Research Article

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Investigation of *GHRL* (rs4684677), *FTO* (rs8044769) and *PGC1A* (rs8192678) polymorphisms in type 2 diabetic Turkish population

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Abstract

Objectives: Diabetes is a chronic group of metabolic disorders those generally present with hyperglycemia hence insulin synthesis defects due to multifactorial causes in beta cells in the Langerhans islets of the pancreas. In the development of diabetes, genetic predisposition is as important as environmental factors. As a result of polymorphism studies in diabetic patients, many genes were associated with the development of diabetes. In our study, we aimed to represent the relationship between diabetes and certain variants of the ghrelin *(GHRL)*, fat mass and obesity-associated protein *(FTO)* and peroxisome proliferator-activated receptor-gamma coactivator *(PGC-1α)* genes which are generally associated with diabetes and obesity.

Methods: One-hundred type 2 diabetes mellitus (T2DM) patients and ninety-four healthy volunteers were enrolled in our study. *GHRL* (rs4684677), *FTO* (rs8044769) and *PGC-1α* (rs8192678) gene polymorphism studies were performed by the real-time PCR method.

Results: The carriers of the TT genotype for the *FTO* (rs8044769) and the GG genotype for the *PGC-1α* (rs8192678) variants were found more frequently in the patient group, while the *GHRL* (rs4684677) did not differ between the groups. For the *PGC-1α* (rs8192678) variant in the patient group, glucose and BMI levels were observed significantly higher in carriers of the GA genotype than those with the GG genotype. There was no statistical difference in the distribution of *GHRL* (rs4684677) alleles among the groups.

Conclusions: We conclude that the *FTO* (rs8044769) and *PGC-1α* (rs8192678) variants are associated with T2DM in the Turkish population. However, there is no association between *GHRL* (rs4684677) and T2DM.

Keywords: genetic polymorphism; ghrelin; single nucleotide polymorphism; type 2 diabetes mellitus.

Introduction

Diabetes mellitus (DM) is a heterogeneous metabolic disorder characterized by the presence of hyperglycemia due to impaired insulin secretion, reduced effect on the target tissue, or both [1]. Major risk factors for the development of type 2 diabetes (T2DM) include age, heredity, ethnicity, obesity, dyslipidemia, visceral steatosis, and sedentary life [2].

T2DM is generally a complex multifactorial polygenic disease. Disease development occurs through the interaction of many different genes, including environmental factors. Studies that include twins and individuals belonging to the same family have shown that the heritability of T2DM varies between 30 and 70% depending on the age of diabetes onset and the glycemic status of the cases. Many studies, including genome-wide association studies (GWAS), have demonstrated the polygenic nature of T2DM and the localization of related genes [3].

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GWAS studies provide estimates and predictions about the genetic nature of T2DM, including the number, frequency and impact power of risky variants in different ethnic communities around the world. With these studies, the polygenic structure of T2DM was revealed, many risky variants were identified in certain loci and allelic heterogeneity was demonstrated [4].

The human ghrelin gene *(GHRL)* is located on chromosome 3p26-p25, encodes the ghrelin-obestatin preproprotein. Ghrelin has many active functions such as including gastric acid secretion, mobility and protein output of the pancreas, modulation of cardiovascular function, osteoblast activation and stimulation of bone formation, stimulation of neurogenesis and myogenesis, learning and memory, thymopoesis, sleep/wake rhythm and neuroprotective effects in neurodegenerative diseases and aging [5, 6].

The fat mass and obesity-associated protein *(FTO)* gene is located on the long arm of chromosome 16 (16q12.2) and encodes a protein that has 2-oxoglutarate and iron-dependent nucleic acid demethylase enzyme activity. As a result of GWAS studies, the *FTO* gene was identified as the most strongly associated gene with all types of obesity. The *FTO* gene is expressed in the appetite control region in the hypothalamus. *FTO* genetic variants were found to increase the risk of weight gain, cause more food consumption, and affect the appetite-control center [7].

The *PPARGC1A* gene encodes the peroxisome proliferator-activated receptor-gamma co-activator 1-alpha (PGC-1α) protein that acts as a metabolic cell regulator in various tissues and is frequently investigated in T2DM etiology studies. *PGC-1α* regulates mitochondrial biogenesis, exercise adaptation and fiber type change in skeletal muscle, cold-induced thermogenesis in brown adipocytes, starvation adaptation, and maintenance of glucose homeostasis [8].

