BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, June 2019. Vol. **16**(2), p. 351-357

Association Study of Candidate Gene Uncoupling Protein 2 (UCP2) with Type 2 Diabetes Mellitus in the Different Population Groups of Jammu Region

Sunil Raina[1] and Roopali Fotra[2]

[1]University of Jammu- 180006, India.
[2]Institute of Human Genetics, University of Jammu- 180006, India.

http://dx.doi.org/10.13005/bbra/2751

(Received: 31 May 2019; accepted: 18 June 2019)

Diabetes Mellitus is a group of metabolic disorders characterized by hyperglycaemic resulting from the defects of insulin secretion, insulin action or both. The present study was conducted in order to know the molecular genetic cause of the T_2DM patients belonging to the Jammu region of J&K State. Many genes have been known to be linked with the onset and progression of the T_2DM therefore the present data represents the role of one of the genes Uncoupling protein 2 (UCP2) known to be strongly associated with T_2DM was selected. A total of 250 confirmed cases & controls samples belonging to four population groups (Hindu, Muslim, Sikh & Christians) of Jammu region were also screened for UCP2 -866G/A promoter polymorphism (rs659366). The allelic odds ratio (OR) as observed for UCP2 -866G/A polymorphism in the four population groups showed significant association with Muslim & Sikh population groups. The study undertaken supports the findings of the previous investigations and thus is an addition to the existing literatute in support of UCP2 and T_2DM.

Keywords: Diabetes Mellitus, polymorphism and UCP2.

Diabetes Mellitus is a group of metabolic disorders characterized by hyperglycaemic resulting from the defects of insulin secretion, insulin action or both[1]. Diabetes Mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe form ketoacidosis or a non–ketotic hyperosmolar state develop that may lead to stupor, coma and in the absence of an effective treatment it may lead to death.

The disease diabetes has been broadly classified into two types:

1. Type 1 Diabetes Mellitus
2. Type 2 Diabetes Mellitus

Type 1 Diabetes Mellitus (T_1DM) also known as Insulin Dependent Diabetes Mellitus (IDDM) is caused by auto-immune destruction of the â-cells of the pancreas, rendering the pancreas unable to synthesize and secrete insulin. Type 2 Diabetes Mellitus (T_2DM), also known as Non-Insulin Dependent Diabetes Mellitus (NIDDM) is a heterogeneous syndrome resulting from the defect of both insulin secretion and insulin action.

Compared to Type 1 Diabetes, T_2DM is the most common form of diabetes, as it accounts for over 90% of all diabetic cases worldwide[3]. The disease usually occurs after the age of 35-40 years but may be diagnosed earlier, especially in the

*Corresponding author E-mail: roopali_sidhharth@yahoo.co.in

populations with high prevalence of the disease. T_2DM can remain undetected (asymptomatic), for several years and the diagnosis is often made from the associated complications or through an abnormal blood or urine glucose test. Type 2 diabetes mellitus is an extremely complex disease with a tremendous social and economical burden. Consequently, diabetes is rapidly emerging as a global health care problem that threatens to reach pandemic levels. By 2030, the number of people with diabetes worldwide is projected to increase from 171 million in 2000 to 366 million by 2030[4]. This will be the most noticeable increase in the developing countries, where the number of people with diabetes is expected to increase from 84 million to 228 million. According to World Health Organization, Southeast Asia and the Western Pacific regions are at the forefront of the current diabetes epidemic with India and China facing the greatest challenges.

Patients that suffer from T2DM have a reduced quality of life and decreased life expectancy. The patients suffer from conditions like retinopathy, kidney malfunctioning, heart and blood vessel diseases and nerve damage in some cases.

