Research Article

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www.imedpub.com

Health Science Journal
ISSN 1791-809X

2021
Vol.15 No. 8:4272

Evaluation of Clinical and biochemical characteristics and Genetic Association Between the Polymorphic loci in Diabetic mellitus 2 patients in Urmia

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Received date: May 25, 2020; **Accepted date:** August 13, 2021; **Published date:** August 23, 2021

Citation: Fatemeh Keshavarzi (2021 Evaluation of Clinical and biochemical characteristics and Genetic Association Between the Polymorphic loci in Diabetic mellitus 2 patients in Urmia. Health Sci J. Vol.15 No.8.

Abstract

Background

Predisposing factors of type 2 diabetes are multi-factorial and factors such as genetics and the environment are involved in its outbreak. The aim of the current study was to analyze the genetic relationships between ATF6rs2070150: G> C and FTOrs8050136: A> C gene variants with clinical and biochemical parameters belong to the patients of type 2 diabetes in Imam Khomeini Hospital of Urmia.

Methods

The present study is a case-control study. 100 patient's blood samples along with 100 healthy control samples for ATF6rs2070150: G> C and FTOrs8050136: A> C variants were tested using Tetra-arms PCR method.

Results

Although the frequency of genotypes were comparable between patients and healthy control subjects, the logistic regression analysis showed no significant relationship between FTO rs8050136 A allele and ATF6rs2070150 C allele with an increased risk of type 2 diabetes. Also,the A allele from FTO and C allele from ATF6 were significantly associated with increased levels of HbA1c, insulin, HOMA-IR, diastolic blood pressure and obesity markers (BMI, waist and hip circumference). In the present study, there was a significant relationship between FTO rs8050136 A> C and ATF6rs2070150: G>C with main markers of type 2 diabetes such as insulin resistance, obesity and inflammation.

Conclusions

These findings can provide new opportunities for the prevention, diagnosis and customization of type 2 diabetes in the future. In particular, the prevention of obesity in some dangerous genetic subgroups may be a valuable contribution to the prevention of type 2 diabetes.

Keywords: Type 2 Diabetes, FTO rs8050136, ATF6rs2070150, Urmia

Background

Type 2 diabetes is a metabolic disorder that in advanced and developing countries is considered as a growing health problem. This disease leads to the disability and early death. The incidence of diabetes mellitus in 2011 was 366 million people worldwide, and is expected to reach 552 million by 2030 (**1**). Type 2 diabetic patients account for 90 to 95% of diabetic patients. Several factors are involved in the development of type 2 diabetes, including obesity, which is considered as the most important risk factor for type 2 diabetes (**2**). Some of the biological products which are produced by adipocytes (i.e leptin, tumor necrosis factor-α, free fatty acid) are causing interference in stages such as insulin secretion, insulin function, and body weight adjusment, and may have a role in insulin resistance. Another factor is impaired glucose tolerance (**3**). The effect of stress, because of the effect on the metabolic status and changes in hormones such as catecholamine and growth hormone, can contribute to insulin secretion and resistance to it. Regarding the cause of hypertension, an underlying hypothesis is that hypertension is an endothelial dysfunction marker, which is also a risk factor for insulin resistance, type 2 diabetes and cardiovascular disease (**3-6**). Genetic relation with type 2 diabetes is stronger than its relation with type 1 diabetes (**7**).

It is possible that activating transcription factor 6 (ATF6) which is located on 1q21-q23, be involved in susceptibility to the disease. This gene in the liver glycolysis and beta-pancreatic cells through glucokinase dephosphorylation plays a role in the regulation of blood glucose in the cytoplasm. Historically, genomic studies in multi-racial populations have shown that the position of 1 q21-q25 has a strong association with diabetes (8-13). ATF6 is associated with various physiological processes, such as cell differentiation, apoptosis and inflammation, all of which play an important role in tumorigenicity (**8**). Molecular