The main purpose of this study is to examine certain polymorphisms that could be seen in the *GHRL, FTO and PGC-1α* genes, which are known to be involved in carbohydrate and lipid metabolism, in patients diagnosed with T2DM.

Materials and methods

One hundred T2DM patients and ninety-four healthy volunteers enrolled on this study. The study was conducted at Istanbul Education and Research Hospital, Istanbul, Turkey. Individuals who were diagnosed with T2DM according to the established American Diabetes Association criteria were included in the patient group. We excluded individuals with a history of chronic diseases, including DM, cancer, hypertension, anemia, thyroid diseases, hepatic diseases, kidney disease and autoimmune diseases for control group. All individuals were Caucasian and of Turkish origin. Venous blood samples were collected and incubated at room temperature for 20 min and then centrifuged at 3,000×*g* for 15 min and the serum was stored immediately at –80 °C until the working day. All samples were assayed of total cholesterol, HDL-C, LDL-C, triglycerides, glucose, HbA1c, ALT, AST, urea and creatinine on AU5800 Series Clinical Chemistry Analyzers (Beckman Coulter Inc., Brea, CA, USA. Genotyping by real-time polymerase chain reaction was used to detect *GHRL* (rs4684677), *FTO* (rs8044769) and *PGC-1α* (rs8192678) gene polymorphisms. Primers that were used for amplification of related single-nucleotide polymorphisms (SNP's) were given in Table 1.

Genomic DNA was extracted from the whole blood using the Qiagen FlexiGene DNA kit (Qiagen, CA, USA). The quality and purity of the isolated DNA were evaluated according to the ratio of absorbance at 260 and 280 nm. PCR was performed in a final volume of 9.95 µL, containing 2 µL of template-DNA, 0.025 µL of each primer, 2 µL of 2xqPCR Mix, Sybr Green and ultra-pure distilled water. All reactions were performed on the BioRad CFX96 (Bio-Rad, Hercules, CA) and evaluated by CFX Maestro Manager Software (Bio-Rad, Hercules, CA). Post-amplification melting curve analysis was applied to detect the allelic type of SNP's according to their characteristics of melting point temperature.

The distribution of genotypes and allele frequencies of the three gene polymorphisms were compared among the patient and control groups. The SPSS 21.0 program (SPSS Inc., Chicago, IL, USA) was used for the statistical comparisons of the demographic information of the groups. One-way ANOVA test, Post-Hoc test and Kruskal-Wallis test were performed to show the significance of difference among the groups for related alleles and biochemical parameters. The study was approved by the Ethics Committee of Istanbul University Faculty of Medicine (13/10/2017, 1174).

Results

Demographic characteristics of each group are summarized in Table 2. In the patient group, fasting blood glucose ($p<0.001$, 95% confidence interval=78.33–102.66), urea ($p<0.001$, 95% confidence interval=4.51–10.82), creatinine ($p<0.001$, 95% confidence interval=0.08–0.23), body mass

Table 1: Primer sequences for PCR amplification of *GHRL, FTO, and PGC-1α genes*.

Primer	Sequence
GHRL (rs4684677), F1	5′-TGGCTGTGCTGCTGGTACT-3′
GHRL (rs4684677), F2	5′-TGGCTGTGCTGCTGGTACC-3′
GHRL (rs4684677), R	5′-AGATGGTGAGTGGGAAGGTG-3′
FTO (rs8044769) F1	5′-AGTGTCATTAGCAGCATAATCTTG-3′
FTO (rs8044769) F2	5′-AGTGTCATTAGCAGCATAATCTTC-3′
FTO (rs8044769) R	5′-CAGAAACTACACCAGCCCTA-3′
PGC-1α (rs8192678) F	5′-TGTCATCAAACTGGCCATACA-3′
PGC-1α (rs8192678) R1	5′-CGACGAAGCAGTCAAGAACG-3′
PGC-1α (rs8192678) R2	5′-CGACGAAGCAGTCAAGAACA-3′

GHRL, ghrelin; *FTO*, fat mass and obesity-associated protein; *PGC-1α*, peroxisome proliferator-activated receptor-gamma coactivator.

Table 2: Demographic characteristic of type 2 diabetes mellitus patient and control groups.

