Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI: 10.26479/2019.0503.18**

CORRELATION OF 45BP I/D POLYMORPHISM OF UCP2 GENE WITH TYPE 2 DIABETES RECOGNITION IN A NORTH INDIAN POPULATION

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ABSTRACT: The objective of the study was to evaluate the association of 45 bp I/D (insertion/deletion) polymorphism in exon 8 of *UCP2* gene in diabetic patients of north Indian origin. A total of 850 subjects from Jammu and Kashmir region of India, including 425 diabetic (211 men and 215 women), and 425 non-diabetic (211 men and 215 women) were included in this case–control study. Anthropometry, fasting lipids, fasting glucose, HbA1c and BMI were estimated using standard protocols. Genotyping of *UCP2* gene polymorphism for all subjects was performed by PCR method. The frequency of ID (insertion-deletion) and II (insertion-insertion) genotypes of *UCP2* was found higher in diabetic subjects in comparison to controls. The levels of biochemical parameters chosen for diagnosis were genotype dependent except for insulin and HDL. We found that the genotypic & allele frequency of 45 bp I/D polymorphism (ID & II & I allele) was more in obese individuals & subjects above 50 years of age. The 45 bp I/D *UCP2* gene polymorphism appears to be an important genetic determinant in the progression of T2DM. This polymorphism may contribute to diabetes susceptibility in this population.

KEYWORDS: UCP2, T2DM, diabetes, insulin, HbA1c, BMI, PCR.

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1.INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by hyperglycemia and causes due to defects in insulin secretion and action as well [1]. Diabetes mellitus is a serious health issue in both developed and developing countries. T2DM is a condition of impaired glucose regulation due to dysfunctional pancreatic β-cells and insulin resistance [2]. The majority of T2DM patients (80–90%) are overweight living a sedentary lifestyle. These are the prime reasons for uncontrolled diabetes [3]. Insulin resistance is inversely proportional to body weight, thus insulin resistance of T2DM patients improves when they start to lose weight [4]. In T2DM patients lipid peroxidation, lipid levels [5] and antioxidant levels have been found to be altered [6]. Further, a decreased in vitamin D and an increase in cytokine levels was observed [7]. Uncoupling proteins (UCPs) are mitochondrial family proteins that are present on inner mitochondrial membrane and encoded by nuclear DNA. They play role in transporting the protons back into the mitochondrial matrix and thus dissipate the proton gradient, decrease ATP production and diminish superoxide production. Therefore, UCPs play major role in redox regulation, mitochondrial and metabolic processes [8,9]. Currently, delineating the role of uncoupling proteins and targeting them from therapeutic purposes is gaining focus of researchers worldwide. UCP2 is abundantly present in the mitochondria of skeletal muscle, adipose tissue, liver, spleen, lung, and macrophages [10]. It is often dysregulated in various metabolic conditions, lipid and fatty acid metabolism, glucose metabolism and transportation of TCA cycle metabolites [11]. Mutations in UCP2 gene are known to be associated with congenital hyperinsulinemia, obesity and aggressive cancers where UCP2 is overexpressed [12]. Its functions are tissue-dependent including regulation of fatty acid metabolism in skeletal muscle and white adipose tissue [13] and regulation of insulin secretion in pancreatic β-cells [14].

UCP2 locus has been linked to obesity, hyperinsulinemia and resting energy expenditure, suggesting that variation in UCP2 expression could influence the development of obesity and its associated metabolic disorders such as T2DM, hypertension and atherosclerosis [15]. The dysregulation of uncoupling proteins, which translocate protons into the mitochondrial matrix resulting in heat generation without ATP synthesis [16], may contribute to the pathogenesis of obesity & T2DM. The UCP2 insertion/deletion (I/D) polymorphism is a 45 bp insertion in exon 8 at 3'untranslated region of the UCP2 gene. Insertion/deletion of the 45 bp I/D polymorphism at 3'untranslated region (3'UTR) in the exon 8 UCP2 gene plays an important role in some metabolic diseases, causing changes in the rate of metabolism and increased body mass index (BMI) [17]. This polymorphism leads to the decrease of UCP2 protein expression and lower energy expenditure. It causes an imbalance of the ratio between intake and expenditure of energy that can lead to obesity and polymorphism depending on the population [18]. However, it has also been shown that the UCP gene cluster variation may not be useful predictor for type 2 diabetes mellitus (T2DM) risk assessment [19]. The present study evaluated the association of the 45 bp insertion/deletion

polymorphism of *UCP2* gene with T2DM in Kashmiri population of north India. We also observed the effect of this *UCP2* gene polymorphism on gender and age basis. The data analysis was performed to determine whether there are any associations with obesity, T2DM and age.