genetic studies have shown polymorphisms in ATF6 that are associated with type 2 diabetes, plasma cholesterol levels, and body mass index (**9-13**). İn a study, Wu et al. studied the role of single-nucleotide polymorphisms in hepatocellular carcinoma (HCC).The results of this study indicated that rs2070150 polymorphism has a significant relationship with HCC (**13**). In another study, 1892 patients with type 2 diabetes and 1808 controls were examined. The results of this study indicated that none of the polymorphisms including rs2070150 showed a significant relationship with type 2 diabetes (**14**). Various studies performed on humans and animal models such as mice indicated the association between Fat mass and obesity-associated (FTO) gene and various diseases such as type 2 diabetes (**11**). This gene has 9 exons and is located on chromosome 16 (**15**). For the first time, the association between the FTO gene and the risk of type 2 diabetes was identified by carrying out genome-wide studies in the white European population (**16-40**). The FTO gene has variants that indicate close correlation with the body fat mass and is a very good candidate for a lipid metabolism marker, especially abdominal fat. Subsequent studies using bioinformatics and animal models indicated that this gene has a potential role in nucleic acid demethylation and is expressed in the brain's hypothalamic nucleus, which monitors the balance of energy and appetite (**17-20**). According to the research, rs8050136 polymorphism increases the chances of developing diabetes (**18**).

In the study of Boissel et al., on the association of polymorphism rs16953002 FTO with type 2 diabetes and obesity indicated that FTO rs16953002 A / G polymorphism had a significant correlation with type 2 diabetes and obesity (**21**). Polymorphisms of this gene have been studied in countries such as China, India, and the United States (**15-21**) which shows the highest incidence of type 2 diabetes. Therefore, in the current study, the genetic link between polymorphic locus of FTO and ATF6 genes with type 2 diabetes in patients referred to Imam Khomeini Hospital of Urmia has been investigated.

Methods

The present research is a case-control study. The study population is 100 patients and 100 healthy controls. Patients were selected among diabetics patients older than 35 years old whom treated with oral medication and had medical records in the diabetes department of Imam Khomeini Hospital of Urmia. In the selection of patients, there were no restrictions in terms of gender and race. Healthy subjects, with the same number as patients, were selected as controls among companions of patients and other people who referred to Imam Khomeini Hospital, by matching age and gender. Healthy people were tested for other systemic diseases and healthy people were selected. This study was conducted in compliance with the Helsinki Declaration and the Ethical Guidelines for Medical and Health Research Involving Human Subjects by the Iran government and has been approved by the ethics committee of department of Internal Medicine, Urmia University of medical Sciences, Urmia, Iran.

Sample size and sampling method

Based on the latest criteria of the American Diabetes Association (ADA), there must be 4 characteristics for the presence of diabetes mellitus in the patient. These four criteria are as follows:

1. Glucose hemoglobin or HbA1C equal to or greater than 6.5% based on NGSP standard. It should be noted that this criterion has already been used to monitor the treatment but is currently considered a diagnostic criterion.

2. Plasma glucose in fasting state equal to or greater than 126 mg / dL. Fasting means that the person has not received any calories for at least 8 hours ago. It should be noted that in the absence of explicit symptoms of hyperglycemia, polyurea, poly dipsia, and weight loss, this finding should be confirmed by repeating the test on the next day.

3. 2-hour plasma glucose equal to or greater than 200 mg / dl, which is determined after eating 75 g of glucose dissolved in water during standard glucose tolerance testing.

4. If hyperglycemic symptoms are present, as well as plasma glucose in a random sample equal to or greater than 200 mg/ dl .After selecting patients and control, they were informed verbally and in writing about the research plan. Written informed consent was received from the patients and control subjects and informed them that sampling was for research purposes only and assuring them of confidentiality of their information, they participated in the research.

Then, two ml of blood was taken from each patient (healthy and patient) and kept in an EDTA vial for PCR in a 20 ° C freezer.

Gene Sequencing and primer designing

The sequence of the FTO and ATF6 genes was obtained by referring to the NCBI International website. Then, using the online software Primer 3, four primers (2 internal primers and 2 two external ones) were designed for the desired SNP of each gene (Table 1).

