Association of 45-bp Ins/del Polymorphism of Uncoupling Protein 2 (UCP2) and Susceptibility to Nonalcoholic Fatty Liver and Type 2 Diabetes Mellitus in North-west of Iran

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Research note

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Abstract

Objective

Uncoupling protein 2 (UCP2) is a regulator of insulin secretion, free fatty acid (FFA) concentrations and lipid metabolism that plays crucial roles in energy homeostasis. Last decade reports have described close association between UCP2 polymorphisms and nonalcoholic fatty liver disease (NAFLD) and diabetes mellitus type 2 (T2DM).

Results

A higher prevalence of insertion/insertion genotype has been observed in T2DM patients compared with the control group (p value 0.05). But, there was no difference in genotype distribution between NAFLD patients and control groups (p value > 0.05). NAFLD patients with D/D, D/I genotype had higher triglyceride, ALT and AST levels, and lower HDL level than healthy controls. Patients with T2DM together with D/D or D/I genotype also had significantly higher fasting serum glucose (FSG) level. While we found association between the 45 bp I/D polymorphism in 3UTR of UCP2 and T2DM, existence of correlation between this polymorphism and NAFLD was not identified.

Introduction

The existence of the robust associations between NAFLD and obesity, type 2 diabetes mellitus (T2DM), dyslipidemia, hypertension, cardiovascular disease and sometimes even hepatocellular carcinoma have been presented based on a wide range of studies [1–3]. NAFLD is considered a significant problem that affects approximately 10–30% of the general population of variable ethnicities across all regions of the world [4, 5]. Intriguingly, the prevalence of NAFLD is 49–62% in patients with T2DM and 18–33% of patients with NAFLD have T2DM [6–8]. Whereas a large number of studies suggested that T2DM is an independent risk factor for NAFLD, T2DM has the potential to produce NAFLD condition in the presence of TG in liver tissue [9, 10].

Uncoupling protein 2 (UCP2) is a mitochondrial inner-membrane anion carrier protein involved in energy homeostasis, regulation of the insulin secretion, free fatty acid (FFA) concentrations as well as lipid metabolism [11, 12]. The UCP2 is widely expressed in human tissues, containing white adipose tissue, skeletal muscle, pancreatic islets and the central nervous system [13, 14]. Due to UCP2 notable capabilities to promote lipid gathering in the liver organ, stimulate protective neural mechanisms in acute ethanol intake [15, 16] and reinforce insulin resistance [17], its basic role in pathophysiology of liver disease and obesity has been accepted. Interestingly, researches have demonstrated that there is correlation between polymorphisms within the UCP2 gene and metabolic diseases, particularly T2DM and obesity [18]. Based studies, modifying of the expression of genes which regulate UCP-2 expression and functions is promising therapeutic approach for controlling insulin resistance, obesity and body-weight gain or body mass index (BMI) [19]. Importantly, genetic polymorphisms in UCP2, in particular 45 bp deletion/insertion (D/I), have been reported to be associated with obesity, BMI and T2DM in the general population [20].

Because of their high prevalence, increased morbidity and mortality, and social and economic burden, NAFLD and T2DM constitute an important public health problem [21–23]. Accordingly, recognizing of the molecular base of the NAFLD and T2DM is required to open new landscape toward novel and effective therapeutic approaches. To our knowledge, there are no data on the relationship between the 45-bp D/I polymorphism in the UCP2 gene and NAFLD and T2DM in population of North-West of Iran. The main purpose of the current study is response to this query whether there are associations between 45-bp ins/del polymorphism and susceptibility to NAFLD and T2DM in a North-West of Iran population or not.

