Original Article

Association Study of the -866G/A UCP2 Gene Promoter Polymorphism with Type 2 Diabetes and Obesity in a Tehran Population: A Case Control Study

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Abstract

Background: A functional polymorphism in the uncoupling protein 2 (UCP2) gene promoter has been associated with obesity and type 2 diabetes (T2D) in some populations. The impact of UCP2 polymorphisms on diabetes and obesity is still under debate. Contradictory results have been reported in different populations world-wide. To clarify the contribution of the UCP2 gene -866 G/A polymorphism in the Iranian population, we studied its association with obesity and T2D.

Methods: A total of 225 unrelated subjects were studied: 75 T2D patients without obesity, 75 obese patients without diabetes and 75 control subjects. The UCP2 -866 G/A polymorphism was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results: In the normal Iranian population, GG polymorphism was significantly associated with an increased HDL-C level (*P*=0.027). G/A polymorphism was not associated with obesity and T2D in our study population, but the odds ratio (OR) between GG and G/A polymorphism was 0.61 with a confidence interval (CI) range of 0.34 – 1.08 in obese patients. Subjects with AA genotypes in all of the studied groups showed a lower body mass index (BMI) than subjects with the GG genotype.

Conclusion: Although the data in our study population is not statistically significant, the A allele in the UCP2 gene promoter seems to be protective against obesity. This may suggest the possibility of UCP2 as a target molecule for studies on the etiology and treatment of obesity.

Keywords: Iranian, -866G/A polymorphism, obesity, PCR-RFLP, type 2 diabetes, uncoupling protein-2

Introduction

Obesity and type 2 diabetes (T2D) are two common multifactorial diseases with a major burden on health care systems worldwide. A major focus on the genetics of these complex disorders studies the role of single nucleotide polymorphisms (SNPs) on the pathogenesis and complications of these diseases.

Uncoupling protein-2 (UCP2) is a recently identified member of the mitochondrial transporter superfamily. Identified and cloned in 1997, this gene is expressed in many tissues, including adipose tissue.[1–9] All members of this superfamily are located in the inner mitochondrial membrane. UCP2 mediates mitochondrial proton leakage, releasing energy stored within the proton motive force as heat that ultimately results in reduced ATP synthesis. Therefore, the UCP2 gene is a candidate gene for obesity as well as diabetes. Controversy in the effects of a common G/A polymorphism at position -866 of the UCP2 promoter has been shown in different populations and ethnicities.[3–8]

This SNP has been studied by Esterbauer et al. in the Austrian population.[3] They concluded that the wild-type common G allele is associated with reduced adipose tissue mRNA expression *in vivo*, reduced transcriptional activity *in vitro* and an increased risk of obesity in middle-aged humans.

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Among obese middle-aged humans in the Austrian population, the G allele has been shown to be associated with a reduced risk of T2D.[4] This was supported by demonstrating that UCP2 -866G/A polymorphism contributes to variations in insulin secretion in glucose-tolerant subjects in the Italian population.[5] However, Schauble et al. have shown that UCP2 -866G/A polymorphism was not associated with early-onset obesity in young German subjects.[6]

The UCP2 is an excellent candidate gene for T2D and obesity.[9] The distribution of UCP2 polymorphism in the healthy Iranian population is similar to Caucasians but different from the Japanese population.[10] In the present study, we evaluate the association of the common -866G/A SNP of the UCP2 gene with T2D and obesity in an Iranian population.

Materials and Methods

Patient populations

There were 225 unrelated Iranian subjects randomly selected for this study. Subjects volunteered to participate in a study design based on the Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (WHO MONICA) project from a Tehran urban district population.[11,12] This study was designed for the assessment of cardiovascular risk factors in Tehran inhabitants.[13] Cases were divided into two groups; 75 diabetic patients without obesity (34 females and 41 males) and 75 obese patients without diabetes (57 females and 18 males). Control and case groups were from the same community and all subjects were unrelated. There were no married couples.