Table 1: Designed Primers for the Polymorphisms of ATF6rs2070150 and FTOrs8050136 Genes

SNP	system	Primer sequence (5'-3')	Allele
rs2070150	Forward inner primer	ATGGTGAAAA CTCTAATAGTC TCTCTTGAC	G
	Reverse inner primer	AGGCTTATCTT CCTTCAGTGG CTCGGC	C
	Forward outer primer	TTCCTTTGGTA TAGATGGATCA TGCTGA	
rs8050136	Reverse outer primer	AAAAAACAGC AAGCCAGCCT AATAAACA	
	Forward inner primer	TAGGTTCCTTG CGACTGCTGT GAATTTT	A

	Reverse inner primer	AGCCTCTCTA CCATCTTATGT CCAAACA	C
	Forward outer primer	GGTTCTACAG TTCCAGTCATT TTTGACAG	
	Reverse outer primer	GAGTAACAGA GACTATCCAA GTGCATCACA	

DNA extraction from the blood using DNAfast solution

At first, 900 µl of DNAfast solution was poured into a microfluidic tube (1.5 ml), and then 200-100 µl of blood was added and vortex vigorously for one minute. The sample was hold in laboratory temperature for 10 minutes. The amount of 400 ml of chloroform was added to the sample and the tube was slowly shaken for 15 seconds (not vortexed) (About 6 times the tube was slowly upside-down). Then, the sample was hold in laboratory temperature for 3 minutes. The sample was centrifuged at 12000 rpm for 15 minutes at 4 ℃. At this stage, 3 distinct phases were formed in the tube, the supernatant (upper phase) solution was slowly separated and transferred to a new microfiber tube. Isopropanol solution equal to the isolated volume (approximately µl 500-600) was added and well vortexed. The sample was then placed on crushed ice for 15 minutes. The sample was centrifuged at 12 ° C for 10 minutes at 4 ° C. Slowly, the outer solution was removed and the precipitate remained at the bottom of the tube. The supernatant was removed slowly and the precipitate remained at the bottom of the tube. The amount of 1ml of 75% ethanol was added to the precipitate and the tube was slowly shaken to rinse the precipitate. The sample was centrifuged at 7500 rpm for 5 minutes at 4 ° C. The supernatant was removed gently and the precipitate DNA was kept in the tube. The tube hold for 10 to 10 minutes in lab temperature, until DNA deposition was dried slightly. The DNA deposition was dissolved in 20 µl sterile distilled water. To ensure the DNA quality, the amount of 3 µl of the sample was placed on the agarose gel 0.7%, and evaluated using anidium bromide and UV radiation staining. After extracting DNA from the whole blood, its quantity was measured by spectrophotometer. The DNA and protein of each sample were determined in micrograms per millimeter at wavelengths of 260 nm, 280 nm. The ratio of 260 nm, 280 nm is desirable if it is between 1.6-1.9. Lower ratios indicate contamination with protein and aromatic compounds (phenol) and higher ratios representing RNA contamination. To evaluate the quality of the samples, agarose gel was also used in current study. For this purpose, 3 µl of the extracted DNA was electrophoresed on a 1.5% agarose gel.

Tetra- ARMs PCR

The tetra-ARMS PCR technique was used to identify the polymorphic locus. The basis of this technique is the design of four primers for each position locus. next, each of the four primers for each sample is poured simultaneously in a microtube, and PCR was performed by adding the required materials include double distilled water, primers (A pair of genaral external primers and a pair internal Allele-specific primer), DNA pattern and polymerase enzyme, buffer and MgCl2.

After boiling 1.5% agarose in buffer (TBE (1x) or TAE), it was allowed to reach the temperature of the gel to 55 ° C and then ethidium bromide was added at a concentration of 0.1 mg/ml and pour into the gel-tray in which corresponding comb is also embedded. After 10 to 15 minutes, the gel was set, the comb was removed, and the gel was placed inside a tank where it was filled with a buffer called TBE (1X) (buffer, which the gel make with). The buffer is as much as to covere the surface of the gel. The samples were loaded in a gel with a 1 microliter loading buffer. To determine the size of the PCR bands and ensure the proliferation of the desired piece, size marker or Ladder were used. After that the color of Bromophenol Blue migrated sufficiently, the power supply was turned off and the gel was examined in a trans-luminator device or a Gel Doc.