2. MATERIALS AND METHODS

This case-control study was conducted in the Department of Biochemistry, Govt. Medical College (Research Centre University of Kashmir). Study participants involved 825 subjects from Jammu and Kashmir region of India, including newly diagnosed 425 (211 men and 215 women) unrelated T2DM patients and 425 (211 men and 215 women) non-diabetic individuals/controls who came for normal routine checkup and did not fulfill any of the criteria used to diagnose diabetes. The diagnosis was made as per the ADA criteria 2010. Exclusion criteria were overt renal or hepatic diseases, autoimmune disorders, chronic use of steroids or non-steroidal anti-inflammatory drugs, statin therapy & subjects from non-Kashmiri origin. The study was approved by the Institutional Ethics Committee. All participants signed an informed consent.

Sample collection

Blood samples were collected from the subjects (cases and controls) in EDTA vials using standard protocol. The whole blood samples were separated into two vials, one part was used for DNA extraction/genotyping & the serum collected from the other part to measure basic biochemical parameters by standard chemical and enzymatic commercial methods in the Department of Biochemistry and hospital laboratory.

BMI calculation

Height (cm) was noted using a measuring tape to the nearest 0.1cm. Weight (Kg) was measured to the nearest 0.1 kg using a weighing machine simultaneously. Body mass index (BMI) was calculated as the ratio of body weight in Kg and height in meter square (kg/m^2). Participants with BMI $\geq$30.0 kg/m^2 were considered as obese.

Genotyping I/D polymorphism of 3'UTR of UCP2 gene

Genomic DNA was isolated from whole blood using Gen ELUTE Blood Genomic DNA Kit (Sigma-Aldrich, USA). The I/D polymorphism located at 3'UTR of *UCP2* gene was examined by allele specific PCR with the following pair of primers: Forward Primer 5'-GTTCATGCCCTCCTTTCTCCGC -3' Reverse Primer 5'- GACGCCAAGGTTGAGCTTGCTT -3'. A total of 20 µl of reaction volume was carried out with 1 µl of each primer, 5 µl of DNA template, 10 µl of ready to use reaction mixture (Blackbio Biotech, India) & 3 µl of millique water. Each reaction was denatured at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 58.7 °C for 30 s, and 72 °C for 1min. The reactions were given a final 10 min extension at 72 °C.

Statistical analysis

Mean ± standard deviation was calculated by applying student-test and analyzed using appropriate statistical tests by using Statistical Package for Social Sciences (SPSS version 16 for Mac. IBMInc.

Chicago). The allelic and genotype frequencies between cases and controls were performed by Chi-square test. Associations between SNPs and T2DM risks were assessed using odds ratios (ORs) with 95% confidence intervals (95% CIs). p value < 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

In this study, we evaluated a genetic polymorphism (45 bp I/D) in the *UCP2* gene and studied its association with anthropometric parameters (fasting glucose, post prandial glucose, insulin, lipid profile, HbA1c and BMI).

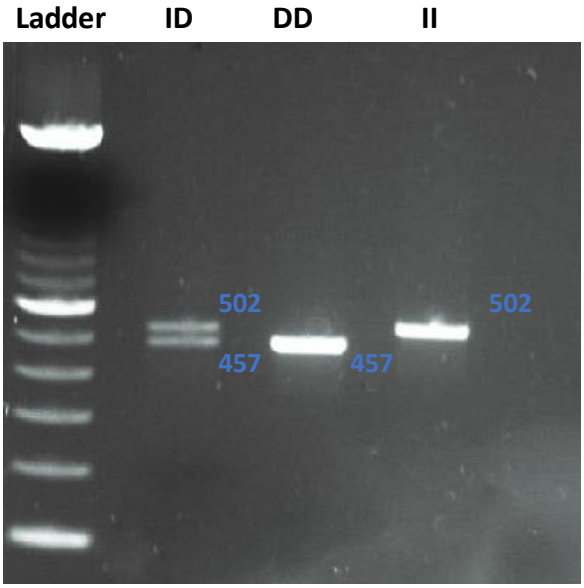

Figure 1. Representative picture showing genotyping of individuals for 45 bp I/D polymorphism of *UCP2* gene.

