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# Original Research Article

## Gene Polymorphisms of Human *FTO* and Obesity in Part of Iranian Population

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# Gene Polymorphisms of Human *FTO* and Obesity in Part of Iranian Population

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**Abstract**
As obesity is a multifactorial characteristic, identifying genetic risk factors can help to prevent obesity prevalence. In 2007, *FTO* (fat mass and obesity associated) gene was identified according to genome-wide association study. Several studies revealed that *FTO* polymorphisms are responsible for the risk of obesity. The primary objective of our study was assessment of *FTO* rs9939609 and rs9926289 polymorphisms as risk factors of obesity in part of the Iranian population. In our case–control study, 62 patients with obesity and 75 controls were selected according to their BMI (obese: BMI $\geq$ 30 kg/m$^2$ and control: BMI: 18.5-24.9 kg/m$^2$). Blood samples were collected from individuals for biochemical parameters assessment and genotyping analysis. After genotyping by high resolution melting (HRM) technique, the odds ratio was used to examine the relationship between the risk factors and the disease; 95% of confidence interval was used for these calculations. The difference in the age, FBS, triglycerides, total cholesterol, and BMI between individuals with obesity and the control group was significant. The analysis of rs9939609 *FTO* gene showed significant association between the AA+TA genotype and TT according to the dominant model. No association was observed for genotypes of the *FTO* rs9926289 polymorphism. In addition, there was no significant correlation of biochemical parameters and dominant model genotypes of rs9939609 polymorphisms. Our study suggests that *FTO* rs9939609 polymorphisms appear to be associated with obesity in part of the Iranian population. AA+TA genotype of rs9939609 polymorphism is a risk factor of obesity. However, further examination should be carried out on large populations.

**Keywords:** Obesity; Polymorphism; *FTO* gene; BMI.

## 1. INTRODUCTION

Over the past 30 years, overweight and obesity have become one of the world's leading health concerns. Obesity establishes a major public health and clinical challenge, as it is associated with an increased risk of metabolic abnormalities such as dyslipidemia, hypertension, type 2 diabetes (T2D), and cardiovascular diseases (CVD) [1]. Obesity is not a monogenic trait. Similar to many complex diseases, obesity results from the interaction between genes and environmental factors [2].

In 2007, a genome-wide association study for T2D revealed a strong association between common single nucleotide polymorphisms (SNPs) in the fat mass and obesity–associated gene (*FTO*) region and the risk of T2D [3]. *FTO* is placed on the long arm of chromosome 16 (16q12.2), which encodes 2-oxoglutarate-dependent nucleic acid demethylase that controls appetite and energy expenditure [4]. Research on *FTO* rs9939609 polymorphism revealed a significant correlation between risk of obesity and type 2 diabetes mellitus [5-9]. The results of Rosskopf research showed that rs9926289 *FTO* polymorphism affects BMI in younger persons (under 55) [10]. Interestingly, the rs9939609 and rs9926289 FTO polymorphisms are closely linked in the *FTO* gene, and some researchers verify their joint effects [7, 11].

High-resolution melting (HRM) analysis is a closed-tube method for rapid analysis of genetic variation within PCR amplicons [12]. Upon completion of PCR in the presence of a saturating intercalating dye such as EvaGreen, which binds to double-stranded but not single-stranded DNA, the PCR product is heated while the level of fluorescence is measured. As the temperature increases and the duplex melts, the dye is released, and fluorescence intensity is reduced. Genetic variants with differences in base sequencing result in different melting temperatures, which are detected by monitoring fluorescence during

an increase in temperature and discriminated by their characteristic melting curves, visualized by a loss of fluorescence as the DNA duplex melts [13].

The aim of our study was verification (by means of a case–control association analysis) of *FTO* gene rs9939609 and rs9926289 polymorphisms and their involvement in the genetic predisposition to obesity in part of the Iranian population.

## 2. METHOD(S)

### 2.1. Patient Study

This case–control study population consisted of 137 individuals (62 with obesity and 75 controls), from central Iran, assessed and collected at the Abol-Abbas Clinic of Diet, Khorasgan, Isfahan, Iran. All subjects were evaluated for the presence of obesity based on BMI (BMI $\geq$ 30) and controls (BMI: 18.5-24.9), and then the subjects were grouped. Individuals with the presence of T2DM were excluded. Written informed consent to participation was obtained from each individual.