The etiology of T_2DM is not fully understood, but presumably, T_2DM develops when a diabetogenic lifestyle (i.e. excessive caloric intake, inadequate caloric expenditure, obesity etc.) acts in conjugation with a susceptible genotype. Majority of patients who develop T_2DM are obese[4.] A variety of environmental factors have been implicated in the clinical expression of T2DM. Some of these factors are the degree and type of obesity, sedentarily, malnutrition in fetal and perinatal periods, lifestyle, and different kinds of drugs (e.g, steroids, diuretics, anti-hypertensive agents). People with a family history of the diabetes are at higher risk of developing the disease because they share genetic background and likely share the similar environments. Family studies had revealed that first degree relatives of individuals with T2DM are about three times more likely to develop the disease than individuals without a positive family history of the disease[6,7].

Risk factors associated with T2DM

- First-degree family history of Diabetes Mellitus (i.e., parents or siblings)
- Overweight or obese
- Habitual physical inactivity
- Race or ethnicity (Native American, Latino/Hispanic American, Asian American, African American, and Pacific Islanders)
- Prediabetes (i.e., previously identified with impaired glucose tolerance or impaired fasting glucose)
- Hypertension (e”140/90 mm Hg)
- High-density lipoprotein (HDL) less than 35 mg/dL (0.91mmol/L) and/or a triglyceride level greater than 250 mg/dL (2.83 mmol/L)
- History of gestational diabetes or delivery of a baby weighing greater than 9 pounds (4.09 kg)
- History of vascular disease
- History of polycystic ovary disease
- Other conditions associated with insulin resistance

Various studies through literature have shown that Asian Indians have an increased susceptibility to diabetes and increased insulin resistance; they are a unique population for carrying out genetic studies. Asian Indians develop T_2DM at lower levels of BMI and have stronger heritability factors as compared to Europeans. Recent genetic studies on Asian Indians indicated that certain genes appear to predispose Indians to diabetes while other genes, which afford protection against diabetes and insulin resistance to Caucasians and do not appear to protect Indians. Moreover, a plenty of data has been generated for the genetic susceptibility of various populations to T_2DM but it is scanty for Indian populations and almost absent for Northwest Indian population groups particularly from Jammu region of J&K State.

In order to know the molecular genetic cause of the T_2DM patients belonging to the Jammu region of J&K State, one of the genes known to be strongly associated with T_2DM was selected in the present study. A total of 250 confirmed cases & controls samples belonging to four population groups (Hindu, Muslim, Sikh & Christians) of Jammu region were also screened for UCP2 -866G/A promoter polymorphism (rs659366).

MATERIAL AND METHODS

In molecular genetic analysis, the present investigation was focused to study the association of a candidate gene Uncoupling protein

2 (UCP2) with Type 2 Diabetes Mellitus (T2DM) in the population groups of the Jammu region. The cytogenetic location of UCP2 Uncoupling protein 2 (mitochondrial proton carrier) is 11q13. UCP2 is a regulator of insulin secretion and is highly expressed in the lymphoid system, macrophages, and pancreatic islets & participating in intermediary metabolism and in particular in fatty acid Metabolism.

Genomic DNA isolated from blood samples of 250 T2DM patients was subjected to genotyping by using PCR-RFLP based method. PCR was performed using standard PCR conditions Initial pre-cycling denaturation 94^0C for 4 min, Denaturation for 30 cycles at 94^0C for 1 min followed by annealing (specific to each primer), Extension 72ºC for 1 min, and Final extension 72ºC for 4 min.

(Table 1&2 showing the details of the Genes selected Primers sequences, Annealing temperatures, Amplified gene product, Restriction Endonuclease and product size of the gene after restriction digestion).

Genotype and allele frequencies of UCP2 G/A -866 polymorphism were calculated in the selected controls. Based on the band size, 250 Controls were scored either homozygous (GG) for wild allele

heterozygous (GA) and homozygous (AA) for mutant allele. The data of the allele and genotype frequencies of UCP2 -866 G/A polymorphism studied in the selected Controls has been provided in Table 3.

RESULTS

Genotype and Allele frequencies of UCP2 promoter-866 G/A polymorphism in T_2DM patients

Genotype and allele frequencies of UCP2 promoter G/A -866 polymorphism were calculated in the selected T_2DM patients. Based on the band size, these 250 T_2DM patients were scored either homozygous (GG) for wild allele, heterozygous (GA) and homozygous (AA) for mutant allele. The data of the allele and genotype frequencies of UCP2 -866 G/A polymorphism studied in the selected T2DM patients has been provided in Table 3.