The control group consisted of 75 normal unrelated subjects (41 females and 34 males) who were not diabetic or obese in the same age range (35 – 76 years) as the case group. Selection criteria for controls were as follows: fasting plasma glucose less than 110 mg/dL, no medications known to affect glucose and lipid metabolism, absence of systemic diseases and body mass index (BMI)<30. Clinical and laboratory data of the controls are shown in Table 1.

Diabetic patients met the following criteria: 1) diabetes diagnosed after 30 years, 2) insulin treatment not required for at least 2 years following diagnosis, 3) absence of clinically evident autoimmune diseases and 4) BMI<30. The demographic and laboratory data of the diabetic populations are shown in Table 2. The obese group had BMI>30 and normal fasting plasma glucose. Demographic data of the obese patients are shown in Table 3. All subjects enrolled in the study underwent physical examinations, including measurements of height, weight, and blood pressure. Venous blood of all fasting subjects was sampled from an antecubital vein for measurements of glucose, total cholesterol and high density lipoprotein cholesterol (HDL-C), triglyceride (TG), uric acid and homocystein by an Endocrinology and Metabolism Research Center (EMRC) Biochemistry Laboratory using routine methodology. All subjects provided informed consent. The study was performed according to the Helsinki Declaration and the protocol was approved by the Local Ethics Com-

Table 1. Demographic and laboratory data of control group according to genotype

Variable	Genotype			*P*-value
	-866 AA *n*=7	- 866 GA *n=41*	- 866 GG *n=27*	
Age	34.4±10.7	35.9±12.2	36.2 ± 12.1	0.857
Cholesterol (mg/dL)	194±47.9	198±43	163 ± 28.5	0.157
HDL (mg/dL)	64.7±20	58.3±15.7	45 ± 12.8	0.027
TG (mg/dL)	127±48.8	156±91.7	172 ± 139	0.283
Uric acid (mg/dL)	3.45±0.9	4.1±1.3	3.9 ± 0.7	0.053
FBS (mg/dL)	74.2±16.9	74.6±6.9	72.8 ± 6.9	0.875
Mean SBP (mmHg)	122±20	122±29.6	126 ± 15.7	0.911
Mean DBP (mmHg)	80±11.8	79.3±17.8	85 ± 11	0.677
Waist (cm)	84.6±10.4	84±10.6	80.3 ± 10.4	0.661
WHR	0.84±0.07	0.85±0.07	0.84 ± 0.07	0.912
BMI (kg/m^2)	25.2±2.8	24.8±2.9	23.4 ± 3	0.3

mittee. Sample size was calculated using the following formula:

$$n = [\frac{z_1 - \beta\sqrt{p_1(1-p_1) + p_2(1-p_2)} + z_1 - \frac{\alpha}{2}\sqrt{p(1-p)}}{p_1 - p_2}]^2$$

$$n = [\frac{z(1-0.2)\sqrt{0.3(1-0.3) + 0.5(1-0.5)} + z(_1 - \frac{0.6}{2})\sqrt{0.4(1-0.4)}}{0.3 - 0.5}]^2$$

n=60

P=(P1+P2)/2

P1=0.3 G/G frequency in diabetes group

P2=0.5 G/G frequency in control group3,4

The sample size was increased from 60 to 75 in all three groups.

Genotyping

Peripheral whole blood was collected in EDTA-containing tubes and genomic DNA was extracted by the salt saturated method.[14] The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used for SNP genotyping as reported by the Esterbauer study.[3] The sequences of the forward and the reverse primers were: 5'-CACGCTGCTTCTGCCAGGAC- 3' and 5'-AGGCGTCAGGAGATGGACCG- 3', respectively. The PCR reaction mixture contained 50 – 100 ng DNA, 0.16 µL dNTPs 25 mM, 1 µL of each of primers 10 µM, and 0.2 U Taq DNA polymerase (5 U/1 µL, Gibco BRL) in a 20 µL mixture. PCR