	T2DM (n=100)	Control group (n=94)	p-Values
Sex, female/male	57/43	59/35	0.060
Age, years	56.58 ± 10.23	50.49 ± 12.62	0.058
BMI, kg/m^2	31.04 ± 5.89	27.49 ± 6.92	**<0.001**
Glucose, mmol/L	10.10 ± 3.27	5.07 ± 0.45	**<0.001**
Trigliseride, mmol/L	1.98 ± 1.36	1.33 ± 1.19	**0.001**
Total cholesterol, mmol/L	5.38 ± 1.13	5.36 ± 1.23	0.913
HDL-cholesterol, mmol/L	1.26 ± 0.27	1.40 ± 0.34	**0.002**
LDL-cholesterol, mmol/L	3.27 ± 0.94	3.37 ± 1.02	0.516
HbA1c, %	8.34 ± 1.66	5.35 ± 0.31	**<0.001**
Total cholesterol/HDL-cholesterol	4.39 ± 1.10	3.98 ± 1.13	**0.013**
AST, U/L	20.34 ± 8.34	20.16 ± 9.85	0.894
ALT, U/L	22.04 ± 11.05	22.63 ± 18.28	0.785
Urea, mmol/L	5.67 ± 2.26	4.39 ± 1.31	**<0.001**
Creatinin, µmol/L	71.62 ± 26.53	57.47 ± 14.15	**<0.001**

p-Value among the type 2 diabetes mellitus patients group and control group calculated by One-way ANOVA. Bold font p-Values indicate statistical significance.

index (p<0.001, 95% confidence interval=1.71–5.38), triglyceride (p=0.001, 95% confidence interval=78.00–89.69) and total cholesterol/HDL-C (p=0.013, 95% confidence interval=0.08–0.73) levels were found to be statistically higher than healthy controls. In the control group, HDL-C levels (p=0.002, 95% confidence interval=1.91–8.79) were statistically higher than the patient group.

FTO (rs8044769) genotypes and alleles distributions are shown in Table 3. The frequency of TT genotype increased statistically in the patient group compared to the control group (p=0.021, χ^2=5.30 OR=1.98, 95% confidence interval=1.10–3.57). In the control group, the frequency of C allele statistically increased compared to the patient group (p=0.021, χ^2=5.30 OR=1.30, 95% confidence interval=1.03–1.64).

Table 3: Distribution of *FTO* (rs8044769) genotypes and alleles in type 2 diabetes mellitus patient and control groups.

FTO (rs8044769)	T2DM (n=100)	Control group (n=94)	Odds ratio (95% CI)
Genotype			
CC	18.00%	22.30%	0.76 (0.37–1.54)
TT	47.00%[a]	30.90%	1.98 (1.10–3.57)
CT	35.00%	46.80%	0.61 (0.3436–1.08)
Allele			
C	35.50%	45.70%[b]	0.65 (0.43–0.98)
T	64.50%	54.30%	1.71 (1.14–2.55)

[a]p=0.021, χ^2=5.30. [b]p=0.021, χ^2=5.30. p-Value among the type 2 diabetes mellitus patient group and control group calculated by one-way ANOVA.

Table 4: Distribution of *GHRL* (rs4684677) genotypes and alleles in type 2 diabetes mellitus patient and control groups.

GHRL (rs4684677)	T2DM (n=100)	Control group (n=94)	Odds ratio (95% CI)
Genotype			
AA	95.00%	91.50%	1.76 (0.55–5.60)
TT	–	–	–
AT	5.00%	8.50%	0.56 (0.17–1.79)
Allele			
A	97.50%	95.70%	1.73 (0.55–5.39)
T	2.50%	4.30%	0.60 (0.19–1.89)

GHRL (rs4684677) genotypes and alleles distributions are shown in Table 4. There is no statistical significance among groups. TT genotype was not seen in both patient and control groups.

PGC-1α (rs8192678) genotypes and alleles distributions are shown in Table 5. The frequency of GG carrying increased statistically in the patient group compared to healthy controls (p<0.001, 95% confidence interval=1.19–1.49). In the control group, the frequency of carrying GA genotype (p=0.003, χ^2=8.85, OR=1.24, 95% confidence interval=1.07–1.43) and A allele (p<0.001, 95% confidence interval=1.19–1.49) was found statistically higher than the patient group.

Table 6 shows the distribution of *FTO* (rs8044769) genotypes according to biochemical profiles. Accordingly, LDL levels (p=0.048, 95% confidence interval=1.17–32.54) and total cholesterol/HDL-C ratio (p=0.035, 95% confidence interval=0.03–1.01) were found statistically higher in patients with TT genotype than those with CT genotype. In the control group, triglyceride levels were found statistically

Table 5: Distribution of *PGC-1α* (rs8192678) genotypes and alleles in type 2 diabetes mellitus patient and control groups.