Data analysis

Statistical analysis was performed using SPSS v.19.0 software. In order to investigate the differences between allele frequencies and distribution of genotype between healthy controls and patients, Chi-square test (C2) and Fischer's exact test (in cases where frequencies were less than or equal to 5) were applied. Logistic regression analysis was used to calculate OR odds ratio with 95% confidence interval (CI) along with age and sex adjustment. The significance of differences between biochemical and anthropometric measurements based on genotypes of analyzed polymorphisms, gender and age was determined by ANCOVA (covariance analysis) in terms of age, gender and body mass index. P-value < 0.05 was considered as statistically significant.

Results

In this study, 100 healthy persons and 100 patients with type 2 diabetes from diabetes department of Imam Khomeini Hospital in Urmia, who had records and were over 35 years old, were enrolled. The metabolic and anthropometric characteristics of patients with type 2 diabetes and healthy controls are presented in Table 2.

As seen in the Table, the most methodic and anthropometric parameters measured between patients and controls were different. As can be seen in the Table 2, most of measured metabolic and anthropometric parameters were significantly different between patients and controls.

Table 2: Metabolic and anthropometric characteristics of patients with type 2 diabetes and healthy controls

		Healthy controls n=100	
	T2D patients n=100		p
			T2D/
Male/ Female ratio	36 / 64	36 / 64	ctrl
Age (years)	45.30 ± 0.365	41.55 ± 0.347	<0.001

Fasting glucose (mmol/L)			
	9.30 ± 0.190	5.12 ± 0.503	<0.001
HbA1c (%)	7.61 ± 0.082	5.53 ± 0.055	<0.001
Fasting insulin (mU/L)	15.47 ±0.761	10.21 ± 0.589	<0.001
BMI (kg/m2)	31.02 ± 0.343	26.75 ± 0.389	<0.001
Systolic BP (mm Hg)			
	137.25 ± 1.378	122.33 ± 1.093	<0.001
Diastolic BP (mm Hg)	88.14 ± 0.521	79.85 ± 0.498	<0.001
Triglycerides (mmol/L)	2.43 ± 0.129	1.66 ± 0.565	<0.001
Total cholesterol (mmol/L)			
	5.22 ± 0.076	5.47 ± 0.077	0.01
HDL			
cholesterol (mmol/L)	1.05 ± 0.016	1.30 ± 0.027	<0.001
LDL-cholesterol (mmol/L)	3.34 ± 0.064	3.54 ± 0.059	0.05
VLDL-cholesterol (mmol/L)	1.400 ± 0.159	0.746 ± 0.049	<0.001

The mean ± Mean squared error (SEM), pT2D / ctrl, and p values indicate the importance of clinical and biochemical differences between patients to control ratios.

Also, the results of comparing the clinical and biochemical characteristics of various genotypes of FTO rs8050136: A> C, in healthy subjects and patients are presented in Table 3.

Table 3: Comparison of Clinical and Biochemical Characteristics among various Genotypes of FTO rs8050136: A> C in patients and Healthy subjects.

	FTO rs8050136 A>C					
	p-value	p-value	p-value	p-value	p-value	p-value
	AA/AC	AA/CC	AC/CC	AA/AC	AA/CC	AC/CC
Fasting glucose (mmol/L)	0.124	0.88	0.195	0.996	0.561	0.502
Fasting insulin (mU/L)	0.543	0.178	0.306	0.82	0.801	0.937
Systolic BP (mm Hg)	0.154	0.654	0.052	0.375	0.175	0.108
Diastolic BP (mm Hg)	0.516	0.322	0.621	0.017	0.382	0.183
Triglycerides (mmol/L)	0.39	0.209	0.557	0.781	0.835	0.6
Total cholesterol (mmol/L)	0.484	0.616	0.899	0.006	0.133	0.33
HDL cholesterol (mmol/L)	0.295	0.083	0.341	0.042	0.169	0.585
LDL-cholesterol (mmol/L)	0.841	0.74	0.855	0.426	0.414	0.914
VLDL-cholesterol (mmol/L)	0.012	0.023	0.978	0.152	0.576	0.365
hsCRP (mg/L)	0.182	0.172	0.821	0.896	0.964	0.933
BMI (kg/m2)	0.377	0.98	0.381	0.133	0.221	0.88
Waist circumference (cm)	0.346	0.268	0.731	0.168	0.069	0.63
Hip circumference (cm)	0.139	0.067	0.503	0.414	0.635	0.194
+HOMA-IR	0.532	0.603	0.186	0.744	0.618	0.842
HbA1c (%)	0.639	0.794	0.435	0.122	0.019	0.251

As shown in Table 3(the first three column), in patients group with AA genotype, levels of VLDL- cholesterol were lower than subjects with AC and CC genotypes (p = 0.012, p = 0.023, respectively) (Fig. 1). In addition, increased systolic blood pressure (BP) in patients with genotype AC in comparison to the CC, has a significant relationship (P=0.052) (Fig. 2).

