletion in the untranslated

region of exon-8^{16,17}. The association between these polymorphisms in *UCP2* and various aspects of obesity has been studied intensively. A functional –866G/A single nucleotide polymorphism (SNP) has been described in the *UCP2* promoter¹². The –866A allele has been reported to increase *UCP2* transcriptional activity in transfected cultured cells¹³. However data in human tissues have been conflicting, reporting either increased¹³, or decreased¹⁸ *UCP2* mRNA levels in association with the –866A allele.

The present study aimed to evaluate the association of the common –866G/A SNP of the *UCP2* gene in T2D subjects with and without obesity of Saudi origin.

SUBJECTS AND METHODS

Study Population:

Both cases and controls were of Saudi origin and resident in the Western regions. For the sake of accuracy, this study focused only on those subjects for whom specific clinical information was available. Cases were 387 selected from 491 patients. Permission to perform this study was obtained from the ethical committee of the Faculty of Medicine, Umm Al-Qura University-Makkah. Informed consents from the cases to obtain the data used in this study were taken. The present study comprised three groups of unrelated patients: 81 T2D, 110 obese and 96 T2D-obese. Patients with T2D were consecutively recruited who met the following criteria: 1) diabetes diagnosed after 25 years, 2) insulin treatment not required for at least 2 years after diabetes diagnosed and 3) absence of clinically evident autoimmune disease. Obese

subjects considered if their body mass indexes (BMI) ≥ 30 kg/m² (according to American Obesity Association 2005; (<http://www.obesity.org>). In addition, age-matched healthy volunteers consist of 100 subjects were randomly selected.

Selection criteria of control group were as follows: fasting plasma glucose < 6.1 mmol/L, no medications known to affect glucose and lipid metabolism, and absence of systemic diseases. They did not have a family history for T2D or obesity at first degree relative. Patients,

who were under certain medication (e.g. cortisone) which could be the reason for their obesity, or could lead them to diabetes, were excluded from the study at time of sampling. In addition, patients who had endocrinal disorders that affected their weights (e.g. Cushing disease, hypothyroidism) and the female volunteers who had polycystic ovarian syndrome (PCOS) were also excluded. The clinical features of T2D, obese and T2D-obese, besides the healthy control group of the Saudi subjects \pm standard deviations (SD) were showed in Table 1.

Table 1: Characteristics of phenotypic parameters: Means of age and body mass index (BMI) \pm SD.

| Parameter | Age range (y) (mean \pm SD) | BMI range (mean \pm SD)(kg/m ²) | Female/Male |
|------------------|-------------------------------|---|-------------|
| T2D | 27–67 (51.1 \pm 10.0) | 18–30 (25.3 \pm 2.6) | 30/51 |
| Obesity | 20–67 (32.3 \pm 10.0) | 30–65 (36.2 \pm 5.6) | 29/81 |
| Obesity-T2D | 20–74 (49.7 \pm 10.5) | 30–59 (35.1 \pm 4.7) | 48/48 |
| Healthy Controls | 20–80 (35.3 \pm 9.0) | 18–25 (23.0 \pm 1.9) | 27/73 |

DNA Extraction:

Genomic DNA was extracted from blood lymphocytes using SpinColumn genomic DNA kit (BioBasic Inc., Ontario, Canada).

PCR Assay:

Previously described primer sets¹² were used to amplify regions around the UCP2 –866G/A promoter region (forward primer, 5'-CACGCT-GCTTCTGCCAGGAC-3' and reverse primer, 5'-AGGCGTCAGGAGATG-GACCG-3'). Genomic DNA (200 ng) was added to a 15- μ l reaction mixture containing 0.5 μ M of each primer with 200 μ M of each dNTP's, 67 mM Tris-HCl, 16 mM (NH₄)₂SO₄, 0.01% Tween-20, 1mM MgCl₂, and 0.45 U *Taq*

DNA polymerase. Amplification conditions were 95°C for 5 min followed by 30 rounds of 95° for 30s, 68° for 30s, 72° for 45s. A final extension of 72° for 5 min was included.