PGC-1α (rs8192678)	T2DM (n=100)	Control group (n=94)	Odds ratio (95% CI)
Genotype			
GG	25.00%[a]	–	1.35 (1.19–1.49)
AA	4.00%	11.70%	
GA	7.00%	88.30%[b]	1.24 (1.07–1.43)
Allele			
G	60.50%	44.10%	2.12 (1.40–3.16)
A	39.50%	55.90%[c]	1.35 (1.19–1.49)

[a]p<0.001. [b]p=0.003. [c]p<0.001, OR=1.35 95% CI=1.19–1.49. p-Value among the type 2 diabetes mellitus patient group and control group calculated by one-way ANOVA.

Table 6: Distribution of *FTO* (rs8044769) genotypes according to biochemical profiles in type 2 diabetes mellitus patient and control groups.

Group	T2DM (n=100)			Control group (n=94)		
***FTO* (rs8044769)**	**CC (n=18)**	**TT (n=47)**	**CT (n=35)**	**CC (n=21)**	**TT (n=29)**	**CT (n=44)**
Triglyceride, mmol/L	1.70 ± 0.98	2.23 ± 1.73	1.78 ± 0.83	2.05 ± 2.18	1.19 ± 0.55[c]	1.08 ± 0.59[d]
Total cholesterol, mmol/L	5.41 ± 1.17	5.55 ± 1.23	5.12 ± 0.95	5.61 ± 1.48	5.46 ± 1.36	5.17 ± 0.99
HDL-cholesterol, mmol/L	1.33 ± 0.28	1.22 ± 0.26	1.27 ± 0.26	1.31 ± 0.32	1.50 ± 0.39	1.37 ± 0.30
LDL-cholesterol, mmol/L	3.20 ± 0.77	3.44 ± 0.98[a]	3.02 ± 0.93	3.47 ± 1.19	3.39 ± 1.17	3.28 ± 0.81
Total cholesterol/HDL-cholesterol	4.13 ± 0.89	4.67 ± 1.16[b]	4.14 ± 1.05	4.40 ± 1.19	3.79 ± 1.15	3.90 ± 1.06
BMI, kg/m^2	30.33 ± 5.88	31.84 ± 5.91	30.29 ± 5.88	27.57 ± 3.50	28.06 ± 8.45	27.06 ± 7.14
Glucose, mmol/L	9.69 ± 2.39	10.27 ± 3.31	10.09 ± 3.62	5.07 ± 0.54	4.99 ± 0.52	5.13 ± 0.34
HbA1c, %	8.30 ± 1.79	8.37 ± 1.65	8.33 ± 1.66	5.30 ± 0.32	5.39 ± 0.31	5.33 ± 0.31

[a]p=0.048. 95% CI=1.17–32.54. [b]p=0.035. 95% CI=0.03–1.01. [c]p=0.012. 95% CI=16.99–134.27. [d]p=0.002. 95% CI=31.17–139.58. p-Value among the type 2 diabetes mellitus patient group and control group calculated by one-way ANOVA.

Table 7: Distribution of the *GHRL* (rs4684677) genotypes according to biochemical profiles in type 2 diabetes mellitus patient and control groups.

Group	T2DM (n=100)			Control group (n=94)		
***GHRL* (rs4684677)**	**AA (n=95)**	**TT (n=0)**	**AT (n=5)**	**AA (n=86)**	**TT (n=0)**	**AT (n=8)**
Triglyceride, mmol/L	1.97 ± 1.38	–	2.17 ± 0.98	1.35 ± 1.23	–	1.13 ± 0.65
Total cholesterol, mmol/L	5.35 ± 1.15	–	5.93 ± 0.42	5.31 ± 1.15	–	5.81 ± 1.92
HDL-cholesterol, mmol/L	1.26 ± 0.27	–	1.27 ± 0.23	1.39 ± 0.33	–	1.51 ± 0.36
LDL-cholesterol, mmol/L	3.24 ± 0.95	–	3.65 ± 0.48	3.32 ± 0.96	–	3.76 ± 1.46
Total cholesterol/HDL-cholesterol	4.37 ± 1.12	–	4.74 ± 0.65	3.99 ± 1.16	–	3.79 ± 0.72
BMI, kg/m^2	30.97 ± 5.94	–	32.24 ± 5.04	27.43 ± 6.93	–	28.07 ± 7.28
Glucose, mmol/L	10.24 ± 3.30	–	7.60 ± 1.07	5.08 ± 0.44	–	4.98 ± 0.59
HbA1c, %	8.42 ± 1.67	–	6.92 ± 0.31	5.36 ± 0.31	–	5.20 ± 0.28

Table 8: Distribution of the *PGC-1α* (rs8192678) genotypes according to biochemical profiles type 2 diabetes mellitus patient and control groups.

