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Common Variations in *Perilipin* rs1052700 and *FTO* rs3751812 Gene Variants, and Risk for Obesity and Type-2 Diabetes

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Abstract

Background: *Perilipins* are proteins that coat lipid globules in adipocytes, which are steroid-generating cells that play a central role in lipid storage and breakdown. The FTO gene is associated with type-2 diabetes (T2D) and increased fat mass. In this work the association of *perilipin* and FTO gene polymorphisms in T2D was investigated.

Methods: One hundred eighty-three Iranian men and women with T2D, and 174 healthy controls with no known metabolic diseases, participated in the study. The subjects were genotyped using tetra-primer amplification refractory mutation system-polymerase chain reaction (Tetra-ARMS-PCR) and their clinical traits were analyzed.

Results: A significant association was found between the *perilipin* rs1052700 polymorphism and T2D, and between the *FTO* rs3751812 polymorphism and obesity (P = 0.03 and 0.003, respectively); however, no significant relationship was found between rs3751812 and T2D.

Conclusions: The *FTO* rs3751812 polymorphism is a major genetic determinant of obesity, but not T2D. The *perilipin* rs1052700 polymorphism is related to T2D but not obesity.

Keywords: FTO, Gene polymorphism, *Perilipin*, Type-2 diabetes.

Introduction

The worldwide incidence of type-2 diabetes (T2D) has increased dramatically in recent years and is a major cause of metabolic disorders, heart disease, stroke, diabetic retinopathy, kidney failure, and poor circulation in the hands and feet, which may lead to amputation (1, 2).

In a genome-wide association study (GWAS), the fat mass and obesity-associated (FTO) locus has been recognized to contain *FTO*, a gene associated with increased risk of T2D in humans (3). In mice, *FTO* is expressed in many tissues, including brain. It is located on a section of chromosome 8 that is removed by the fused toes mutation (4). Dina et al. (5) surveyed the expression of *FTO* in human tissues and found it in all tissues tested, and in animal studies *FTO* was expressed in the brain. The dependence between *FTO* genotype and T2D proposes that *FTO* alleles, which enhance adiposity, have detrimental metabolic effects. Obesity is associated with insulin resistance, nonalcoholic fatty liver disease, hyperglycemia, hypertension, and dyslipidemia in the general population (6). A survey of the effects of *FTO* variation on clinical characteristics may improve our understanding of how genetic alterations to *FTO* could predict T2D and other diabetes-related diseases. In this study, the associations between the *FTO* rs3751812 polymorphism and T2D, and metabolic traits,

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including fasting blood glucose (FBG), total cholesterol (TC), triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C) were investigated. It was assumed that the *FTO* variant, through body mass index (BMI), would be related to metabolic traits, and changes in metabolic factors may predispose a person to T2D. However, the effects of the *FTO* genotype on pre-diabetic-mediated characteristics are not known.

The other gene investigated in this study was *perilipin* rs1052700, which encodes the *perilipin* protein, also known as PLIN or lipid droplet-associated protein. *Perilipins* are phosphoproteins that coat intracellular lipid droplets (7). In humans, *perilipin* is located at chromosomal location 15q26.1 (8) and has been shown to be a susceptibility locus for obesity, diabetes, and hypertriglyceridemia (9).

Perilipin is a target of protein kinase A (PKA), and non-phosphorylated *perilipin* may inhibit the hormone-sensitive lipase (HSL) that mediates lipolysis of TAG's in lipid droplets (10). With phosphorylation, *perilipin* may activate HSL function (11). Therefore, *perilipin* A acts to increase cellular TAG storage by decreasing the rate of TAG hydrolysis. This activity may have a role in obesity and changes in lipid metabolism. Several studies have related *perilipin* expression to obesity.

Two studies identified obese individuals with lower levels of *perilipin* than thinner individuals (12); however, another study demonstrated that p*erilipin* mRNA and protein were elevated in obese individuals (13). In this survey, the genetic variability of *perilipin* and *FTO* and their relationship with obesity, T2D, metabolic traits, and related characteristics in a southeast Iranian population were investigated.