In healthy subjects (the next three column of Table 3), FTO rs8050136 gene polymorphisms had a significant relationship with the most important markers of glucose, homeostasis, dyslipidemia and obesity. Carriers of AA genotype had higher levels of HbA1c (p = 0.019) compared to those with CC genotype. In addition, carriers of AA genotype had a higher level of diastolic blood pressure (p = 0.017) (BP) and lower HDL cholesterol than carriers of AC genotype (p = 0.040). Also from other results of Table 3, the correlation between AA genotype and waist circumference was similar to CC genotype (P = 0.069). Another result of Table 3 was the dependence of AA genotype with the size of waist in comparison with CC genotype (P =

0.069).

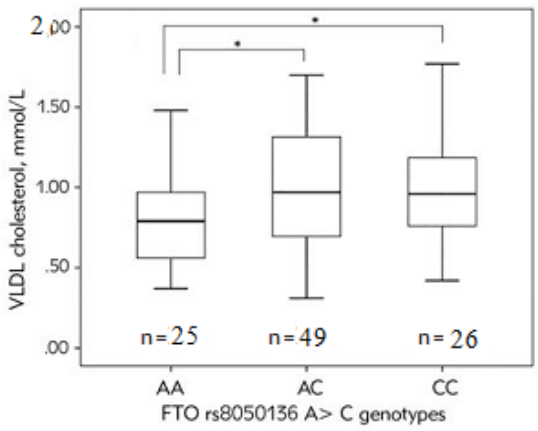

Figure 1: Error bar graph to examine the [* of] genotype

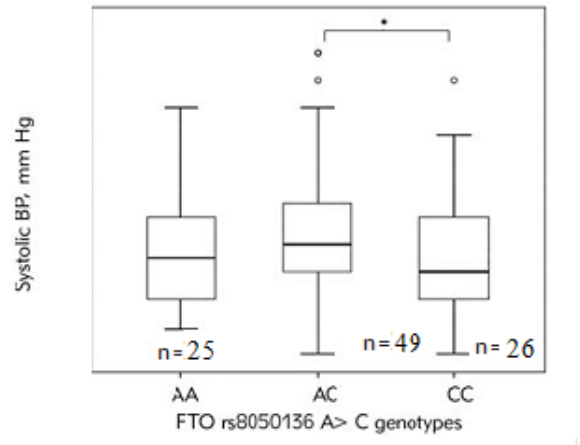

Figure 2: Error bar graph to examine the [* of] genotypes

Also, FTO rs8050136 was significantly associated with glucose homeostasis, insulin resistance, and obesity. Allelic and genotypic frequency of FTO rs8050136 polymorphism for diabetic patients and healthy controls are presented in Table 4. Frequency of alleles for FTO-rs8050136 A> C polymorphism in two groups was in Hardy-Weinberg equilibrium (p> 0.05), i.e. there was no significant difference between the frequencies of observed genotypes between diabetic patients and healthy controls (Table 4). The odds ratio (OR), which adjusted for age and sex, did not show a potential association of FTOrs8050136 with the risk of diabetes (OR = 1.084, 95% CI 0.823-1.294, p = 0.568). In Fig. 3, the typical electrophoretic pattern of PCR products for gene (FTO (rs8050136)) is presented.