Methods

Patient recruitment

72 patients with NAFLD (age range: 20– 50 years), 71 age and race matched healthy controls and 80 patients with T2DM, 77 age and race matched healthy controls were enrolled in the study. The patients were selected from those referred to the outpatient clinics of Tabriz University of Medical Sciences, Tabriz, Iran, and differential diagnosis was confirmed by a physician according to ultrasonography and biochemical tests consequences. The inclusion criteria for NAFLD patients were Iranian ancestry and unrelated, adults between 20 years and 50 years old, having body mass index (BMI) between 25 and 39 and any alcohol consumption. The exclusion criteria for controls were using medications, including metformin, corticosteroids, amiodarone, and/or valproate in the past 3 months, any history of acute and chronic liver diseases, viral hepatitis, hemochromatosis, Wilson disease, any autoimmune or endocrine disorders and having participated in weight loss diets for at least 3 months before the start of the screening process for this study. Diabetic type 2 patients were identified by an endocrinologist based on biochemical tests. The patients and control group were selected with age between 30 and 70 years old. They hadn't type 1 diabetes disease and history of insulin injection. Control group also had no history of diabetic disease. Subjects provided written informed consent after a full explanation of the research outline. The study protocol was reviewed and approved by the Medical Ethics Committee of the Tabriz University of Medical Sciences.

Biochemical Measurements

Fasting serum glucose (FSG), total cholesterol (TC), high-density lipoprotein cholesterol (HDLC), triglycerides (TG), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) concentrations were checked using kits on the Abbott ALCYON 300 auto analyzer (Abbott Laboratories, Inc) after fasting for more than 10 hours. All of the biochemical parameters are listed in Table 1.

DNA isolation and polymerase chain reaction (PCR)

Genomic DNA was extracted from whole blood by using salting-out method [24]. We selected one SNP in UCP2 gene from published literature and the Database of Single Nucleotide Polymorphism (dbSNP) at the NCBI website (http://www.ncbi.nlm.nih.gov/SNP). SNP genotyping was performed by PCR. DNA fragments related to 45-bp ins/del polymorphism were amplified by primers: 5′- TTCTCCGCTTGGGTTCCTG – 3′ as forward primer and 5′- CACTGTCAAATGTCAACTCCACC – 3′ as reverse primer. PCR primer sequences were designed using Gene Runner software (version 3.01), based on GenBank coding DNA reference sequence NG_011478, from the National Center for Biotechnology Information (NCBI) website http:// www.ncbi.nlm.nih.gov.

Statistical analysis

All statistical analyses were conducted by using the SPSS statistical package ver. 22 (SPSS Software, Chicago, IL, USA). The Distributions of categorical variables in groups compared using chi-squared test. Kolmogorov-Smirnov and Shapiro–Wilk tests performed to determine the normal distribution of quantitative variables. We used t-test for comparing quantitative data between two groups that had normal distribution and Mann-Whitney test for data that had abnormal distribution. Odd Ratio calculated by logistic regression for genotypes and alleles that adjusted by sex and age. As well, evaluation of continuous variables changes between different UCP-2 genotypes was completed by analysis of covariance (ANCOVA) with modification effects of age and sex.

Results

Anthropometric and laboratory data of the study population

Table 1 show the clinical and demographic data of patients enrolled (NAFLD; n = 72, controls; n = 71) (T2DM; n = 80, controls; n = 77). Subjects with NAFLD comprised 35 (48.6%) men and 37 (51.4%) women, with a mean age of 40 years. Their mean difference of BMI was 3.164 kg/m and there were no significant differences in age, sex, BMI and BMR between the groups. Subjects with T2DM comprised 36 (45%) men and 44(55%) women, with a mean age of 54 years and their mean difference of BMI was − 1.116 kg/m. Also, there were no significant differences in age or sex between groups. However, we found that patients with NAFLD had significantly higher triglyceride, higher ALT levels, higher AST levels, and lower HDL than healthy controls ($p \leqq 0.05$ for all values). Moreover, patients with T2DM had significantly higher FSG, TG, and lower LDL and HDL levels than healthy controls ($p \leqq 0.05$ for all values).