Table 2. Demographic and laboratory data of T2D group according to genotype

Variable	Genotype			*P*-value
	-866 GG *n*=29	-866 GA *n*=38	-866 AA *n*=8	
Age	52±9.6	51±11.6	54.6±13	0.818
Cholesterol (mg/dL)	203±48	203±53	221±76	0.685
HDL (mg/dL)	58.7±16	62.3±19.3	72.5±31	0.229
TG (mg/dL)	249±18.6	264±23.6	223±12.9	0.873
Uric acid (mg/dL)	4.3±1.5	4.19±1.73	3.69±1.29	0.620
FBS (mg/dL)	148.9±75	129.4±78	169.1±76	0.343
Mean SBP (mmHg)	125±22.4	137±19	134±17.1	0.867
Mean DBP (mmHg)	85±13.2	88.3±19.6	84±14.6	0.470
Waist (cm)	93.1±7.8	88.6±10.8	89.2±11	0.169
WHR	0.9±0.09	0.789±0.07	0.91±0.09	0.177
BMI (kg/m^2)	26.8±2.4	25.7±3	25.7±3.02	0.296
Homocystein* (µmol/L)	2.73±0.44	2.72±0.53	2.72±0.38	0.995

*Geometric Means±SD, HDL=high density lipoprotein; TG=triglyceride; FBS=fasting blood sugar; SBP=systolic blood pressue; DBP=diastolic blood pressue; WHR=Waist-to-hip ratio; BMI=body mass index

Table 3. Demographic and laboratory data of obese group according to genotype

Variable	Genotype			*P*-value
	-866 GG *n*=16	-866 GA *n*=48	-866 AA *n*=11	
Age	44.6±12.4	44.7±10	42.4±15.2	0.834
Cholesterol (mg/dL)	218±39	214±37.6	194±45.7	0.263
HDL (mg/dL)	63.5±15.5	63±14.9	59.2±19.6	0.747
TG (mg/dL)	201±14.2	177±11.3	206±19.4	0.717
Uric acid (mg/dL)	4.31±1.3	4.18±1.1	4.02±1.2	0.826
FBS (mg/dL)	80.8±9.4	80.3±9.6	83.5±12.5	0.631
Mean SBP (mmHg)	135±19.6	131±20.7	122±8.5	0.262
Mean DBP (mmHg)	86.8±12.3	86.4±12.9	82.5±10.7	0.617
Waist (cm)	98.2±14.1	98.1±11.9	102±9.9	0.636
WHR	0.89±0.07	0.86±0.08	0.89±0.07	0.34
BMI (kg/m^2)	34.8±3.1	33.6±3.6	33.7±3.2	0.538
Homocystein* (µmol/L)	2.7±0.4	2.6±0.5	2.8±0.6	0.806

* Geometric Means±SD; HDL=high density lipoprotein; TG=triglyceride; FBS=fasting blood sugar; SBP=systolic blood pressure; DBP=diastolic blood pressure; WHR= Waist-to-hip ratio BMI=body mass index

was performed as follows: DNA denaturation for 5 min at 96°C; 36 cycles of: 30 sec at 95°C, 30 sec at 68°C, 30 sec at 72°C, and a final extension for 7 min at 72°C. The PCR products were run on 1% agarose gel electrophoresis for visualization, then 3 µL of PCR product were mixed with 1.5 U *Mlu*I (10 U/1 µL, Invitrogen) and incubated overnight at 37°C. The digested fragments were separated on 2.5% agarose gel stained with ethidium bromide and visualized under ultraviolet light. The PCR product has a length of 360 bp and if the G allele is present, it is cut into two fragments (290 and 70 bp) by *Mlu*I. The UCP2 -866A allele was identified by a single band of 360 bp.

Data analysis

All statistical analysis was performed using the SPSS software program version 11.5 for Windows (SPSS, Inc., Chicago, IL) and Stata 8 Software Package (Stata Corporation, TX). Multiple comparisons between variables among different genotypes were calculated by the one-way ANOVA method and two to two comparisons were done by the Bonferroni post Hoc tests. Deviation of the frequency distribution of UCP2 -866G/A genotypes from the Hardy-Weinberg equilibrium was examined by Chi-square analysis for heterogeneity Data was expressed as the mean±SD and frequencies were expressed in proportions (percentages). A difference level below 5% was considered to be significant. The same analysis was used to model the effect of each single polymorphism and its effect on the risk of T2D and obesity with adjustment for sex, age and BMI by logistic regression analysis.