The genotype frequencies, allele frequencies and heterozygosity value of Uncoupling proteins 2 (UCP2) -866G/A polymorphism in 250 patients representing the different population groups are as under:

Hindu Population: A total of 104 Hindu individuals were genotypes for UCP2. Out of 104 individuals, 27 were homozygous (GG) for wild type allele, 46 were heterozygous (GA) and 31 individuals were found homozygous (AA) for mutant allele. Out of the total 208 alleles (2x104=208), the frequency of the G allele for UCP2 866G/A Polymorphism was found to be 0.48 where as the frequency of A allele for UCP2 866G/A Polymorphism was found to be 0.52 (Table 3).

Muslim Population: A total of 51 Muslim individuals were genotyped for UCP2. Out of the 51, 29 individuals were found to be homozygous (GG) for wild type allele, 10 were found heterozygous (GA) and 12 were found homozygous (AA) for mutant allele. Out of the total 102 alleles (2x51=102), the frequency of the G allele for UCP2 866G/A Polymorphism was found to be 0.67 where as the frequency of A allele for UCP2 866G/A Polymorphism was found to be 0.33 (Table 3)

Sikh Population: A total of 78 Sikh individuals were genotyped for UCP2. Of the 78 individuals, 40 were found homozygous (GG) for wild type allele, 28 were found heterozygous (GA) and 10 were found homozygous (AA) for mutant allele. Out of the total 156 alleles (2x78=156), the frequency of the G allele for UCP2 866G/A Polymorphism was found to be 0.69 whereas the frequency of the A allele for UCP2 -866G/A Polymorphism was found to be 0.31. (Table 3)

Christian Population: A total of 17 Christian individuals were genotyped for UCP2. Out of the 17 individuals, 08 were found to be homozygous (GG) for wild allele, 05 were found heterozygous (GA) and 04 individuals were found homozygous (AA) for mutant allele. Out of the total 34 alleles (2x17=34), the frequency of the G allele for UCP2 866G/A Polymorphism was found to be 0.62 whereas the frequency of the A allele for UCP2 -866G/A Polymorphism was found to be 0.38 (Table 3) .

The allelic odds ratio (OR) as observed for UCP2 -866G/A polymorphism in the four population groups showed significant association with Muslim & Sikh population groups (Table 4).

The genotype frequencies, allele frequencies and heterozygosity value of UCP2 866G/A polymorphism in the 250 Controls representing the selected population groups are as under:

Hindu Population: A total of 104 individuals selected as Control from Hindus were genotyped for UCP2. Out of 104 individuals, 29 were homozygous (GG) for wild allele, 45 were found heterozygous (GA) and 30 individuals were found homozygous (AA) for mutant allele. Out of the total 208 alleles (2x104=208), the frequency of UCP2 -866G/A polymorphism for G allele was found to be 0.50 whereas the frequency of A allele for UCP2 -866G/A polymorphism was found to be 0.50. (Table 5)

Muslim Population: Out of the total 102 individuals as controls, the frequency of the G allele for UCP2 866G/A polymorphism was found to be 0.53 where as the frequency of A allele for UCP2 866G/A polymorphism was found to be 0.47 (Table 5).

Sikh population: A total of 78 Sikh individuals selected as Control were genotyped for UCP2. Of the 78 individuals, 24 were found homozygous (GG) for wild allele, 31were found heterozygous (GA) and 23 individuals were found homozygous (AA) for mutant allele. Out of the total 156 alleles (2x78=156), the frequency of the G allele for UCP2 866G/A polymorphism was found to be 0.51 whereas the frequency of T allele for UCP2 866G/A polymorphism was found to be 0.49 (Table 5).