Group	T2DM (n=100)			Control group (n=94)		
***PGC-1α* (rs8192678)**	**GG (n=25)**	**AA (n=4)**	**GA (n=71)**	**GG (n=0)**	**AA (n=11)**	**GA (n=83)**
Triglyceride, mmol/L	1.86 ± 1.61	2.02 ± 1.50	2.02 ± 1.28	–	1.03 ± 0.55	1.37 ± 1.24
Total cholesterol, mmol/L	5.28 ± 0.93	6.23 ± 1.38	5.34 ± 1.17	–	5.45 ± 1.50	5.33 ± 1.20
HDL-cholesterol, mmol/L	1.28 ± 0.27	1.46 ± 0.21	1.24 ± 0.26	–	1.59 ± 0.20	1.37 ± 0.34
LDL-cholesterol, mmol/L	3.22 ± 0.78	3.84 ± 0.63	3.24 ± 1.00	–	3.37 ± 1.34	3.35 ± 0.98
Total cholesterol/HDL-cholesterol	4.29 ± 1.20	4.23 ± 0.56	4.43 ± 1.09	–	3.44 ± 0.89	4.04 ± 1.14
BMI, kg/m^2	28.66 ± 3.95	31.26 ± 2.89	31.87 ± 6.38[b]	–	28.52 ± 13.47	27.36 ± 5.80
Glucose, mmol/L	8.80 ± 3.03	10.16 ± 4.08	10.54 ± 3.24[a]	–	5.00 ± 0.48	5.08 ± 0.45
HbA1c, %	7.97 ± 1.08	7.67 ± 0.51	8.52 ± 1.84	–	5.27 ± 0.43	5.36 ± 0.29

[a]p=0.029. 95% CI=3.08–56.83. [b]p=0.019. 95% CI=0.53–5.88. p-Value among the type 2 diabetes mellitus patient group and control group calculated by one-way ANOVA.

higher in patients with CC genotype when compared to both patients with CT and TT genotype.

The distribution of the *GHRL* (rs4684677) genotypes according to the biochemical profiles are given in Table 7. There is no statistical significance among groups.

The distribution of the *PGC-1α* (rs8192678) genotypes according to the biochemical profiles are given in Table 8. In the patient group, glucose and BMI levels were observed significantly higher in those with the GA genotype. GG genotype was not seen in control group.

Discussion

Although a large number of studies have been made on relation between SNPs and T2DM, a consensus has not been established. In the context of our study, we aimed to investigate the relationship between *FTO* (rs8044769), *PGC-1α* (rs8192678) and *GHRL* (rs4684677) variants and T2DM.

The ghrelin hormone and ghrelin gene are cited as candidates for the development of T2DM. In a study conducted in a French population including 610 individuals with T2DM and 820 healthy control individuals, it was reported that ghrelin hormone and ghrelin gene were not associated with T2DM. They emphasized that this gene might be associated with ovarian cancer, obesity and hypertension [9]. Similarly, Faris et al. reported that Ghrelin (rs4684677) polymorphism was not associated with T2DM [10]. Additionally, Li et al. investigated the relationship between T2DM and GHRL gene *Leu72Met* Polymorphism with 11 centers and 8,194 participants. There were six countries among the participants, including China, Denmark, Finland, France, Germany and Korea. They found a relationship between Leu72Met Polymorphism and T2DM in Chinese individuals. However, they didn't find such a relationship for other country citizens [11]. Rivera et al. reported that Leu72Met Polymorphism prevents the development of T2DM in the Mexican Population [12]. Larsen et al. reported that there was no significant difference for the distributions of genotype and allele of *GHRL* (rs4684677) polymorphism between T2DM and control groups [13]. In our study, there is no difference between T2DM and the control group in terms of *GHRL* (rs4684677) genotype and allele distributions. We also did not determine TT genotype in both group for this SNP. It could be related with ethnicity or relatively lower sample size. Since the development of diabetes is affected by ethnic origin and environmental factors, different results were found in studies conducted in different populations.