Materials and Methods

Subjects and clinical analysis

The study was designed and subjects enrolled according to the American Diabetes Association (ADA) (14) and as described in our previous reports (2, 15). The study population included 137 females and 46 males patients with T2D with a mean age of 54.10 ± 9.33 years, and 123 females and 51 males healthy controls with a mean age of 48.95 ± 9.65 years. The study was performed in Ali-Asghar Hospital in southeast Iran. All subjects provided informed consent before entering the study. The body mass index (BMI) was determined for all subjects and biochemical measurements were performed using standard methods. FBG, HDL-C, TC, TG, and LDL-C were determined for all subjects via enzymatic procedures and the Friedewald formula.

This study was financed by the Zahedan University of Medical Science, and the University Ethics Committee approved this study with code 7179.

DNA extraction and genotyping

Two mL of whole blood were collected from all subjects into CBC tubes containing 0.5M EDTA and stored at -20 ^{0}C until use. Genomic DNA was extracted from all samples using the salting-out method as previously described (16). DNA extracts were analyzed for quality by electrophoresis and DNA concentrations were determined by Nano-Drop. Both *perilipin* rs1052700 and *FTO* rs3751812 single nucleotide polymorphisms (SNPs) were analyzed by tetra primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR). DNA was amplified by PCR in 20 µL volumes containing 10 µL of master mix (Taq 2x Mastermix, Ampliqon, Denmark), 1µL (10pmol/mL) each of forward and reverse primers, 1.5 µL of template DNA, and 4.5 µL of DNase-free water (17). For *perilipin* rs1052700 and *FTO* rs3751812, the amplifications were performed with an initial denaturation step at 95 ^{0}C for 5 min, followed by 30 cycles of denaturation at 95 ^{0}C for 30 sec, annealing 65 ^{0}C for 30 sec, and extension at 72 ^{0}C for 30 sec, with a final extension at 72 ^{0}C for 5 min. The primer sequences were as follows:

for *perilipin*:

forward outer:
CCTCTGCGGATAAATATTTGCCACGAAT
reverse outer:
TAATGATGCAAATGGAAATGTGGCTGTG
forward inner:
CCCTCTGATGAACATCCTCTGATGAACA
reverse inner:
CTTCAAAGTAGCCTGCTGGGAGCGTA
for *FTO*:
forward outer:
TTTGGCTCTCCTTGTGTCTTTTCATGTG
reverse outer:
GCAATGACTGGTAATGAAACCTACTTGGCA
forward inner:
AGACCTGAAAATAGGTGAGCTGTCAGGG
reverse inner:
CCAGAGGAGCCTCTCCCTGCCAATAA

Statistical analyses

SPSS version 21.0 (SPSS, Chicago, IL, USA) was used for all statistical analyses. Means ± standard deviations were used to describe the quantitative data, and the student's t-test was used to compare the quantitative variants of the T2Ds and healthy controls. The Fisher's exact test or the $\chi 2$-test was used to analyze genotypes and data frequencies. The allele frequencies were determined by gene counting. The Hardy–Weinberg equilibrium (HWE) was examined using the $\chi 2$ for each SNP. P values < 0.05 were considered statistically significant.

Results

FTO and *perilipin* polymorphisms of the T2D and control subjects were compared. Significant differences were found between the two groups for TG, FBG, and BMI (P < 0.05, Table 1). Regarding the *FTO* rs3751812, no significant differences were found between the two groups for genotypes or alleles (Table 2); however, significant differences in obesity (BMI ≥ 25) and TC were found between the *FTO* genotypes (Table 3) and between the groups for age, BMI, TG, and FBG in the TT genotype and for age, BMI, LDL-C, and FBG in the GT-GG genotype (Table 4). The genotype and allele frequencies of the *perilipin* rs1052700 polymorphism in the T2D and control groups are summarized in Table 5. Significant differences were observed between the three *perilipin* genotypes and *perilipin* alleles (genotypes, P = 0.03 and alleles, P < 0.001). Significant differences were observed for age ≤ 50 years, females, FBG < 110, and TG ≥ 130 between the three *perilipin* rs1052700 polymorphism genotypes (Table 6), and significant differences were found between T2D and control groups with TT genotypes for age, gender, BMI, FBG, and TG, and with the TA-AA genotype between T2D patients and controls for age, BMI, and FBG (Table 7).