Table 1
Demographic and biochemical characteristic of NAFLD and Diabetic type 2 study group

Variable	*NAFLD Group*	*Control Group*	*Mean difference (95% CI)*	*P value*
Sex, No. (%)	0.402			
Female	37 (51.4%) 42 (59.2%) NA			
Male	35 (48.6%) 29 (40.8%) NA			
Age	42.00 (35.5 – 49)	40 (33 – 45)	NA	0.167
BMI (kg/m)	31.69 (4.174)	31.37 (3.96)	3.164 (-1.0357 – 1.6686)	0.644
BMR(kcal/d)	1732 (1502 – 2017)	1600 (1426 – 1994)	NA	0.329
FAT (%)	33.6 (24.9 – 39.6)	35.6 (27.35 – 39.75)	NA	0.297
FFM (%)	56.9 (49.35 – 68.2)	52.7 (46.25 – 68)	NA	0.298
FSG (mg/dl)	91.21 (11.35)	89.77 (9.91)	1.352 (-2.153 – 4.857)	0.424
Cholesterol (mg/dl)	183.28 (36.64)	188.17(30.72)	-4.741 (-15.923 – 6.441)	0.391
TG (mg/dl)	152 (114 – 225)	130 (84 – 207)	NA	0.039
HDL (mg/dl)	45 (33.5 – 51)	48 (38.5 – 58)	NA	0.036
LDL (mg/dl)	104.16 (35.98)	111.74 (28.27)	-7.5706 (-18.328 – 3.186)	0.166
ALT (IU/L)	44 (26.5 – 66.5)	25 (19 – 33.5)	NA	0.00
AST (IU/L)	32 (20.5 – 38.5)	23 (19 – 28)	NA	0.00
Variable	Diabetic Group	Control Group	Mean difference (95% CI)	P value
Sex, No. (%)	0.351			
Female	36 (45%) 29 (37.7%) NA			
Male	44 (55%) 48 (62.3%) NA			
Age	55.5 (7.02)	53.21 (8.51)	2.29 (-0.16 – 4.75)	0.06
BMI (kg/m)	24.26 (3.78)	25.38 (3.23)	-1.116 (-2.22 – -0.0049)	0.05
FSG (mg/dl)	146 (127.5 – 206.5)	90 (84.0 – 97.0)	NA	0.00
CHOL (mg/dl)	189.32 (50.156)	188.91 (36.496)	0.414 (-13.99 – 14.82)	0.95
TG (mg/dl)	183.0 (122.5 – 255.0)	130.0 (88.5 – 212.0)	NA	0.002
LDL (mg/dl)	95.6 (23.543)	107.54 (29.712)	-12.385 (-24.177 – 0.594)	0.04
HDL (mg/dl)	40.0 (35.0 – 45)	48.0 (39.0 – 58.0)	NA	0.001

Note

NAFLD group: CI, confidence interval; NA, not applicable. P value for sex is based on chi squares; for BMI, FSG, cholesterol and LDL are based on independent t-testing; otherwise, based on Mann-Whitney testing. BMI, FSG, cholesterol, LDL values are presented based on mean (SD) values; data for other variables are presented based on median (P25-P75).

Diabetic group: P value for sex is based on chi squares and for FSG, TG, HDL values are based on Mann-Whitney testing; other parameters are based on independent t-testing using equal variable. FSG,TG, HDL values are presented based on median (P25-P75); data for other variables are presented based on mean (SD) values.

Genotypes and allele frequencies of polymorphism

In study population the frequency of 45 bp D/I genotypes from more to less are belonged to DD homozygote, DI heterozygotes and II homozygotes, respectively. PCR products for deletion and insertion alleles were 310 bp and 355 bp, respectively. There were no significant differences in genotypic distribution or allelic frequency of 45 bp Ins/Del between the NAFLD and control groups (p value > 0.05). However, UCP-2 I/I genotype (p = 0.025) and UCP-2 I allele (p = 0.004) were associated with susceptibility to T2DM. Subgroup analysis revealed that the proportion of subjects with homozygous genotype D/D was higher in control cases (57.7%) than in patients with NAFLD (51.4%) and that the proportions of heterozygous D/I was higher in NAFLD (40.3%) than in control groups (32.4%), although distribution of genotypes and allelic frequency was not significantly different between NAFLD patients and control subjects (see Table 2). Subgroup analysis in T2DM subjects also revealed that the frequency of subjects with homozygous genotype D/D was higher in control cases (64.9%) than in patients with T2DM (46.3%) and frequency of subjects with heterozygotes D/I and homozygous I/I were higher in patients with T2DM than in control cases (33.8% vs. 26.0% and 20.0% vs. 9.1%, respectively).