Genotype Analysis:

Following amplification, PCR amplicon (5 μ l) was incubated with 5 U of *Mlu*I (New England Biolabs) at 37°C for 2 h. The digested PCR fragments were separated on 3% MetaPhor gel (BMA Bioproducts Inc., USA), visualized by ethidium bromide staining and recorded by Gel Documentation and Analysis System (UVitec, Cambridge, UK). The – 866GG genotype was cleaved into two fragments (290- & 70-bp), whereas the AA genotype lack the recognition site and gave rise to a

fragment at 360 bp (Fig. 1). Automated sequencing was used (ABI PRISM 310 Genetic Analyser (Perkin Elmer-ABI, Warrington, UK)) to confirm validation of digest assay and to select positive control samples. Homozygous uncut (AA), heterozygous (GA) and homozygous cut (GG) were determined.

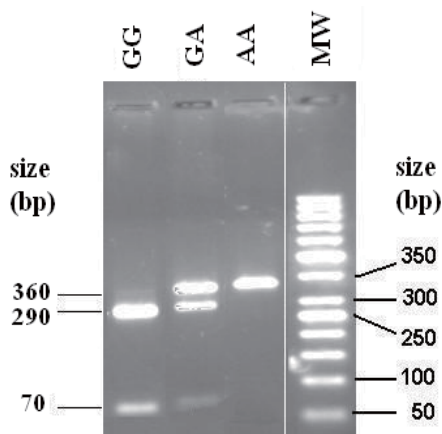


Fig. 1: Gel patterns of *UCP2* –866G/A polymorphism. The *Mlu* I restriction enzyme differentiated the *UCP2*–866G/A polymorphism. Lane 1, represents the GG genotype due to the complete cutting the PCR fragment (360 bp) into 2 fragments (290 and 70 bp). The AA genotype in lane 3 shows the uncut PCR fragment (360-bp), while the heterozygote GA genotype was in lane 2. MW is a 50 bp ladder (lane 4) was separated on 3% agarose gel.

Statistical Analysis:

The frequency distribution of allele and genotypes in the studied population were compared statistically using the Chi-squared test (Minitab version 12.1, Minitab Inc., Coventry, UK). Hardy-Weinberg analysis was done using CLUMP software version 1.2. The odd ratio (OR) for *UCP2* marker was calculated by using STATA 1.0 software. T-test (Minitab version 12.1, Minitab Inc., Coventry, UK) was used to determine if there is a difference between groups regarding the age and BMI.

RESULTS

The inherited genotypes –866G>A in the promoter region of the *UCP2* gene were determined. Genotype and allele frequencies of *UCP2* gene in Saudi subjects were shown in (Tables 2 and 3). In the diabetic group, 45% had the GG genotype, compared with 25% in healthy controls. There was a statistically significant difference between these two groups (p-value = 0.014) and the odd ratio (OR) for risk of developing T2D in patients having GG genotype was 1.97 (95% CI: 0.80-4.87) as shown in (Table 2).

Table 2: Genotype determining risk of *UCP2* –866G>A among obesity, T2D and obese-T2D Saudi subjects.

| Populations | n | UCP2 –866G/A genotypes Subjects (%) | | | p-value* | OD (95% CI) |
|----------------|-----|-------------------------------------|-----------|-----------|----------|-----------------|
| | | A/A (%) | G/A (%) | G/G (%) | | |
| Obese | 110 | 12 (11) | 36 (33) | 62 (56) | 0.0001 | 3.3(1.37-7.98) |
| Diabetic | 81 | 12 (15) | 33 (40) | 37 (45) | 0.014 | 1.97(0.80-4.87) |
| Obese/diabetic | 96 | 19 (20) | 41 (43) | 36 (37) | 0.067 | 1.21(0.52-2.80) |
| Control | 100 | 16 (16.0) | 59 (59.0) | 25 (25.0) | - | - |

* p-value was considered as significant if it is <0.05.

UCP2 genotypes in T2D/Obesity in Saudi population

A similar finding was also found in the allele frequency, where the OR for risk of developing T2D in patients having G-allele was 1.6 (95% CI: 0.90- 2.79) (Table 3). A similar pattern was seen also in the obese group, where the frequency of the GG genotype was 56%, in obese group 25% in healthy controls

(p-value = 0.0001). The OR for risk of developing obesity in patients having GG genotype was 3.3 (95% CI 1.37-7.98) (Table 2). A similar finding was found in the allele frequency and the OR for risk of developing was 2.3 (95% CI: 1.28- 4.16) in those patients having G-allele (Table 3).