Figure 1 shows gel picture of PCR product from individual genotypes of I/D polymorphism located at 3'UTR of UCP2 gene. 1: Ladder 100 bp (mol. weight), Lane 2: ID genotype (502,457bp), Lane 3: DD genotype (457bp), and Lane 4: II genotype (502bp).

Characteristic features of the study participants

Base line parameters were found to be statistically significant between cases and controls except for HDL. Clinical features of study subjects are summarized in Table 1. The independent t-test analysis showed that fasting glucose, insulin, HbA1c, BMI, triglyceride, cholesterol, HDL and LDL levels in T2DM patients were significantly higher than those of the control group ($p < 0.05$). The parameters like fasting glucose, insulin, HbA1c, BMI, triglyceride, cholesterol, HDL and LDL are known to be independent risk factors for T2DM development.

Table 1. Clinical features of study participants

Parameter	Case	Control	p-value
Age (years)	51.02±13.50	51.24±13.58	0.80
Male/Female (%)	211/214	211/214	
FG	117.67±5.19	81.56±8.36	<0.0001
PP	167.81±15.55	112.92±16.13	<0.0001
HbA 1c (%)	10.97±4.17	4.82±0.86	<0.0001
BMI (kg/m^2)	31.47±11.33	20.19±2.3	<0.0001
Insulin (%)	39.18 ±4.89	14.62 ± 4.29	<0.0001
TG (mg/dL)	172.40±12.20	105.55±32.44	<0.0001
TC (mg/dL)	256.3±33.48	154.3±40.53	<0.0001
HDL (mg/dL)	51.66±9.37	51.36±11.10	0.386
LDL (mg/dL)	149.18±8.60	68.10±26.90	<0.0001

Data are represented as mean ± SD and analyzed by chi square. $p<0.05$ was considered as statistically significant.

Characteristic features of the study population according to the UCP2 genotype

Table 2 shows the clinical and biochemical characteristic profile of studied subjects categorized according to UCP2 genotype i.e. DD, ID and II. It was noted that the levels of fasting glucose, LDL, plasma protein, HbA1C %, cholesterol and triglycerides in blood were significantly different from each other in diabetic patients except for HDL. The BMI of these patients followed the same pattern. The levels of insulin were high but independent of UCP2 genotypes. The upregulation of these parameters in diabetic patients indicates their association with UCP2 genotypes. However, insulin and HDL seem to follow an independent mechanism of regulation in this population.

Table 2. Clinical and biochemical characteristics of the study population according to the UCP2 genotype

Parameter	DD Genotype	ID Genotype	II Genotype	p-value
FG	113.57 ± 2.98	121.30 ± 2.92	126.12 ± 2.36	<0.0001
PP	155.40 ± 4.62	176.25 ±3.59	212.16 ± 2.46	<0.0001
HbA1C %	7.37 ±0 .81	14.07 ± 2.38	19.04±0.57	<0.0001
Insulin	38.86 ± 5.24	38.80 ± 5.83	39.61 ± 4.29	0.288
BMI	24.02 ±5.65	27.68 ± 7.16	30.35± 4.03	<0.0001
Cholesterol	222.68 ± 7.78	260.03 ± 16.43	284.68 ± 34.41	<0.0001
TG	162.94±4.084	179.51 ± 7.15	201.16 ± 6.27	<0.0001
LDL	144.63 ± 5.53	153.94 ± 1.53	156.93 ± 6.30	<0.0001
HDL	51.24 ± 9.11	51.99 ± 9.57	51.84 ± 10.18	0.587

Data are analyzed by chi square. $p<0.05$ was considered as statistically significant.