Results

The proportion of individuals carrying the three possible genotypes (-866GG, -866GA, and -866AA) was not significantly different between controls and T2D or obese patients in our study (Tables 4 and 5). Overall, no significant association between the UCP2 -866G/A genotype with T2D risk was observed [odds ratio (OR), 0.94; 95% confidence interval (CI), 0.54 – 1.71; *P*=0.882; age, gender and BMI adjusted].

No significant association between the UCP2 -866G/A genotype with obesity was observed (OR=0.61; 95%CI: 0.34 – 1.08; *P*=0.119; age, gender, and BMI adjusted).

No difference was observed across the three genotype groups for glucose, lipid profile, and homocystein levels in T2D patients and obese patients. Also, no difference in BMI was observed in controls (25.2±2.8, 24.8±2.9, and 23.4±3 in -866GG, -866GA, and -866AA individuals, respectively; *P*=0.3) and T2D subjects (26.8±2.4, 25.7±3, and 25.7±3.02 in -866GG, -866GA, and -866AA indi-

Table 4. Distribution of the UCP2-866 G/A polymorphism in T2D patients and controls

Genotype	Diabetes		Control		*P value	OR (95%CI)	Adjusted ˙OR (95%CI)
	n	٪	*n*	٪			
- 866 AA	8	10.7	7	9.3	0.882	1	1
- 866 GA	38	50.7	41	54.7		0.81 (0.26–2.45)	0.98 (0.24–3.94)
- 866 GG	29	38.6	27	36		0.94 (0.54–1.71)	0.85 (0.36–2.03)

*By χ^2 analysis (2×3) between obese patients and controls. Adjusted for age, gender and BMI.

Table 5. Distribution of the UCP2-866 G/A polymorphism in obese patients and controls

Genotype	Diabetes		Control		*P value	OR (95%CI)	Adjusted ˙OR (95%CI)
	n	٪	*n*	٪			
- 866 AA	11	14.7	7	9.3	0.119	1	1
- 866 GA	48	64	41	54.7		0.74 (0.26–2.09)	0.77 (0.24–2.48)
- 866 GG	16	21.3	27	36		0.61 (0.34–1.08)	0.59 (0.31–1.13)

*By χ^2 analysis (2×3) between obese patients and controls; ˙Adjusted for age, gender

viduals, respectively; *P*=0.296).

In controls, HDL-C values of 64.7±20, 58.3±15.7 and 45±12.8 mg/dL were observed in -866GG, -866GA, and -866AA individuals, respectively (*P*=0.027). The GG genotype was associated with high serum HDL-C levels in the control groups.

Discussion

UCP2 is one of the candidate genes in diabetes and obesity. This gene is expressed in many tissues such as fat tissue and pancreatic β cells and plays a role in body metabolism regulation as well as insulin secretion. Animal studies have shown the role of UCP2 in insulin secretion. UCP2 knock-out mice show increased ATP levels and insulin secretion.[9] Human studies of the UCP2 gene promoter, T2D, and obesity have shown controversial results among different populations.[3–8]

As diabetes and obesity have a multifactorial inheritance, more studies must be done to clarify the role of different genes and SNPs. To the best of our knowledge, this is the first association study between the UCP2 gene, obesity, and T2D in an Iranian population. In this study, the associations between the UCP2 gene, T2D, and obesity in addition to risk factors of cardiovascular events were analyzed.

D'Adamo et al. did not detect any association between BMI, waist-hip ratio (WHR), cholesterol, HDL-C and TG, and G/A polymorphism in a diabetic Italian population.[15] This finding was similar to our results.

In the obese group, there was no association between age, BMI, WHR, lipid profile, and G/A polymorphism. Serum HDL-C levels were higher in the GG genotype but were not statistically significant. Mancini et al. did not report any association between G/A polymorphism and lipid profile, fasting plasma glucose and BMI in an obese Italian population[16] which was compatible with our study.