Table 3: UCP2 –866G>A alleles determining risk in obesity, T2D and obese-T2D.

| Populations | n | UCP2 –866G/A Alleles | | OD (95%, CI) |
|----------------|-----|----------------------|----------|-----------------|
| | | G-allele | A-allele | |
| Obese | 110 | 0.73 | 0.27 | 2.3 (1.28-4.16) |
| Diabetic | 81 | 0.65 | 0.35 | 1.6 (0.90-2.79) |
| Obese/diabetic | 96 | 0.59 | 0.41 | 1.2 (0.7-2.15) |
| Control | 100 | 0.54 | 0.46 | |

The GG genotype frequency showed no statistical significant differences between healthy and obese-diabetic groups (25% and 37 %, retrospectively; p-value = 0.067). The OR for risk of developing obesity and T2D in patients having GG genotype was 1.21 (95% CI: 0.52-2.80) (Table 2). A similar finding was found in the allele frequency, where the OR for risk of developing obesity and T2D in patients having G-allele was 1.2 (95% CI: 0.7-2.15) (Table 3).

DISCUSSION

Obesity and T2D are major health problems all over the world¹⁹. Both conditions are considered as a polygenic disorder in which environmental factors interact with genetic variation in the predisposition to these diseases²⁰⁻²². Identification of candidate genes responsible for obesity and T2D is an important to understand the molecular mechanism underlying these conditions. The *UCP2* which expressed in pancreatic β -cells and adipose tissue, has led to the suggestion that such

molecule may be able to play an important role in the etiology of obesity and T2D.^{12,23}

Previous studies have reported that there is an association between – 866G/A polymorphism in the promoter region of *UCP2* with obesity and T2D.^{11-13, 24-31} This is the first study trying to identify the association between –866G/A polymorphism in *UCP2* with obesity and T2D in Saudi population. The results of this study showed that the frequencies of GG genotype were significantly higher in obese populations compared to the control. The risks of developing obesity in individuals having GG genotype was increased 3-fold than in individuals having other genotypes. These results are consistent with previous findings in which a higher frequency of G allele in obese population was founded^{12,13}. Esterbauer et al.¹² and Krempler et al.¹³ have previously reported that the G allele was associated with reduced adipose tissue mRNA expression *in vivo*, reduced transcriptional activity *in vitro* which increased the risk of obesity.

However, other studies showed no association of this polymorphism with obesity.¹¹ The results of this study showed that the frequencies of GG genotype were significantly higher in T2D populations compared to the control. The risks of developing T2D in individuals having GG genotype was increased 2-fold than individuals having other genotypes^{18,32}. A lack of association between obesity and T2D according to *UCP2* polymorphism genotypes was observed in this study. Where the results of this study showed no statistically significant association between G allele and obese-T2D population, although the G allele was most frequent in this population. This finding might be due to sample size or this polymorphism might be modified by differences in environmental factors such as caloric intake which make the effect of the polymorphism less pronounced than in other populations. Moreover, it could be due to the presence of other polymorphism in the promoter region of *UCP2* or close located gene which might have alternated the effect of this polymorphism. There has been relatively little pharmacogenetic analysis in Arab populations. This study was extended to include the *UCP2* polymorphism analysis in different ethnic populations that were previously reported and its correlation with the results of this study.

The results of this study demonstrated that the frequency of the G allele was much higher in Saudi obese and T2D individuals than in the other ethnic groups^{11-13,24-28,30,31}. The differences in frequency distribution of G allele among different ethnic populations indicated the importance of ethnic studies for this polymorphism. Therefore, it is important to determine the association of the -866G/A polymorphism in

the promoter region of *UCP2* with the T2D, obesity and obesity-T2D in Saudi population.

CONCLUSION

G allele in the promoter region of *UCP2* might be associated with increase risk of obesity and T2D in Saudi population. These data suggest the possibility of using *UCP2* as a predictive marker to identify those people at high risk of developing obesity and T2D in the future. Further studies with larger population are required to confirm this finding.

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