Association of *UCP2* (I/D) gene polymorphism with T2DM

Genotypic and Allele frequencies of T2DM patients vs controls for I/D polymorphism of UCP2 gene

We evaluated the association of I/D gene polymorphism of *UCP2* with T2DM. The frequency of genotypes DD, ID and II differ significantly among cases and controls and were found to be 49.1 %, 44.2%, 6.5% and 64.4%, 32.7% & 2.8% respectively. This analysis evidently indicates an association of I/D polymorphism with T2DM in the present population. We also analyzed the frequency of a variant allele ID+II was comparatively more in diabetic patients as compared to controls (p=0.00012).

Table 3. Genotypic and Allele frequencies of T2DM patients vs controls for I/D polymorphism of UCP2 gene

Genotype	Cases (n=425) %	Controls (425) %	OR (95% CI)	p-value
DD	209 (49.1)	274 (64.4)	Ref	
ID	188 (44.2)	139 (32.7)	1.7 (1.30-2.30)	0.0008
II	28 (6.5)	12 (2.8)	2.6 (1.59 -7.63)	0.0032
ID +II	216 (50.8)	163 (38.3)	1.8 (1.29-2.23)	0.00012
D Allele	606 (71.2)	687 (80.8)	Ref	
I Allele	244 (27.6)	167 (19.6)	0.60 (0.48-0.75)	<0.0001

Data are analyzed by chi square. P<0.05 was considered as statistically significant.

Genotypic and allele frequencies of T2DM males vs control males for I/D polymorphism of *UCP2* gene

When the subjects were classified according to gender, the frequency of DD, ID and II alleles among T2DM male cases and controls was 51%, 42.1 %, 6.1% and 68.7%, 27.9% and 3.3% respectively. The genotypes ID was significantly higher in diabetic population (p = 0.0008). Also, the variant allele ID + II was higher in diabetic population (p=0.0004) (Table 4).

Table 4. Genotypic and Allele frequencies of T2DM males vs control males for I/D polymorphism of UCP2 gene.

Genotype	Male cases (n=211) %	Male Controls(n=211) %	OR (95% CI)	p-value
DD	109 (51.6)	145 (68.7)	Ref	
ID	89 (42)	59 (27.9)	2.00 (1.32 -3.03)	0.0008
II	13 (6.1)	7 (3.3)	2.47 (0.95 -6.39)	0.006
ID +II	102 (48.3)	66 (31.2)	2.05 (1.3-3.05)	0.0004
D Allele	307 (72.7)	349 (82.7)	Ref	
I Allele	115 (27.2)	73 (17.2)	1.79 (1.28-2.49)	0.0006

Data are analyzed by chi square. p<0.05 was considered as statistically significant.

Genotypic and allele frequencies of T2DM females vs control females for I/D polymorphism of *UCP2* gene

The frequency of DD, ID, II among T2DM female cases & controls were 46.7%, 46.2%, 7.0% and 60.2%, 37.3%, 2.2 % respectively (table 5). The frequency of genotypes ID and II was comparatively higher in diabetic females (0.0021 and 0.009 respectively). Also, frequency of the variant allele ID+II and mutant allele 'I' was higher in cases in comparison to controls (0.006 and 0.0028 respectively) (table 5).

Table 5. Genotypic and allele frequencies of T2DM females vs female controls for I/D polymorphism of UCP2 gene.

Genotype	Female cases (n=214) %	Female Controls(n=214) %	OR (95% CI)	p-value
DD	100 (46.7)	129 (60.2)	Ref	
ID	99 (46.2)	80 (37.3)	1.56 (1.07- 2.36)	0.0021
II	15 (7.0)	5(2.29)	3.87 (1.36 -11.0)	0.009
ID +II	114 (53.2)	85 (39.7)	1.73 (1.17-2.53)	0.006
D Allele	299(69.8)	338 (78.9)	Ref	
I Allele	129 (30.1)	90 (21.0)	1.62 (1.18-2.22)	0.0028

Data are analyzed by chi square. P<0.05 was considered as statistically significant.

Genotypic and allele frequencies of T2DM patients vs controls when categorized on the basis of age (>50 and <50 years)

The subjects were categorized according to their age group of less or greater than 50 years to assess the impact of frequency of UCP2 genotypes on development of T2DM. It was noted that the frequency was not much altered between cases and controls below 50 years of age. However, the cases above 50 years of age had significantly increased frequency of ID and II alleles (p= 0.00017 and 0.004 respectively) (table 6). The frequency was also much higher for mutant allele 'I' (p=0.000048). So, it can be inferred that the present population is prone to development of T2DM above 50 years of age due increase in frequency of above alleles. So, the risk of T2DM is higher in Kashmiri population above 50 years of age.