Table 4. Genotype and frequency of different alleles for FTO rs8050136: A> C gene polymorphism

		T2D	Mutated allele frequency	Healthy controls	Mutated allele frequency	
Polymorphism	Genotype	patients				
						pT2M/ctl
	AA	25 (25%)	0.48	24 (24%)	0.53	
	AC	49 (49%)		48 (48%)		
rs8050136	CC	26 (26%)		28 (28%)		
	Total	100	P=0.976	100	P=0.453	p=0.247

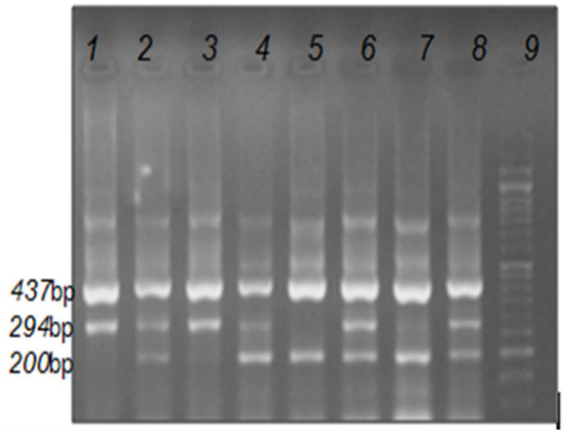

Figure 3: The electrophoretic pattern of PCR products of the gene (FTO (rs8050136))

Also, in Tables 5, the results of the comparison of clinical and biochemical characteristics between different genotypes of ATF6rs2070150: G>C are observed in healthy and patient subjects. In patients group, subjects with CG genotype had a higher insulin level than those with CC and GG genotype (p = 0.014). Also, the rate of insulin resistance index in people with C allele was higher than those with G allele (p = 0.011). The index of insulin resistance in individuals with C allele was more than those with G allele (p = 0.011). In addition, the relationship between CG genotype and obesity index was higher than other genotypes. The frequency of alleles and genotypes for the polymorphism of the ATF6rs2070150 gene for diabetic patients and healthy controls are presented in Table 5(the first three column).

Also as outlined in the Table5, ATF6rs2070150: G>C polymorphisms were significantly associated with glucose homeostasis, insulin resistance, and obesity in control group (the next three column of Table 5).

Table5: Comparison of clinical and biochemical characteristics among various genotypes of ATF6rs2070150: G> C in patients and Healthy subjects.

	ATF6rs2070150: G> C					
	p-value	p-value	p-value	p-value	p-value	p-value
	GG / GC	GG /CC	GC /CC	GG / GC	GG /CC	GC /CC
Fasting glucose (mmol/L)	0.357	0.297	0.232	0.996	0.561	0.502

Fasting insulin (mU/L)	0.244	0.412	0.014	0.82	0.801	0.937
Systolic BP (mm Hg)	0.218	0.106	0.49	0.375	0.175	0.108
Diastolic BP (mm Hg)	0.897	0.863	0.745	0.017	0.382	0.183
Triglycerides (mmol/L)	0.986	0.155	0.091	0.781	0.835	0.6
Total cholesterol (mmol/L)	0.284	0.091	0.34	0.006	0.133	0.33
HDL cholesterol (mmol/L)	0.452	0.302	0.628	0.042	0.169	0.585
LDL-cholesterol (mmol/L)	0.218	0.103	0.456	0.426	0.414	0.914
VLDL-cholesterol (mmol/L)	0.149	0.601	0.405	0.152	0.576	0.365
hsCRP (mg/L)	0.559	0.94	0.659	0.896	0.964	0.933
BMI (kg/m2)	0.768	0.141	0.053	0.133	0.221	0.88
Waist circumference (cm)	0.178	0.071	0.661	0.168	0.069	0.63
Hip circumference (cm)	0.409	0.641	0.201	0.414	0.635	0.194
+HOMA-IR	0.262	0.218	0.011	0.744	0.618	0.842
HbA1c (%)	0.901	0.912	0.731	0.122	0.019	0.251

Frequency of alleles for ATF6rs2070150 G> C polymorphism in two groups was at Hardy-Weinberg equilibrium (p> 0.05), i.e. there was no significant difference between the frequency of observed genotypes between diabetic patients and healthy controls (Table 6). The odds ratio (OR) that was adjusted for age and sex did not indicate a possible association of ATF6rs2070150 with the risk of diabetes (OR=1.084, 95% CI 0.749–1.641, p=0.638). Figure 4 shows an example of an electrophoretic pattern of PCR products of the gene (ATF6rs2070150: C> G).