The subjects' loci were analyzed for Hardy-Weinberg equilibrium (HWE). The genotype distribution of *FTO* rs3751812 was in HWE for healthy controls (P = 0.8462, $\chi 2$ = 0.04) and T2Ds (P = 0.1284, $\chi 2$ = 2.31); however, *perilipin* rs1052700 was not in HWE for both groups (P < 0.05, $\chi 2$ = 174 and P < 0.05. $\chi 2$ = 166.41 for healthy controls and T2Ds, respectively).

Table 1. Clinical and demographic characteristics in control and T2D subjects.

Characteristics	Healthy Control (n = 174)	T2D (n = 183)	P value
Age in years ± SD	48.95 ± 9.65	54.10 ± 9.33	0.543
Gender, No (%) Female	123 (67)	137 (79)	0.222
TC (mg/dL)	180 ± 36.64	183.39 ± 43.95	0.533
TG (mg/dL)	124 (89)	148 (90)	**0.005**
HDL-C (mg/dL)	52.92 ± 16.58	47.63 ± 21.66	0.603
LDL-C (mg/dL)	104.15 ± 33.33	93.92 ± 24.71	0. 190
FBG (mg/dL)	93 (14)	180.23 (104)	**P < 0.001**
BMI (kg/m2)	23 (5)	26.95 (5)	**P < 0.001**

TC: total cholesterol; TG: triglycerides; HDL-C: high density lipoprotein-cholesterol; LDL-C: low density lipoprotein-cholesterol; FBG: fasting blood glucose; BMI: body mass index.

Table 2. Genotype and allele frequencies of the *FTO* rs3751812 polymorphism in T2D and control subjects.

	Genotypes			Alleles	
	TT	**GT**	**GG**	**T**	**G**
T2D (n =183) N (%)	63 (34.4)	98 (53.6)	22 (12)	224 (61.2)	142 (38.8)
Control (n = 174) N (%)	61 (35.1)	83 (47.7)	30 (17.2)	205 (58.9)	143 (41.1)
P value		0.320		0.53	

Table 3. Association between the *FTO* rs3751812T/G polymorphism and clinical characteristics.

Variable	*FTO* rs3751812			
	TT	**GT**	**GG**	**P value**
Age (years)				
≤50 n (%)	31 (17)	82 (46)	67 (37)	0.440
>50	21 (12)	99 (56)	57 (32)	0.464
Gender, n (%)				
Female	35 (14)	131 (50)	94 (36)	0.694
Male	17 (18)	50 (51)	30 (31)	0.737
BMI (Kg/m2)				
< 25 n (%)	24 (13)	82 (46)	72 (41)	0.180
≥25	28 (15)	99 (55)	52 (30)	**0.003**
TC (mg/dL)	146.43 ± 50.	132 ± 47	195 ± 61.6	**0.045**
TG (mg/dL)				
<130 n (%)	26 (15)	81 (51)	60 (36)	0.692
≥130	23 (13)	95 (52)	63 (35)	0.897
HDL-C (mg/dL)	52.26 ± 15.47	51.26 ± 12.57	55.49 ± 17.7	0.131
LDL-C (mg/dL)	107.45 ± 26	104.47 ± 32.32	101.83 ± 42.22	0.601
FBG (mg/dL)				
< 110 n (%)	30 (16)	93 (51)	61 (33)	0.056
≥110	20 (12)	83 (50)	62 (38)	0.468

TC: total cholesterol, TG: triglycerides, HDL-C: high density lipoprotein-cholesterol, LDL-C: low density lipoprotein-cholesterol, FBG: fasting blood glucose, BMI: body mass index.