Table 2
Association of the 45-bp I/D polymorphism of UCP2 gene in NAFLD and Diabetes mellitus type 2 in the Study Population

45 bp Ins/Del	***NAFLD***				***T2DM***			
Genotypes	Case	Control	OR (95% CI)	P Value	Case	Control	OR (95% CI)	P Value
D/D	37 (51.4%)	41(57.7%)	Ref	-	37 (46.3%)	50 (64.9%)	Ref	-
D/I	29 (40.3%)	23(32.4%)	1.39 (0.69 – 2.81)	0.35	27 (33.8%)	20 (26.0%)	1.82 (0.89 – 3.73)	0.10
I/I	6 (8.30%)	7 (9.9%)	0.95 (0.29 – 3.08)	0.93	16 (20.0%)	7 (9.1%)	3.088 (1.153 – 8.26)	0.025
Alleles								
D	103(71.53%)	105(73.94%)	Ref	-	101(63.12%)	120 (77.92%)	Ref	-
I	41(28.47%)	37 (26.05)	1.13 (0.67 – 1.90)	0.647	59 (36.87%)	34 (22.07%)	2.06 (1.252 – 3.39)	0.004

Note

OR; odds ratio, CI; confidence interval. P value is based on logistic regression analysis.

Relationship between genotypes and laboratory data

Evaluation of continuous variables changes among different UCP2-45 bp D/I genotypes in NAFLD study group showed that HDL level in ones with D/D genotypes was significantly lower in NAFLD patients in comparison with control group. In addition, the level of ALT and AST in ones with D/D and D/I genotypes were significantly higher in NAFLD patient group compared to control group (Table 3). Moreover, in ones with D/D and D/I genotype the serum level of FSG was significantly higher in T2DM patients compared to healthy control. In individuals with I/I genotype the concentrations of cholesterol and LDL in serum were significantly lower in T2DM patients in matched with the healthy group (Table 3). No significant differences were observed between other clinical or laboratory characteristics and genotypes.

Table 3
Assessment of study variables based on UCP2 45 bp Ins/Del genotypes in NAFLD and T2DM patients with healthy groups

Variable	*Genotypes*	*NAFLD Group*	*Control Group*	*Mean Difference (95% CI)*	*P Value*
BMI (kg/m)	D/D	31.95 (4.28)	31.01 (3.52)	0.69 (-1.02 – 2.41)	0.424
	D/I	31.39 (4.12)	32.17 (4.79)	-0.15 (-2.65 – 2.34)	0.901
	I/I	31.56 (4.33)	31.52 (5.07)	-0.56 (-6.34 – 5.20)	0.829
BMR(kcal/d)	D/D	1658.0 (1488.0 – 1955.0)	1600 (1403.5 – 2022.5)	NA	0.52
	D/I	1812.0 (1592.5 – 2023.5)	1670.0 (1434.5 – 1999.0)	NA	0.803
	I/I	1580.00 (1462.5 – 2124.5)	1576.0 (1426.0 – 1830.0)	NA	0.241
FAT (%)	D/D	36.3 (24.7 – 41.6)	32.4 (27.7 – 38.15)	NA	0.83
	D/I	30.70 (25.25 – 36.25)	38.60 (26.65 – 42.95)	NA	0.246
	I/I	38.7 (22.5 – 39.65)	36.00 (26.30 – 39.5)	NA	0.39
FFM (%)	D/D	55.4 (47.7 – 65)	53.2 (45.8 – 68.3)	NA	0.71
	D/I	60.90 (52.35 – 68.30)	53.60 (46.05 – 67.70)	NA	0.983
	I/I	52.20 (48.05 – 72.10)	50.90 (46.20 – 61.90)	NA	0.66
FSG (mg/dl)	D/D	92.34 (11.45)	90.22 (10.26)	1.569 (-3.35 – 6.49)	0.52
	D/I	90.76(10.263)	90.29 (9.52)	-0.717 (-6.65 – 5.22)	0.809
	I/I	88.40 (16.11)	86.86 (9.20)	2.861(-16.03 – 21.7)	0.736
Cholesterol (mg/dl)	D/D	188.14(41.79)	187.51(29.750)	-0.66 (-17.28 – 15.94)	0.936
	D/I	179.79 (28.74)	191.52 (31.73)	-10.61 (-28.93 – 7.699)	0.249
	I/I	165.00 (13.26)	185.00 (38.30)	-27.17 (-71.13 – 16.78)	0.192
TG (mg/dl)	D/D	154.00 (114 – 225)	117.00 (77 – 213)	NA	0.066
	D/I	152.00 (100.0 – 234.5)	142.00 (103.0 – 192.50)	NA	0.913
	I/I	144.0 (135.0 – 184.5)	140.0 (69.0 – 214.0)	NA	0.762
HDL (mg/dl)	D/D	45.00 (33.00 – 54.0)	48.00 (38.5 – 58.0)	NA	0.026
	D/I	45.00 (34.0 – 48.0)	45.00 (38.0 – 58.0)	NA	0.505

