Progress in Nutrition 2017; Vol. 19, N. 4: 430-435 DOI: 10.23751/pn.v19i4.6124

Original Article

UCP2 866 G/A gene (rs659366) polymorphism associated with diabetes type 2 in Turkish population

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Summary. Type 2 diabetes mellitus (T2DM) is a major health problem in worldwide and uncoupling protein-2 (UCP2) that have a role in the regulation of insulin secretion and emergence of diabetes mellitus. In this study we have investigate whether an association between UCP2 866 G/A polymorphism and T2DM. In this study we have collected peripheral blood samples from 50 type 2 diabetes mellitus patients and 50 healhty control individuals. Genomic DNA was isolated from blood samples and UCP2 866 G/A polymorphism was investigated by polymerase chain reaction restriction fragment lenght polymorphism assay (PCR-RFLP). In our results we have not observed any statistically significant association between UCP2 866 G/A polymorphism and risk of T2DM.

Key words: diabetes mellitus type 2, UCP2 gene, polymorphism

Introduction

Diabetes mellitus is a chronic disease that is characterized by insulin secretion and action disorders and hyperglysemia. T2DM is a complex and multifactorial metabolic disease which is characterized by failure of insulin secretion of βcells in the pancreas and resistance or reduced response of peripheral tissues to insulin. Regardless of other known genetic or environmental risk factors, obesity is a major risk factor in the emergence of the T2DM. Obesity is a prevalent characteristic in the patients who has T2DM and it appears approximately 80% of patients. In addition to this decreasing of energy expenditure could increased T2DM risk in the obese people (1). Uncoupling proteins (UCPs) regulate proton gradient and ATP synthesis in the mitochondrial membranes and they works as a membrane transport proteins. UCP2s has shown widespread distribution in humans such as adipose tissue, skeletal muscle, kidney, pancreas, heart, placenta, liver, and brain tissues.

It has been shown that UCP2 has neuroprotective and neuromodulatory effects in the central nervous system and it was shown that UCP3 has a neuron protective effects against to glucose induced degeneration via preventing of reactive oxygen species (ROS) formation (2-4). Because of this important functions UCP gene polymorphisms were evaluated frequently in some diseases such as body composition and resting energy expenditure (5), energy metabolism (6), obesity (7, 8), multiple sclerosis (9, 10), diabetic neuropathy (11, 12), coronary artery disease (13) and schizophrenia (14). Studies have shown that UCP2 has a very important role in the continued clearance of apoptotic cells (15) and also UCP2 can affect the function of β cells (16-18). It was also shown that UCP2 gene expression level is affected by glucose metabolism in pancreatic islets of mice (19) for this reason overexpression of UCP2 causes decreased insulin secretion (20). Some studies have been shown that micro RNAs have some regulatory effects on UCP2 gene expression. For example, microRNA-15a positively regulates insulin

secretion via inhibition of UCP2 expression in mouse cells (21). The 866 G/A (rs659366) polymorphism is existed in the promoter region of UCP2 gene and this polymorphism effect binding of the transcription factors IPF1 and PAX6 (22). Many studies have examined association between UCP2 gene polymorphism and risk of T2DM. Most of them have focused on UCP2 866 G/A polymorphism and contradictory results were reported (23- 33). The aim of this study was to investigate association of the 866 G/A polymorphisms in the UCP2 gene with T2DM risk in Turkish population.

Materials and Methods

Participants

The study was approved by the ethics committee of Firat University Medical Faculty (ethics committee date/number 16.02.2016 / 04-05). A total of 50 patients with T2DM were consecutively recruited who met the criteria of World Health Organization and followed up in the Internal Medicine Department of the Firat University Hospital in Turkey. Age matched healthy volunteers consist of 50 individuals were randomly selected. Fasting plasma glucose <6.1 mmol/L, no medications which affect the glucose and lipid metabolism, and absence of systemic diseases and no family history for T2DM at first degree relatives were used as selection criteria for control group.