Table 4. Baseline characteristics of the study population according to the *FTO* gene rs3751812T/G

Characteristics	TT			GT-GG		
	Patients (n = 63)	Controls (n = 61)	P value	Patients (n = 120)	Controls (n = 113)	P value
Age in years ± SD	52 (13)	48 (11)	**0.003**	55 (13)	47 (14)	**< 0.001**
Gender, No (%) Female	48 (76)	46 (75)	0.921	89 (74)	77 (68)	0.312
BMI (Kg/m^2)	26.29 (4)	21 (6)	**< 0.001**	27.38 (6)	23.42 (4)	**< 0.001**
TC (mg/dL)	187.6±55.65	181±41.06	0.455	181 ± 36.28	181 ± 33	0.980
TG (mg/dL)	148 (99)	106 (69)	**0.004**	148 (96)	126 (92)	0.379
HDL-C (mg/dL)	53.30 ± 18.23	55.89 ± 15.37	0.450	54.75 ± 21.30	52.91 ± 13.70	0.507
LDL-C(mg/dL)	101±41.57	100.54±29	0.860	97±28.52	107±29.95	**0.022**
FBG (mg/dL)	186 (11)	94 (14)	**< 0.001**	159.50 (96)	93 (14)	**< 0.001**

TC: total cholesterol, TG: triglycerides, HDL-C: high density lipoprotein-cholesterol, LDL-C: low density lipoprotein-cholesterol, FBG: fasting blood glucose, BMI: body mass index.

Table 5. Genotype and allele frequencies of the *perilipin* rs1052700 polymorphism in T2D patients and controls.

	Genotypes			Alleles	
	TT	**TA**	**AA**	**T**	**A**
Patients(n=183) N (%)	171 (93.4)	1 (0.6)	11 (6)	343 (93.7)	23 (6.3)
Control (n=174) N (%)	150 (86.2)	0 (0)	24 (13.8)	300 (86.2)	48 (13.8)
P value		**0.03**		**P < 0.001**	

Table 6. Association between the *perilipin* rs1052700 gene polymorphism and clinical characteristics.

Perilipin	**Variable**			**p-value**
	AA	**AT**	**TT**	
Age (years)				
≤50 n (%)	21 (12)	0 (0)	159 (88)	**0.027**
>50	14 (8)	1 (0.5)	162 (91.5)	0.312
Gender, n (%)				
Female	27 (10.5)	1 (0.3)	232 (36)	**0.011**
Male	8 (7)	0 (0)	89 (92)	0.586
BMI (Kg/m2)				
< 25 n (%)	22 (12)	0 (0)	156 (88)	0.098
≥ 25	13 (7.3)	1 (0.5)	165 (92.2)	0.249
TC (mg/dL)	185.91 ± 44.96	134	176.46 ± 37.95	0. 478
TG (mg/dL)				
< 130 n (%)	20 (12)	0 (0)	147 (88)	0.406
≥ 130	15 (8)	1 (0.5)	165 (91.5)	**0.010**
HDL-C (mg/dL)	54.02 ± 12.73	44	53.33 ± 17.76	0.116
LDL-C (mg/dL)	98.98 ± 26.55	63	102.98 ± 35.78	0.724
FBG (mg/dL)				
< 110 n (%)	23 (12)	0 (0)	161 (88)	**0.01**
≥ 110	12 (7.2)	1 (0.6)	152 (92.2)	0.490

TC: total cholesterol, TG: triglycerides, HDL-C: high density lipoprotein-cholesterol, LDL-C: low density lipoprotein-cholesterol, FBG: fasting blood glucose, BMI: body mass index.

Table 7. Baseline characteristics of the study population according to the *perilipin* rs1052700 gene

Characteristics	**TT**			**TA-AA**		
	Patients (n = 171)	Controls (n = 150)	P value	Patients (n = 12)	Controls (n = 24)	P value
Age in years ± SD	53 (13)	48 (13)	**< 0.001**	54.5 (14)	45.5 (12)	**0.025**
Gender, No (%) Female	128 (55)	104 (45)	**< 0.001**	9 (75)	19 (79)	0.051
BMI (Kg/m2)	26.95 (5)	23 (5)	**< 0.001**	26.85 (8)	21 (6)	**0.008**
TC (mg/dL)	184.2 ± 44.4	180.9 ± 34.9	0.451	170.8 ± 36.8	179.3 ± 46.6	0.586
TG (mg/dL)	149 (98)	125 (91)	**0.021**	126.50 (47)	94.50 (14)	0.717
HDL-C (mg/dL)	169 ± 54.71	99 ± 54.02	0.763	47.63 ± 21.6	52.9 ± 16.6	0.498
LDL-C (mg/dL)	99 ± 34.17	105 ± 29.64	0.149	93.3 ± 24.7	104.15 ± 33	0.368
FBG (mg/dL)	173 (110)	93 (14)	**< 0.001**	179 (84)	94.5 (14)	**< 0.001**

TC: total cholesterol, TG: triglycerides, HDL-C: high density lipoprotein-cholesterol, LDL-C: low density lipoprotein-cholesterol, FBG: fasting blood glucose, BMI: body mass index.