Table 6: Genotype and frequency of different alleles for polymorphism of ATF6rs2070150: G>C

SNP	Genotype	T2D patients	Mutated allele frequency	Healthy controls	Mutated allele frequency	pT2M/ctl p=0.224
ATF6(rs2070150)	CC	65(65%)	0.41	63 (63%)	0.49	
	CG	31(31%)		31 (31%)		
	GG	4(4%)		6 (6%)		
	Total	100	P=0.127	100	P=0.424	

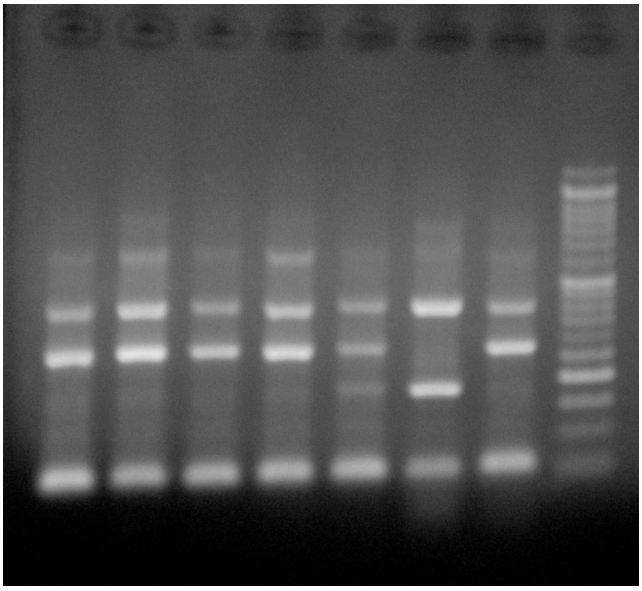

Figure 4: The electrophoretic pattern of PCR products of the gene (ATF6rs2070150: G> C)

1: genotype GG, 5: genotype GC, 6: genotype CC, 8: ladder50 bp

Discussion

Today, obesity is one of the biggest global problems (**4**). Overweight in the pathophysiology of type 2 diabetes is very important and is one of the main causes for the prevention of this complex disease (**37**). The increased global outbreak of type 2 diabetes is tied to increased labor rates, which is partly due to social trends toward energy consumption and energy consumption reduction (**38**). However, the mechanisms that create individual susceptibility for obesity are still unknown (**1**). This is an important reason to find out the relationship between different types of unknown genes with obesity and subsequently with diabetes. In this study, the genetic link between ATF6 (rs2070150) and FTO (rs8050136) gene mutations and type 2 diabetes in a population of 100 healthy individuals and 100 diabetes patients in Urmia Imam Khomeini Hospital was investigated. Also, the relationship between rs8050136 polymorphism genotypes with clinical and biochemical characteristics of patients, particularly the levels of glucose, insulin, BMI, waist circumference as an indicator of neurological obesity, were evaluated. The results indicated that there was a significant difference in the frequency of analyzed genotypes

between patients with type 2 diabetes and healthy controls(Table 2), but there was no significant relationship between genetic variation of FTO (rs8050136: A> C) and the risk of diabetes(Table 4). In several studies conducted in different populations, this type of genetic variant was effective in increasing the risk of developing type 2 diabetes (**15-19**). However, studies similar to this study which have been conducted in the populations of Russia, Mexico, the Netherlands, and Lebanon have not confirmed this increase, and the results are similar to the results of this study (**29-32**, 40). Another result of the current study was the observation that there was a significant correlation between FTO rs8050136 A> C polymorphism and the most important markers of obesity. The high values of BMI, waist circumference and hip circumference in both groups of patients and control indicated the relationship between the risk of altering the single-nucleotide variation of rs8050136 A> C gene with these indices and the incidence of obesity(Table 3). In a large study in the Indian population, also a significant relationship between rs8050136 A> C with high BMI, waist circumference, and also with the waist and intestinal ratio were found. Similar results are also seen in Han Chinese teens (**24**). Xiao et al. indicated a significant relationship between the rs805016 gene allele with high BMI values in the Uyghurs population of northwest China (**23**).