Table 6. Genotypic and allele frequencies of T2DM patients vs controls when categorized >50 & <50 0f age

Age: <50 years				
Genotype	**cases (n=210) %**	**Controls(n=210) %**	**OR (95% CI)**	**p-value**
DD	125 (59.5)	138 (65.7)	Ref	
ID	73 (34.6)	67 (31.9)	1.20 (0.79-1.81)	0.40
II	12 (5.7)	5 (2.3)	2.64 (0.90-7.73)	0.08
ID +II	85 (40.4)	72 (34.2)	1.30 (0.87-1.93)	0.22
D Allele	323 (76.9)	343 (81.6)	Ref	
I Allele	97 (23.0)	77 (18.3)	1.33 (0.95-1.87)	0.10
Age: >50 years				
Genotype	**Cases (n=215) %**	**Controls (n=215) %**	**OR (95% CI)**	**p-value**
DD	84 (39.0)	136 (63.2)	Ref	
ID	115 (53.4)	72 (33.4)	2.5 (1.73-3.86)	0.00017
II	16 (7.4)	7 (3.2)	3.7 (1.46-9.36)	0.004
ID +II	131 (60.9)	79 (36.7)	2.6 (1.18-3.96)	<0.001
D Allele	283 (55.3)	344(80)	Ref	
I Allele	147(34.1)	86 (20)	2.07 (1.5-2.83)	0.00081

Data are analyzed by chi square. $p<0.05$ was considered as statistically significant.

Association of *UCP2* (I/D) gene polymorphism with obese T2DM

Genotypic and allele frequencies of obese T2DM patients vs controls when categorized according to BMI

When T2DM subjects were further classified according to BMI, no significant difference was noted in allele frequency among T2DM lean subjects in comparison to controls. However, a remarkable difference was noted in frequency of ID and II alleles between T2DM obese subjects and controls (p=0.0001 and 0.0004 respectively). The variant allele ID + II and the mutant allele 'I' were also significantly higher in obese subjects (p=0.0001 and 0.0001 respectively) (table 7). The results indicate that the UCP2 alleles are more frequent in obese subjects and this possibly leads to development of diabetes. So, obesity poses a risk towards the development of T2DM in Kashmiri population.

Table 7. Genotypic and allele frequencies of T2DM patients vs controls when categorized according to BMI

Lean subjects				
Genotype	**cases (n=210) %**	**Controls(n=425) %**	**OR (95% CI)**	**p-value**
DD	119 (57.6)	274 (63.5)	Ref	
ID	79 (38.0)	139 (34.3)	1.30 (0.92-1.85)	0.14
II	12 (4.4)	12 (2.8)	1.9 (0.88-4.39)	0.12
ID +II	91 (42.3)	151 (35.5)	1.36 (0.97-1.91)	0.069
D Allele	317 (75.4)	687 (80.8)	Ref	
I Allele	103 (24.5)	163 (19.1)	1.36 (0.97-1.91)	0.06
Obese subjects				
Genotype	**Cases (n=215)%**	**Controls (n=425)%**	**OR (95% CI)**	**p-value**
DD	94 (43.4)	274 (63.5)	Ref	
ID	105 (49)	139 (34.3)	2.38 (1.68 -3.37)	<0.0001
II	16 (7.4)	12 (2.8)	3.8 (1.77-8.51)	<0.0001
ID +II	121 (56.2)	151 (35.5)	2.3 (1.67-3.26)	<0.0001
D Allele	293 (69.7)	687 (80.8)	Ref	
I Allele	137 (32.6)	163 (19.1)	2.00(1.54-2.61)	<0.0001

Data are analyzed by chi square. $p<0.05$ was considered as statistically significant.