Genotyping analysis

Blood samples were taken from all the participants into the tubes which containing ethylenediamine tetraacetate (EDTA). DNA was extracted with commercially available genomic DNA isolation kit (Promega Corporation, Madison, WI) according to the manufacturer's recommendations. Afterwards quality control of DNA samples were performed by Nanodrop UV spectrophotometer (UV-Visible NanoDrop 1000, Thermo Fisher Scientific Inc.) and concentrations were adjusted to 50 ng μL-1 and all DNA samples were stored at -20°C until analysis of the UCP2 polymorphism. The 866 G/A single nucleotid polyomophism was genotyped by PCR-restriction fragment length polymorphism (RFLP) with the following primers: 5'-CAC GCT GCT TCT GCC AGG AC-3' (forward) and 5'-AGG CGT CAG GAG ATG GAC CG-3' (reverse) (33). The PCR conditions were: initial denaturation at 95°C for 5 min; followed by 35 cycles of 95°C for 30 seconds, 65°C for 40 seconds, 72°C for 50 seconds, a final extension of 72°C for 5 minutes. The PCR products were digested at 37°C for 4 hours with 5.0 U of HaeIII restriction enzyme (Promega). After enzymatic digestion, PCR products were loaded in the 3% of agarose gel and visualized by SYBR Safe staining.

Statistical analysis

Statistical analyses were performed with SPSS software version 21 (SPSS Inc. Chicago IL USA). The genotype distribution was tested for Hardy-weinberg equilibrium with chi-square (χ^2) test in T2DM patients and controls. The student *t*-test was used to compare differences in the clinical characteristics between the T2DM and non diabetic control groups, $p< 0.05$ was considered to be statistically significant. The distributions of 866 G/A polymorphism between T2DM patients and control groups were compared using the Fisher's exact test. $p< 0.05$ was considered significant.

Results

We have studied UCP2 866 G/A (rs659366) gene polymorphism on T2DM patients in Turkish families. Clinical characteristics of subjects are summarized in Table 1. The statistical analysis showed that BMI (kg/m^2), Creatinine (mg/dL), Urea (mg/dL), diastolic blood pressure (mmHg), sistolic blood pressure (mmHg) levels of T2DM patients were significantly higher than those of the control group ($p<0.05$) (Table 1). These results suggested that BMI, creatinine, urea, diastolic blood pressure, sistolic blood pressure were independent risk factors for T2DM patients in the Turkish population. Also the statistical analysis showed that sex, age, AST, ALT values of T2DM patients were not significantly higher than those of the control group ($p>0.05$) (Table 1). Totally, 50 subjects with T2DM and 50 controls were enrolled in our study. In this study, the genotype distributions of all groups were found in Hardy-Weinberg equilibrium. Genotypes and alleles frequencies of the 866 G/A polymorp-

Table 1. Clinical characteristics of the patient and control groups.

Clinical characteristics	Patient	Control	p value
Sex (n) (Male/Female)	20/30	13/37	0.614
Age (years)	58.20 ± 13.06	33.32 ± 12.88	0.754
Duration of T2DM (years)	11.10 ± 7.78		
BMI (kg/m^2)	29.66 ± 6.60	25.67 ± 6.38	0.018
AST (U/L)	23.36 ± 8.71	21.62 ± 4.64	0.962
ALT (U/L)	23.54 ± 9.11	20.72 ± 8.94	0.761
Creatinine (mg/dL)	0.83 ± 0.42	0.64 ± 0.15	0.041
Urea (mg/dL)	39.16 ± 18.06	24.98 ± 7.44	0.014
Diastolic blood pressure (mmHg)	70.50 ± 8.40	68.10 ± 8.10	0.023
Sistolic blood pressure (mmHg)	111.90 ± 11.64	105.90 ± 14.55	0.037

± standard deviation

hism of UCP2 gene in T2DM patients and controls are shown in the Table 2.