Variable	*Genotypes*	*NAFLD Group*	*Control Group*	*Mean Difference (95% CI)*	*P Value*
	I/I	45.0 (40.0 – 56.5)	54.00 (34.0 – 59.0)	NA	0.507
LDL (mg/dl)	D/D	103.120 (39.43)	110.62 (26.53)	-9.0 (-24.28 – 6.26)	0.244
	D/I	101.876 (26.44)	113.26 (29.78)	-8.83 (-25.64 – 7.98)	0.296
	I/I	86.08 (8.82)	108.25 (35.60)	-28.82 (-67.73 – 10.08)	0.126
ALT (IU/L)	D/D	44.00 (30.0 – 67.0)	22.00 (17.5 – 33.00)	NA	0.00
	D/I	50.00 (26.00 – 69.5)	29.00 (23.00 – 34.50)	NA	0.002
	I/I	27.00 (19.50 – 42.5)	31.00 (23.00 – 35.00)	NA	0.866
AST (IU/L)	D/D	32.00 (20.0 – 41.0)	21(17.5 – 27.5)	NA	0.00
	D/I	33.00 (22.00 – 38.50)	24.00 (21.50 – 27.50)	NA	0.012
	I/I	19.00 (19.00 – 27.00)	27.00 (21.00 – 34.00)	NA	0.095
Variable	Genotypes	Diabetic Group	Control Group	Mean Difference (95% CI)	P Value
BMI (kg/m)	D/D	24.81 (4.32)	25.30 (3.13)	-0.598 (-3.19 – 1.99)	0.646
	D/I	24.89 (4.344)	26.007 (2.81)	-2.511 (-7.03 – 2.01)	0.263
	I/I	22.95 (2.55)	25.65 (3.45)	-4.527 (-10.644 – 1.59)	0.13
FSG (mg/dl)	D/D	133.0 (126.0 – 193.0)	90.0 (84.0 – 96.0)	NA	0.00
	D/I	146.0 (134.0 – 247.0)	94.50 (85.50 – 98.0)	NA	0.006
	I/I	136.0 (117.0 – 190.0)	88.0 (76.0 – 96.0)	NA	0.148
CHOL (mg/dl)	D/D	167.31 (36.48)	184.40 (35.86)	-23.497 (-50.37– 3.37)	0.085
	D/I	206.73 (59.84)	189.25 (28.23)	10.79 (-40.79 – 62.38)	0.669
	I/I	168.57 (20.065)	185.00 (38.30)	-56.975 (-111.68 – -2.26)	0.043
TG (mg/dl)	D/D	129.0 (107.5 – 179.5)	123.0 (78.0 – 211.0)	NA	0.966
	D/I	278.0 (183.0 – 298.0)	150.0 (108.7– 202.5)	NA	0.444
	I/I	197.0 (115.0 – 215.0)	140.0 (69.0 – 214.0)	NA	0.836
LDL (mg/dl)	D/D	37.46 (5.66)	48.26 (12.37)	-20.36(-41.68 – 0.95)	0.061
	D/I	40.64 (7.46)	45.44 (12.49)	-2.83 (-40.34 – 34.67)	0.877