Christian population: A total of 17 Christian individuals selected as Control were genotyped for UCP2. Out of the 17 individuals, 22 were found to be homozygous (GG) for wild allele, 16 were found heterozygous (GA) and 11 individuals were found homozygous (AA) for mutant allele. Out of the total 34 alleles (2x17=34), the frequency of the G allele for UCP2 866G/A polymorphism was found to be 0.62 whereas the frequency of T allele for UCP2 866G/A polymorphism was found to be 0.38.

Table 1. Table showing details of Primers used during the present study

Gene	Forward primer sequence	Reverse primer sequence	Restriction enzyme & its source	Site of action
UCP2	5'GGCGTCAGGA GATGGACCG3'	5'-CACGCTTCT GCCAGGAC-3'	Mlu I Micrococcus *luteus*	5'…A¼%CGCGT…3' 3'…TCGC²%A…..5'

Table 2. Table showing the PCR conditions

Gene	Annealing Temp.(^{0}C)	PCR Product size	No. of cycles	Product size (restriction Digestion)Wild/Hetero/Mutant
UCP2	65	360	35	290-70bp/360bp

Table 3. Distribution of UCP2 Genotypes and its Allele frequencies of the T_2DM patients

Population		GG	GA	AA	Total	G	A	Total
Hindus	N	27	46	31	104	100	108	208
	Frequency	0.26	0.44	0.30	1.0	0.48	0.52	1.0
Muslims	N	29	10	12	51	68	34	102
	Frequency	0.56	0.19	0.23	1.0	0.67	0.33	1.0
Sikhs	N	40	28	10	78	108	48	156
	Frequency	0.51	0.35	0.12	1.0	0.69	0.31	1.0
Christians	N	08	05	04	17	21	13	34
	Frequency	0.47	0.29	0.24	1.0	0.62	0.38	1.0

DISCUSSION

T_2DM is of major concern to man. In the past 5 years, Genome wide association studies have identified and replicated over 40 single nucleotide polymorphisms (SNPs) that predispose to T_2DM [8&9]. Study of the literature on the genes responsible for the disease T_2DM shows that several genes are behind the origin of the disease, however, some of the candidate gene variations include variants of calpain-10 (CAPN10), Peroxisome Proliferator-Activated Receptor-ã2 (PPARã2), Potassium inwardly rectifying channel, subfamily J, member 11 (KCNJ11), ATP Binding Cassette, subfamily C, member 8 (ABCC8), Hepatocyte Nuclear Factor-1A (HNF1A), Hepatocyte Nuclear Factor4A (HNF4A), Glucokinase (GCK), Plasma Cell Glycoprotein-1/encoding Ectonucleotide Pyrophosphate Phosphodiesterase 1(PC-1/ENPPI), Insulin Receptor Substrate-1 (IRS-1), Protein Tyrosine Phosphatase 1B (PTPNI).

Among all T_2DM susceptibility genes studied till date, only few were found to be convincingly associated with the disease. UCP1, UCP2, PPP1R3A, P12A variant in peroxisome proliferator activated receptor gamma (PPARã) gene [10], E23K in potassium inwardly rectifying channel, subfamily J, member 11(KCNJ11) [11]. All these genes have modest effect on the development of the disease T_2DM. The TCF7L2 gene is the most important T_2DM susceptibility gene found to date. Since its discovery, the association has been replicated in a variety of studies in subjects of different ethnicities [12&13].

UCP2 -866 G/A POLYMORPHISM: Uncoupling protein 2 is an attractive candidate gene for obesity and T_2DM. UCP2 is a member of the mitochondrial inner membrane carrier family that is highly expressed in adipose tissue and pancreatic islets. UCP2 mediates mitochondrial proton leak releasing energy stored within the proton motive force as heat that, ultimately, results in a decrease in ATP production. â-cells sense glucose through its oxidative metabolism and the resulting increase in the ATP/ADP ratio plays a central role in glucose-induced insulin secretion by causing closure of the membrane ATP-sensitive potassium channel, membrane depolarization, influx of calcium, and finally, insulin granule exocytosis [14&15]. Uncoupling glucose metabolism from ATP generation would be expected to impair â-cell ability to secrete insulin in response to glucose. Thus, an increased expression or activity of UCP2 in pancreatic â-cell may contribute to impair insulin secretion.