The *FTO* gene is associated with increased food intake, obesity and related metabolic results. Czajkowski et al. observed that individuals carrying CT genotype had high weight, high BMI value and a high proportion of adipose tissue for the *FTO* (rs8044769) variant. In addition, high insulin levels were detected in the second hour of glucose load for oral glucose tolerance test in individuals carrying homozygous CC and TT. Likewise, they obtained high HOMA-IR in homozygous groups. In our study, the frequency of carrying the TT genotype in the patient group was statistically significant compared to the control group. Similar to this study, we found higher LDL and total cholesterol/HDL ratios in individuals with TT genotype compared to the individuals with other genotypes [14].

In a study involving the *FTO* (rs8044769) variant with the participation of 238 healthy individuals, the volunteers have completed 30 min of submaximal aerobic exercise. The metabolic response of the volunteers was investigated through several physiological tests such as VO2Max, heart rate, blood pressure, temperature, lactate and norepinephrine measurements. Karoli et al. observed that individuals carrying the TT genotype gave the most positive response to exercise. The fact that this gene, which is associated with obesity and high BMI, gives the most positive response shows that the study should done including obese individuals [15].

Younus et al. reported that *FTO gene polymorphisms* (rs9939609 and rs17817449) were associated with the development of diabetes in the Iranian population. They emphasized that the *FTO* (rs17817449) variant increases weight gain directly and the risk of developing T2DM indirectly. Although we conducted our study on the same gene and same patient groups, our variant was different. However, we have shown that another variant of the *FTO* gene may also be associated with T2DM [16].

The association of the *FTO* (rs8044769) variant with osteoarthritis has been investigated and related due to its effect on BMI [17, 18]. On the contrary, several studies have shown that the *FTO* (rs8044769) variant is not associated with increased BMI and osteoarthritis [19, 20]. Differences in geography, nutrition styles, and ethnicity may have caused the results to differ from each other.

PGC-1α gene is investigated in studies of insulin resistance, diabetes and metabolic syndrome due to its effect on control of cellular energy metabolism. Xia et al. conducted a meta-analysis that investigate the relationship between *PGC-1α* (rs8192678) polymorphism and T2DM. This meta-analysis included 20 different studies and 16,000 participants from East Asian, Caucasian, Indian and African subgroups. They reported that individuals with the A allele in the Caucasian and Indians have a higher risk to develop T2DM. They also reported that individuals with the AA genotype have a higher risk for the development of T2DM. However, since there were no individuals with the AA genotype in the African group, they could not detect a relationship between the AA genotype and T2DM in this group [21].

Jeema et al. conducted a study involving 889 people with same ethnic origin and living in the same geographical area in Tunisia. They found a relationship between T2DM and *PGC-1α* (rs8192678) polymorphism. They also stated that there was no relationship between the *PGC-1α* (rs8192678) polymorphism and T2DM in those studies

conducted on Japanese, Chinese, French and Pima natives. They tried to explain this situation by mentioning that there may be different allele distribution frequencies in different ethnic groups [22].

Fanelli et al. conducted a study investigating the relationship between insulin resistance and *PGC-1α* (rs8192678) polymorphism in normal and glucose-intolerant obese subjects. They found higher HOMA-IR and insulin values in individuals carrying this variant than those who were not. They concluded that this variant goes along with the increased insulin resistance in obese individuals [23]. In our study, although we did not measure insulin, we found that individuals carrying the GG genotype had high fasting blood glucose and body mass index values.

Sharma et al. reported that the A-allele of *PGC-1α* (rs8192678) polymorphism played a protective role against T2DM in the Bania group, whereas it increased 1.5-fold risk towards T2DM development in the Jat Sikh group [24]. The controversial effect of the same alleles on diseases is called the flip flop phenomenon. The flip flop phenomenon explained by population differences, genetic background and environmental factors [25].

In conclusion, we observed that the carriers of the TT genotype for the *FTO* (rs8044769) variant and the GG genotype for the *PGC-1α* (rs8192678) variant were found more frequently in the T2DM group, while the *GHRL* (rs4684677) did not differ between the T2DM and control groups. For the *PGC-1α* (rs8192678) variant in the T2DM group, glucose and BMI levels were observed significantly higher in carriers of the GA genotype than those with the GG genotype. LDL levels and total cholesterol/HDL-C ratios were found statistically higher in patients with TT genotype than those with CT genotype for the *FTO* (rs8044769) variant. There was no statistical difference in the distribution of *GHRL* (rs4684677) alleles according to biochemical profiles.

Controversial results could be occurred due to the sample size, ethnic differences, study design, inclusion/exclusion criteria, patient and control group differences, age and environmental factors. There is a need for repeating the study between different or specific ethnic groups and further studies involving large numbers of patient and control groups.

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