In addition, our results indicated that ATFrs8050136 A>C polymorphism was significantly associated with lowering HDL / cholesterol levels in control subjects(Table 5). Several studies, including a large meta-analysis that investigate the relationship between the rs8050136 A>C polymorphism and Increased risk of developing metabolic syndrome, indicated a direct relationship between this polymorphism and higher levels of glucose, waist circumference, triglyceride and total cholesterol, and lower levels of HDL cholesterol (**16**, **22**, **41**, **42**) the results of our research also indicated such a relationship. It should be noted that most patients with type 2 diabetes have developed metabolic syndrome. Also, the results of the current study indicated that there is a significant relationship between this polymorphism and high diastolic pressure, which is one of the diagnostic parameters and one of the most important risk factors in metabolic syndrome(Table 5). In addition, in the group of patients with type 2 diabetes, allele A was associated with low systolic pressure, which can be interpreted by the fact that most patients in this study probably used anti-hepatitis drugs. Therefore, the results for this group of patients need to be interpreted with particular caution. The other result was that there was a significant relationship between A rs8050136 A> C and high levels of HbA1c in the healthy control group. Increasing the level of insulin in the early stage of developing type 2 diabetes represents the body's defense mechanism against glucose levels and HbA1c. Our findings are also consistent with the results of studies that investigated the association of rs8050136 A> C polymorphism with insulin sensitivity.

In a study that investigated the effect of insulin sensitivity rs8050136 A> C in women with advanced polycystic ovary syndrome (PCOS), there was no significant relationship between insulin sensitivity in women without PCOS and PCOS(Table 5). In a study that investigated the relationship between rs8050136 A> C and insulin sensitivity in women with advanced polycystic ovary syndrome (PCOS), there was no significant relationship between insulin sensitivity in women without PCOS and those with PCOS (**26**).

Studies of Wing et al. have confirmed the effects of rs8050136 A> C on the parameters of glucose obesity and homeostasis in different populations in the United States (**27**). In the current study, the relationship between rs8050136 A> C and the important markers of glycemic control and insulin sensitivity, such as increased levels of HbA1c, insulin and HOMA IR, was confirmed.

The results of the current study also indicated significant effects of rs8050136 A> C polymorphism on the increase of inflammatory markers (fibrinogen and number of leukocytes). In a large meta-analysis, it has been found that the candidate genes of the metabolic syndrome play an important role in inflammation, In fact, an important link between the FTO gene and the CRP level was determined (**41**). Wu et al. in their study examined 1082 patients with chronic hepatitis B (CHB) and 816 patients with hepatitis B virus (HBV) in Han Chinese. The results of their study indicated that rs2070150 polymorphism has a significant relationship with HCC (**14**). Hu et al. in a study entitled "Lack of association between genetic polymorphisms within DUSP12-ATF6 locus and glucose metabolism related traits in a Chinese population" investigated the relationship between the genetic polymorphism and type 2 diabetes. In this study, 1892 patients with type 2 diabetes and 1808 control samples with normal glucose regulation were used. The results of this study indicated that none of the polymorphisms and haplotypes had a significant relationship with type 2 diabetes(Table 6). There was no relationship between polymorphisms and quantitative characteristics. The results of this study indicated that polymorphisms in the DUSP12-ATF6 locus do not play an important role in glucose metabolism in the samples (**13**). Tamim et al. investigated 900 subjects with diabetes. The results of the study indicated a partial relationship (**10**). In the study of Winston et al., the relationship between this polymorphism and diabetes in 353 African-American patients was not observed (**8**) which was similar to the results of the current study. The major limitation of our research was the relatively low number of controls and patients, especially in regard diabetic patients and newly diagnosed diabetes patients who do not take any medication. This group of patients is very important in analyzing and identifying different types of genotypes with pathophysiological biomarkers associated with type 2 diabetes.

Conclusion

In the present study, there was a significant relationship between FTO rs8050136 A> C and ATF6rs2070150: G>C with main markers of type 2 diabetes such as obesity, inflammation. These results outline new procedures for many questions in the pathogenesis of this disease.

Acknowledgments

The authors would like to thank the volunteers who contributed samples for this study and Clinical research development unit of Imam Hospital in Urmia, Iran.

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