Table 4. Allelic odds ratio (OR) of UCP2 in the four selected population groups

Population	Cases		Controls		P value	Odds Ratio
	G	A	G	A		(95% Confidence Intervals)
Hindus	0.48	0.52	0.50	0.50	0.769	0.94 (0.64, 1.39)
Muslims	0.67	0.33	0.53	0.47	0.045	1.78 (1.01, 3.13)
Christians	0.69	0.31	0.51	0.49	0.000	2.19 (1.38, 3.48)
Sikhs	0.62	0.38	0.62	0.38	0.329	1.62 (0.62, 4.24)

Table 5. Distribution of UCP2 Genotypes and its Allele frequencies of selected Controls

Population		GG	GA	AA	Total	G	A	Total
Hindus	N	29	45	30	104	103	105	208
	Frequency	0.28	0.43	0.29	1.0	0.50	0.50	1.0
Muslims	N	17	20	14	51	54	48	102
	Frequency	0.33	0.39	0.28	1.0	0.53	0.47	1.0
Sikhs	N	24	31	23	78	79	77	156
	Frequency	0.31	0.40	0.29	1.0	0.51	0.49	1.0
Christians	N	06	05	06	17	17	17	34
	Frequency	0.35	0.30	0.35	1.0	0.62	0.38	1.0

Studies on UCP2 -866 G/A polymorphism and its association with T_2DM studies have been performed by various researchers seeking for an association between UCP2 866G/A gene polymorphism with T_2DM [16,17,18,19,20&21] in the different population and ethnicities]. These studies have demonstrated association of the -866A allele with increased [22&23] and decreased risk of T_2DM [24] as well as no association at all [25,26&27].

During the present findings UCP2 -866 G/A gene polymorphism was found to be less common in the selected population groups. Suggesting thereby that in the present study the UCP2 -866 G/A gene polymorphism was insignificant as far as the disease T2DM is concerned.

Workers like [28,29&30] in their studies on different population groups of their respective regions studied the genotype (-866 GG, -866 GA & 866 AA) of UCP2 -866 G/A polymorphism and observed the frequency of the -866 GA genotype found to be less common as compared to remaining two genotypes (GG and AA). Present study did not record the high prevalence of -866 G/A genotype in the selected population groups. Present study supports the previous findings investigated by various workers however it is an addition to the existing literature.