Variable	*Genotypes*	*NAFLD Group*	*Control Group*	*Mean Difference (95% CI)*	*P Value*
	I/I	88.29 (10.193)	108.0 (35.42)	-54.004 (-100.93 – -7.07)	0.028
HDL (mg/dl)	D/D	38.00 (34.50 – 42.5)	49.00 (39.0 – 58.0)	NA	0.263
	D/I	42.0 (34.00 – 47.00)	44.0 (34.5 – 57.5)	NA	0.939
	I/I	40.0 (38.0 – 43.0)	54.0 (34.0 – 59.0)	NA	0.653

Discussion

The UCP2 is the most broadly distributed UCP gene family member which highly expressed in pancreatic β-cells. The three prominently evaluated polymorphisms in the UCP2 gene are Ala55Val polymorphism in the exon 4, the − 866G/A polymorphism in the promoter region and the 45 bp D/I polymorphism in the 3’ UTR of exon 8. Importantly, these variants have been revealed to be associated with different metabolic traits in several populations from various ethnic groups [25]. However, there exist some researches which failed to present notable association between these variants and metabolic disorders [26, 27]. The 45 bp D/I polymorphism was first associated with energy balance in Pima Native Americans. Whereas biological effect of the 3'UTR D/I is not well recognized, its location in the 3'UTR may possibly involve in the transcript stability or in mRNA processing [28].

A large number of studies in different populations have verified that carriers of the I-allele of UCP-2 gene had a significantly higher BMI and promoted possibility of the obesity [27, 29]. Besides, it was found that individuals with I/D genotype had an increased rate in basal metabolic, high energy expenditure and lower BMI [30]. Another study revealed that patients with D/D genotype had a remarkable enhancement in total and truncal fat mass and body weight [31]. An investigation carried out on Korean female population by Yong Hwan Lee et al. displayed that subjects with a 45 bp I allele of UCP2 might have a higher risk of developing obesity [32]. Other investigations noticed a significant association between this polymorphism and BMI mean differences in European population [33]. Additionally, correlation between 45-bp I/D polymorphism of UCP2 and metabolic syndrome (MeS) has been demonstrated following case control study on 151 patients with MeS [34]. On the other hand, one study on 268 obese and 185 nonobese children and adolescents showed that the ins allele of the UCP2 45-bp D/I polymorphism may contribute to low HDL cholesterolemia [35]. Besides, various studies have presented UCP-2 45-bp D/I polymorphism association with higher degree of obesity, insulin resistance, dyslipideamia and lower adjusted metabolic rate [36, 37]. Conversely, other studies did not describe any correlation between UCP2 45-bp I/D polymorphism and obesity [38, 39]. These inconsistencies are likely due to small sample size and incomplete coverage of the UCP2 gene variations or potential population-specific influences on metabolic traits [40, 41]. The important consequence of the current study is that the UCP2 45 bp D/I polymorphism has the potential to affect liver cells function (as measured by HDL, AST, TG and ALT levels). On the other hand, we found that patients with NAFLD and UCP2 45 bp D/D or D/I genotype had significantly higher TG, ALT and AST, and lower HDL levels than healthy controls. Besides, patients with T2DM and UCP2 45 bp D/D or D/I genotype had meaningfully higher FSG and lower cholesterol and LDL levels than healthy controls, respectively.

Conclusion

According results, there was association between UCP2-45 bp I/I polymorphism and elevated risk for T2DM, in a North-West population of Iran. Moreover, we found that there is no significant association between UCP2-45 bp D/I polymorphism and NAFLD.

Limitations

These results are specifically valid for the study population and its generalization to other population needs further studies.

Abbreviations

NAFLD, nonalcoholic fatty liver disease;; BMI, body mass index; BMR, basal metabolic rate; FFM, fat-free mass; FSG, fasting serum glucose; HDL, high-density cholesterol; LDL, low-density cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TG, triglyceride.

Declarations

Ethics approval and consent to participate

The subjects were informed about the purpose of the study and written informed consent was obtained. The study was approved by the Ethics Committee of Tabriz University Of Medical Sciences.

Availability of data and materials

All data used or analyzed during this study are included in this published article.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SR performed the experiments and wrote the first draft of the manuscript, SF involved in themanagement of the project and supervised the study, SA and FM analyzed data and co-wrote the paper, HF involved in data collection and analysis and MK prepared the revised version of the manuscript. All authors read and approved the final manuscript.

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