Distribution of G/A polymorphism between the normal and diabetic groups was not statistically significant in our population. The OR between AA and GG was 0.94 (0.54 – 1.71), which suggests a lack of association between G/A polymorphism and T2D in our study population.

D'Adamo et al. studied the association of G/A polymorphism with T2D and found that the AA genotype was associated with an increased risk of T2D in women.[15] In another study, Bulotta et al. have reported that the UCP2 polymorphism is associated with T2D risk and the A allele renders a protective effect on the risk of T2D.[17] This is not compatible with the D'Adamo study conducted in the same country, and none of the above mentioned data was confirmed by our study. The UCP2 -866G/A SNP might have no effect per se on the risk of T2D; rather, it may represent only a marker in linkage disequilibrium with another causative genetic variant(s).

An intriguing possibility to partially explain some of these inconsistencies is that the contribution of the UCP2 -866G/A SNP to T2D risk may be different in distinct samples depending on their genetic backgrounds. Bulotta et al. studied PPARγ2 P12A genotypes. The risk of T2D for subjects carrying the UCP2 -866GG genotype was significantly increased in PPARγ2 P12/P12 homozygous subjects, whereas no effect was observed in individuals who carried the PPARγ2 A12 variant.[17] There is the possibility of a combined effect of the two genes on the risk of T2D.

Distribution of G/A polymorphism between the normal and obese groups has no statistical significance and the OR between AA and GG is 0.61 in our study population (0.34 – 1.08). A number of sequence variants of the UCP2 gene exist, but only the -866 G/A polymorphism seems to be important for obesity. Dalgaard et al. have investigated the variants of this gene for association with changes in BMI or body fat content in three different Danish populations. No association between these variants and increased BMI, body fat percentage, or weight gain was found in their study.[18] Otabe et al. did not detect any polymorphisms in the coding region of the UCP2 gene associated with morbid obesity nor did they detect an association between G/A polymorphism and obesity in French Caucasians.[19] Lack of association between other UCP2 polymorphisms and obesity was detected in an Italian Caucasian population that studied morbidly obese patients.[20] In contrast, Esterbauer et al. found that the AA genotype was associated with a lower BMI.[3] Our finding is similar with a lower BMI in the AA group but with no statistical significance. Based on our data, we can consider a slight protective effect rendered against obesity in the A allele.

We have shown that the G/A SNP at position -866

in the promoter region of UCP2 has no association with TG, total cholesterol and LDL cholesterol values in T2D subjects.[10] However, GG polymorphism was significantly associated with an increased HDL-C level (P=0.027) in our normal study population. Multiple factors are involved in the dyslipidemia associated with T2D, including the effects of insulin on liver apo-protein production, actions of cholesteryl ester transfer protein, the regulation of lipoprotein lipase and hormone-sensitive lipase, and other peripheral actions of insulin on adipose tissue and skeletal muscle.[21]

Reis et al. have reported a common polymorphism in the promoter region of the UCP2 gene that modulates TG and cholesterol levels in French Caucasian T2D subjects.[22] Homozygosity for the G-allele, which is found in 39% of this population, is associated with a protective effect against both high TG, high total cholesterol and LDL cholesterol levels. Conversely, the presence of the A allele either in the homozygous or heterozygous form is associated with an unfavorable effect on these parameters. In our diabetic group, there was no association between G/A polymorphism and lipid profile. Cha et al. found that -866G/A SNP accounted for 8.09% of the variation in serum HDL-C levels independent of BMI. They concluded that this result may provide clues to the association of the UCP2 gene with a risk of atherosclerosis through the effects on HDL-C.[23] Our data shows an agreement with their results.

Small sample size is a limitation of our study. We recommend further studies with larger sample sizes in order to have a better understanding on the effect of this SNP in diabetes and obesity.

Conclusion

In the Iranian population, there are no associations between -866G/A polymorphism in the UCP2 gene promoter with T2D. In obese patients, the A allele has some protective effect for obesity but was not statistically significant. Further studies may be indicated with larger sample sizes and linkage disequilibrium with other genetic variants must be considered, such as the PPARγ2 gene.

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