REFERENCES

1. American Diabetes Association. Standards of Medical Care in Diabetes—2007. *Diabetes Care*, **30**; Supplement 1, S4-S41.
2. Gonzalez, E.L., Johansson, S., Wallander, M.A., Rodriguez, L.A. Trends in the prevalence and incidence of diabetes in the UK: 1996-2005. J Epidemiol Community Health, 2009; **63**; 332-6.
3. Mohan, V., Ganesan, A., Vijayachandrika, V., Weber, M.B., Gokulakrishnan, K., Venkat Narayan, K.M., Anjana, R.M. A1c Cut Points to Define Various Glucose Intolerance Groups in Asian Indians. *Diabetes Care,* 2010a; **33**; 515–519.
4. Mohan, V., Jeremy, D. Goldhaber-Fiebert, Radha, V., Gokulakrishnan, K. Screening with OGTT alone or in combination with the Indian diabetes risk score or genotyping of TCF7L2 to detect undiagnosed type 2 diabetes in Asian Indians. *Indian J Med Res.,* 2011; **133**; 294-299.
5. Eckel, R.H., Kahn, S.E., Ferrannini, E., *et al*. Obesity and type 2 diabetes: what can be unified and what needs to be individualized? *Diabetes Care,* 2011; **34**; 1424-30.
6. Flores, J.C., Hirschhorn, J., Altshuler, D. The inherited basis of diabetes mellitus: implications for the genetic analysis of complex traits. *Annu Rev Genomics Hum Genet.,* 2003; **4**; 257-291.
7. Hansen, L. Candidate genes and late-onset type 2 diabetes mellitus. Susceptibility genes or common polymorphisms? *Dan Med Bull.,* 2003; **50**(4); 320-4
8. McCarthy, M.I. Genomics, type 2 diabetes, and obesity. *N Engl J Med.,* 2010; **363**;2339– 50.
9. McCarthy M.I. From hype to hope? A journey through the genetics of type 2 diabetes. *Diabet Med*., 2011; **28**; 132–40.
10. Altshuler, D., Hirschhorn, J.N., Klannemark, M., Lindgren, C.M., Vohl, M.C., Nemesh, J., Lane, C.R., Schaffner, S.F., Bolk, S., Brewer, C., Tuomi, T., Gaudet, D., Hudson, T.J., Daly, M., Groop, L., Lander, E.S. The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet.,* 2000; **26**(1); 76-8.
11. Gloyn, A.L., Michael, N.W., Katharine, R.O., Martina, J.T., Bridget, A.K., Graham, H., Mark, W., Jonathan, C.L., Mike, S., Stephanie, H., Mark, I.M., Andrew, T.H., and Timothy, M.F. Large-scale association studies of variants in genes encoding the Pancreatic â-Cell KATP Channel Subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) confirm that the KCNJ11 E23K variant is associated with Type 2 Diabetes. *Diabetes*, 2003; **52**; 568–572.
12. Zhang, C., Qi, L., Hunter, D.J., Meigs, J.B., Manson, J.E., van Dam, R.M., Hu, F.B. Variant of transcription factor 7-like 2 (TCF7L2) gene and the risk of type 2 diabetes in large cohorts of U.S. women and men. *Diabetes,* 2006; **55**; 2645-8.
13. Chandak, G.R., Janipalli, C.S., Bhaskar, S., *et al*. Common variants in the TCF7L2 gene are strongly associated with type 2 diabetes mellitus in the Indian population. *Diabetologia,* 2007; **50**; 63-7.
14. Fleury, C., Neverova, M., Collins, S., Raimbault, S., Champigny, O., Le- vi-Meyrueis C., *et al*. Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. *Nat Genet*., 1997; **15**(3); 269-72.
15. Gimeno, R.E., Dembski, M., Weng, X., Deng, N., Shyjan, A.W., Gimeno, C.J., Iris, F., Ellis, S.J., Woolf, E.A., Tartaglia, L.A. Cloning and characterization of an uncoupling protein homolog: a potential molecular mediator of human thermogenesis. *Diabetes,* 1997; **46**; 900–906.
16. Esterbauer, H., Schneitler, C., Oberkofler, H.,

Ebenbichler, C., Paulweber, B., Sandhofer, F., Ladurner, G., Hell, E., Strosberg, A.D., Patsch, J.R., Krempler, F., Patsch, W. A common polymorphism in the promoter of UCP2 is associated with decreased risk of obesity in middle aged humans. *Nat Genet.,* 2001; **28**(2); 178-183.