Discussion

Type 2 diabetes is recognized when any of the following criteria are met: FBG ≥ 126 mg/dL (7.0 mmol/L), 2-hour plasma glucose ≥ 200 mg/dL (11.1mmol/L), or a random blood glucose measurement ≥ 200 mg/dL (11.1mmol/L) in the presence of classic symptoms such as polydipsia and polyuria(17). A hemoglobin A1C measurement of 6.5% is also been considered as an indicator of T2D (18). All these conditions were confirmed in the current study on diabetic patients. However, obesity and T2D are increasing worldwide. In the present study, the impact of the genetic variability of *perilipin* and *FTO* on obesity, T2D, and related clinical and demographic characteristics in a sample of southeastern Iranian men and women with T2D (n = 183) was assessed. Our results provide evidence that *FTO* genetic variants are associated with obesity risk and related clinical traits in this population. Evidence from GWAS and meta-analyses supported the relationship of *FTO* variants with obesity and related traits in Caucasians (19, 20). *FTO* variants increased obesity risk by 1.20 to 1.32-fold in Europeans (21) and by 1.25-fold in Asians (22). Our findings indicate that the lack of association between *FTO* variants and T2D in Iranians is due to relativity small sample size and low allele frequency in the Iranian population. However, no association was found between *FTO* genotypes and T2D in the study population. In agreement with our results, Scuteri et al. found no significant association between *FTO* rs3751812 and T2D in African individuals (25). In addition, Hennig et al. found no relationship between several FTO SNPs, including rs3751812 and rs9931494 at this locus, and diabetes in a Gambian population (23). Bressle et al. (24) reported that the risk of T2D and obesity was differentially associated with variation in *FTO* rs9939609 in whites and African-Americans. Perhaps another cause of the lack of association between *FTO* and T2D in our study was the relatively low number of subjects. To achieve more accurate results, further studies are required. Another gene in the current survey was *perilipin* rs1052700. In contrast to *FTO* rs3751812, *perilipin* rs1052700 was significantly associated with T2D; however, no correlation was observed between the *perilipin* rs1052700 polymorphism and BMI. Wang (25) detected an association between *ENPP1* and *perilipin* with an increased risk of T2D in a Taiwanese population. In clinical practice, the World Health Organization (WHO) classifies a person with a BMI ≥ 25 kg/m2 as obese (26). Because obesity is considered a pre-diabetic condition, the aim of the present study was to assess the association between the obesity-related genes *perilipin* and *FTO* and an individual's diabetes risk. Gender and age are associated with obesity and body composition; for example, women tend to store more fat subcutaneously than in visceral adipose tissue, and carry more body fat than men (27). Yu et al. (28) reported two genetic variants of *Perilipin* Gene (PLIN) rs1052700 and rs894160; the results detected that fasting glucose concentration and diabetes risk increased significantly with increase of BMI also they found adult with allele T in rs1052700 or with allele A in rs894160 of *Perilipin* Gene may have a high risk for diabetes. However, Bergmann et al. (29) found no association between *perilipin* polymorphisms and obesity and weight variations in people at high risk for T2D. In a survey of an Asian population, the *perilipin* polymorphism was associated with increased obesity risk (30).

The reason for the deviation from HWE in our study is not clear but may be due to small sample size, migration, and/or subjects who were offspring of consanguineous marriages, which are commons in southeast Iran.

In conclusion, the *FTO* rs3751812 polymorphism is a major genetic determinant of obesity, but not T2D. The *perilipin* rs1052700 polymorphism is related to T2D but not obesity.

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The authors declare that there is no conflict of interests to disclose.

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