In the T2D, GG genotype was found in 26 patients (52%), GA genotype in 23 (46%) patients, AA genotype in 1(2%) patient and significant differences were noted in comparison with the frequencies in the genotype subjects ($p<0.05$, Table 2). In the control group GG genotype was found in 19 (38%) subjects, GA genotype in 28 (56%) subjects, AA genotype in 3 (6%) subjects and significant differences were noted in comparison with the frequencies in the genotype subjects ($p<0.05$, Table 2). The frequency of the G allele in T2DM patients was 0.75, while A allele frequency was 0.25, and significant differences were noted in comparison with the frequencies between G and A alleles in the T2DM patients ($p> 0.05$, Table 2). The frequency of the G allele in control group was 0.66, while A allele frequency was 0.34, and significant differences were noted in comparison with the frequencies between G and A alleles in control groups ($p> 0.05$, Table 2).

Discussion

T2DM and its complications are complex diseases associated with both genetic and environmental risk factors (34, 35). T2DM a major public health problem in Turkey like the worldwide (35-37). The discovery that UCP2 is present in pancreatic β cells and adipose tissues has led to the suggestion that such molecules may be able to play an important role in etiology of T2DM (38, 39). Considering the important role of UCP2 in ROS formation in mitochondria, the relationship between UCP2 locus and susceptibility for T2DM and its complications has been investigated (40- 43). Over the past few years, many publications have suggested that there was an association between UCP2 866 G/A (rs659366) polymorphism and the risk of T2DM (40-44). The allele frequency analysis demonstrated that the A allele of rs659366 has not statistically significant higher frequency in T2DM compared with control group. Several studies exami-

Table 2. Genotype and allele frequency of the 866 G/A (rs659366) polymorphism of UCP2 gene patient and control groups.

	Genotype				Allele			
Subjects (n)	GG (%)	GA (%)	AA (%)	p	G (%)	A (%)	p	HWE (P)*
Control (50)	19 (38%)	28 (56%)	3 (6%)	0.0173	66	34	0.037	0.0018
Patients (50)	26 (52 %)	23 (46%)	1(2%)	0.0246	75	25	0.011	0.0021

** HWE (P) is the significance of correspondence to Hardy-Weinberg proportions according to chi-square test.*

ned the UCP2 866 G/A polymorphism in relation to T2DM with inconsistent results (25-31). Two studies (25, 29) found statistically significant associations. It is noteworthy that in the two above mentioned studies, the frequencies of the A allele in the controls were the lowest. But we have observed that A allele frequency was higher in our control group. Further studies are necessary to better define if the 866 G/A polymorphism has a synergistically effect on UCP2 gene expression. Alternatively, there is a possibility that the 866 G/A polymorphism is not themselves responsible for the observed association with T2DM only being a still unknown functional polymorphism. Nevertheless, previous studies indicate that the 866 G/A polymorphism could be directly leading to changes in UCP2 gene expression (45, 46). As a limitation of this study, the sample sizes of the experimental groups in this study were not large enough to evaluate a small impact from very low penetrance genes or single nucleotid polymorphism (SNPs). Further studies with larger cohorts of Turkish population are needed to clarify the etiopathophysiological and functional role of UCP2 gene expression on T2DM.

In conclusion, we examined the frequencies of one common polymophism in UCP2 rs659366 in the T2DM patients and also control subjects without T2DM. The UCP2 rs659366 polymorphism was not found statistically significant for relationship with T2DM. Some other SNPs in the UCP2 gene can play important role in the pathogenesis of T2DM in Turkish population. However, this is a preliminary study and further functional studies are required to determine the association of this polymorphism with T2DM.

Acknowledgements

The authors are acknowledge financial support by Firat University vide project No. BAP – TF. 16.1 for carrying out these study.

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