17. Sesti G., Marina Cardellini, Maria Adelaide Marini, Simona Frontoni, Monica D'Adamo, Silvia Del Guerra, Davide Lauro, Pierluigi De Nicolais, Paolo Sbraccia, Stefano Del Prato, Sergio Gambardella, Massimo Federici, Piero Marchetti, and Renato Lauro. A Common Polymorphism in the Promoter of UCP2 Contributes to the Variation in Insulin Secretion in Glucose-Tolerant Subjects. *Diabetes,* 2003; **52**; 1280-1283.
18. D'Adamo, M., Lucia Perego, Marina Cardellini, Maria Adelaide Marini, Simona Frontoni, Francesco Andreozzi, Angela Sciacqua, Davide Lauro, Paolo Sbraccia, Massimo Federici, Michele Paganelli, Antonio E. Pontiroli, Renato Lauro, Francesco Perticone, Franco Folli, and Giorgio Sesti. The -866A/A Genotype in the Promoter of the Human Uncoupling Protein 2 Gene Is Associated With Insulin Resistance and Increased Risk of Type 2 Diabetes. *Diabetes,* 2004; **53**.
19. Sasahara M., Nishi M., Kawashima H., Ueda K., Sakagashira S., Furuta H., Matsumoto E., Hanabusa T., Sasaki H., Nanjo K. Uncoupling protein 2 promoter polymorphism -866G/A affects its expression in beta-cells and modulates clinical profiles of Japanese type 2 diabetic patients. *Diabetes*, 2004; **53**(2):482-485.
20. Bulotta, A., Ludovico, O., Coco, A., Paola, R.D., Quattrone, A., Carella, M., Pellegrini, F., Prudente, S., and Trischitta, V. The Common -866G/A Polymorphism in the Promoter Region of the UCP-2 Gene Is Associated with Reduced Risk of Type 2 Diabetes in Caucasians from Italy. *The Journal of Clinical Endocrinology & Metabolism,* 2005; **90**(2); 1176–1180.
21. Heidari, J., Akrami, S. M., Heshmat, R., Amiri, P., Fakhrzadeh, H., Pajouhi, M. Association Study of the -866G/A UCP2 Gene Promoter Polymorphism with Type 2 Diabetes and Obesity in a Tehran Population: A Case Control Study. *Archives of Iranian Medicine,* 2010; **13**; 384-390.
22. Krempler F., Esterbauer H., Weitgasser R., Ebenbichler C., Patsch J.R., Miller K., Xie M., Linnemayr V., Oberkofler H., Patsch W. A functional polymorphism in the promoter of UCP2 enhances obesity risk but reduces type 2 diabetes risk in obese middle-aged humans. *Diabetes,* 2002; **51**(11); 3331-3335.
23. Gable, D.R., Stephens, J.W., Dhamrait, S.S., Hawe, E., and Humphries, S. E. European differences in the association between the UCP2 -866G > A common gene variant and markers of body mass and fasting plasma insulin," *Diabetes, Obesity and Metabolism*, 2007; **9**(1): pp. 130–131, 2007.
24. Lyssenko V., Almgren P., Anevski D., Orho-Melander M., Gren M.S., Saloranta C., Tuomi T., Groop L. Genetic Prediction of Future Type 2 Diabetes. *PLoS Med.,* 2005; **2**(12); e345
25. Reis A.F., Dubois-Laforgue D., Bellanne-Chantelot C., Timsit J., Velho G. A polymorphism in the promoter of UCP2 gene modulateslipid levels in patients with type 2 diabetes. *Mol Genet Metab.,* 2004; **82**(4); 339-344.
26. Kovacs P., Ma L., Hanson R.L., Franks P., Stumvoll M., Bogardus C., Baier L.J. Genetic variation in UCP2 (uncoupling protein-2) is associated with energy metabolism in Pima Indians. *Diabetologia*, 2005; **48**(11); 2292-2295.
27. Zee, R.Y., Ridker, P.M., Chasman, D.I. Mitochondrial uncoupling protein gene cluster variation (UCP2-UCP3) and the risk of incident type 2 diabetes mellitus: the Women's Genome Health Study. *Atherosclerosis,* 2011; **214**(1): 107-9.
28. Tayeb M T. (2009). Association of the UCP2 –866G/A polymorphism with type 2 diabetes and obesity in Saudi population. *Egypt. J. Med. Hum. Genet.,* **10**(2); 228-236.
29. Rai E., Sharma S., Koul A., Bhat A.K., Bhanwer A.J.S., Bamezai R.N.K. Interaction between the *UCP2*–866G/A, mtDNA 10398G/A and *PGC1á* p.Thr394Thr and p.Gly482Ser polymorphisms in type 2 diabetes susceptibility in North Indian population. *Human Genetics,* 2007; **122**(5): 535-540.
30. Oktavianthi S., Trimarsanto H., Febinia C. A., Suastika K., Saraswati M. R., Dwipayana P., Arindrarto W., Sudoyo H., Malik S.G. Uncoupling protein 2 gene polymorphisms are associated with obesity. *Cardiovascular Diabetology,* 2012; **11**; 41.