DISCUSSION

UCPs play vital roles in regulation of human energy metabolism by dissipating proton gradients, uncoupling respiration from oxidative phosphorylation, and converting fuel to heat [16]. UCP2 is widely expressed in pancreatic β-cell, adipose tissue and skeletal muscles of mostly adult humans [20]. In present study, we analyzed the possible association of 45-bp I/D polymorphism of *UCP2* gene and T2DM in Kashmiri population. DD, ID & II alleles of 45bp I/D polymorphism demonstrated the clinical and biochemical features of diabetes. We observed that fasting glucose, BMI, LDL, plasma protein, HbA1C%, cholesterol and triglycerides levels [21,22] were significantly variable but pathologically high in all three genotypes, particularly with ID & II allele except for insulin (p-value = 0.587) and HDL (p-value = 0.288) levels indicating that insulin secretion in T2DM in this population is independent of UCP2 polymorphism and its transcription. The frequency of variant allele ID + II and mutant allele 'I' was significantly higher in T2DM subjects compared to controls for both males and females. Several studies have shown that I/D polymorphism is not associated with T2DM [23,24]. But our results are in contrast with these studies as we found that

45bp I/D polymorphism at 3' UTR of exon 8 of *UCP2* gene was strongly associated with T2DM in Kashmiri population. The frequency distribution of I/D genotype as well as I allele were significantly higher in T2DM subjects than that of controls. This is the first study till date in which 45bp I/D polymorphism was found to be associated with T2DM. This difference in results may be due to difference in ethnical background, sedentary life style, stress due to increasing unemployment & present situation in our population. In our study, we found a significant association of 45bp I/D polymorphism in T2DM subjects greater than 50 years of age. The I/D allele & II allele was found to be significantly high in T2DM subjects & I allele was found to be a risk factor for T2DM development in subjects more than 50 years of age. Age is considered as a risk factor for development of diabetes. Metabolic disorders including T2DM and cardiovascular diseases are closely related with the aging process. Decline in lean body mass and increase in body fat, particularly visceral adiposity that often accompanies aging, may contribute to the development of insulin resistance. It has been recently proposed that an age-associated decline in mitochondrial function contributes to insulin resistance in the elderly [25] and UCP2 a mitochondrial protein, has a direct relevance. So, increasing age could be considered as risk factor for development of T2DM in our study population. Indian population suffer from an increased susceptibility to T2DM, insulin resistance and cardiovascular diseases due to an increased prevalence of obesity and high body fat deposition, even at low body mass indexes in comparison to western populations [26]. In present study, the frequency of UCP2 genotypes was not much variable between control population and lean T2DM patients. In contrast, we found that the frequency was significantly higher in obese diabetic subjects in comparison to controls ($p = 0.0001$). Our results are consistent with a study conducted in Spanish population in which obese T2DM subjects carrying 'I' allele had a higher risk of developing obesity [27]. In some studies, also, the frequency of the I-allele of 45 bp I/D polymorphism was significantly comparatively higher in obese subjects as compared to lean, and carriers of the I-allele have been reported to have a significantly higher BMI, raising the possibility that carriers of the I-allele may have a greater risk of developing obesity [28-30]. However, some studies have shown no associations of this polymorphism with obesity, resting energy expenditure, BMI and insulin secretion [31-34]. The biological significance of the 3'UTR I/D is not well known. However, its location in the 3'UTR may be related to involvement in mRNA processing or in the stability of the transcript [28]. Any reduction in UCP2 mRNA stability could compromise the ability to remove excess calories through thermogenesis, especially in a person with a propensity to obesity from other genetic or environmental influences [34]. Our results suggested that ID & II genotypes had higher BMI compared to DD genotype in obese T2DM subjects but not in lean T2DM subjects inferring that these variants of 45bp I/D polymorphism in obese subjects may be responsible for development of T2DM in our study population.

4. CONCLUSION

In the present study, we found that allele frequency of 45 bp I/D polymorphism present on exon 8 of *UCP2* gene was more in T2DM patients in comparison to control subjects. We noted that I/D & II allele was significantly high in T2DM subjects with I allele as a risk factor for T2DM development in subjects more than 50 years of age. It can be concluded from the present study that 45bp I/D polymorphism of *UCP2* gene plays a role in development of T2DM in our population especially when age and BMI were considered. This polymorphism appears to be an important genetic determinant in the progression of T2DM.

ACKNOWLEDGEMENT

The authors highly acknowledge the Department of Biochemistry for the financial support. The authors also acknowledge the technical and non-technical staff for their help.

CONFLICT OF INTEREST

The authors have not conflicts of interest to declare.

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