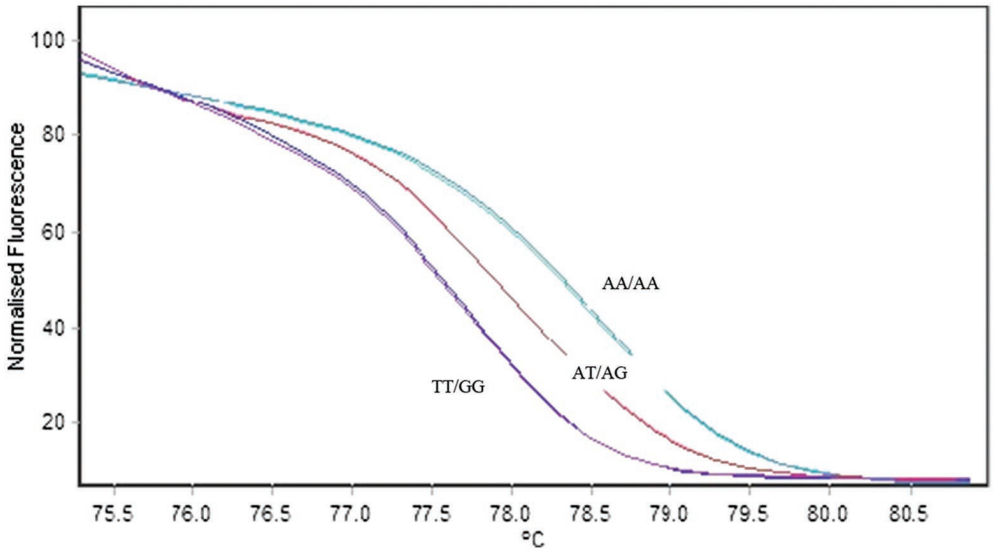
### 2.2. Genotyping

Blood samples were collected from fasting subjects for determination of biochemical parameters (fasting blood sugar, triglycerides, and total cholesterol), and genomic DNA was isolated by means of an Irazol kit (RNABiotech, Isfahan, Iran). Blood biochemistry analyses were carried out in the laboratory. Two SNPs (rs9939609 [A/T] and rs9926289 [A/G]) of *FTO* gene were genotyped using a PCR-HRM analysis that was validated by direct sequencing. PCR-HRM was performed in 15 µL reaction volume, which included 10 ng genomic DNA, 5X HOT FIREPol, EvaGreen HRM Mix, no ROX (Solis BioDyne), and 10 pmol of each primer. A 95-bp fragment was amplified with the primers 5′-CATCAGTTATGCATTTAGAATGTCTG-3′ and 5′-AGAGTAACAGAGACTATCCAAGTGC-3′. All genotyping tests were performed in duplicate. PCR was performed with initial denaturation at 95°C for 3 min, followed by 45 cycles of denaturation at 95°C for 15 s, and annealing at 60°C for 30 s. Following PCR amplifications, the samples were heated to 90°C for 1 min and then rapidly cooled down to 40°C for 1 min by Rotor-Gene 6000. HRM was carried out over the temperature range of 60-85°C, with an increment of 0.2°C every 10 s, and each amplicon cluster was determined using Precision Melt Analysis Software (BioRad, USA) (Figure 1). For testing two SNPs, 21 samples representing control and obese patients were randomly chosen for sequencing to verify genotyping results. A 695-bp product was amplified with the primers 5′-TGGTTTCAGAGGCTTGTGTG-3′ and 5′-GCCCAAGGATGGTGTTTCTA-3′ for sequencing.

### 2.3. Data Analysis

Statistical analysis was conducted using SPSS statistical software (version 20.0). Descriptive data were presented by mean and standard deviation and frequency of allele and genotype. The Kolmogorov–Smirnov test was used to analyze the normality of distribution of each variable. For qualitative data Chi square test and for quantitative data independent samples T-test were used to compare groups. Odds ratios (ORs) were calculated using logistic regression and were given with 95% confidence intervals (CI). A *p*-value less than 0.05 was considered statistically significant.

**Figure 1: Sample results of normalized graph for HRM analysis for studied *FTO* SNPs. Genotypes of two examined SNPs (rs9939609, rs9926289).**



## 3. RESULTS

The major anthropometric and biochemical characteristics of obese subjects and controls are summarized in Table 1. Obese individuals had significant high level of FBS, total cholesterol, and triglyceride. Compared with the controls, BMI was significantly higher in individuals with obesity.

Differences in the frequency of genotypes between the group of normal-weight subjects and the group of obese patients were evaluated using the dominant model and the recessive model (Table 2). The frequency of allele A in the obese group of two polymorphic sites was 51.6%. The frequency of allele T (G in rs9926289) in the obese group was 48.4%. The distribution of genotypes AA, TA (GA in rs9926289), and TT (GG) in the obese group was 16 (25.8%), 32 (51.6%), and 14 (22.6%), respectively. Subjects in the control group differed in the frequency of AA and TT (GG in rs9926289) genotypes and then A and T (G) alleles of two polymorphic sites.

Assuming a recessive inheritance model, carriers of the rs9939609 TT+TA genotype and rs9926289 GG+GA genotype in *FTO* were not found to be a risk factor for obesity (Table 2).

Assuming a dominant inheritance model, carriers of the AA+TA genotype in *FTO* rs9939609 were characterized by a higher risk of obesity (OR = 3.429, 95% CI: 1.14-10.35, $p = 0.026$). In turn, assuming a dominant model, the GA+AA genotype in *FTO* rs9926289 was not found to be a risk factor or protective factor for obesity.

Figure 2 presented the distribution of some serum factors according to the genotypes observed in individuals with obesity and without obesity. As carriers of the AA+TA genotype in FTO rs9939609 dominant model were risk factors of obesity,

**Table 1: Characteristics of the control group and obese group**

| Variable | Normal weight (control group) | Obese (case group) | *p*-value |
|---|---|---|---|
| **Number *n* (%)** | 75(100.0) | 62(100.0) | 0.195 |
| **Male *n* (%)** | 10(13.3) | 2(3.2) | |
| **Female *n* (%)** | 65(86.7) | 60(96.8) | |
| **Age (years) (Mean ± SD)** | 47.90 ± 11.39 | 33.63 ± 8.91 | 0.008 |
| **FBS (Mean ± SD)** | 98.37 ± 14.95 | 112.00 ± 22.75 | 0.006 |
| **TG (Mean ± SD)** | 133.87 ± 64.17 | 186.97 ± 93.79 | 0.013 |
| **Total Cholesterol (Mean ± SD)** | 163.13 ± 40.44 | 194.65 ± 43.02 | 0.005 |
| **BMI (Mean ± SD)** | 22.18 ± 1.78 | 36.54 ± 4.10 | <0.001 |

FBS: fasting blood sugar; TG: triglyceride; BMI: body mass index; SD: standard deviation.

**Table 2: Distribution and association analysis of *FTO* genotypes among obese subject and controls**

| | | | Control (%) | Obese (%) | *p*-value | OR | 95% CI |
|---|---|---|---|---|---|---|---|
| ***FTO*** rs9939609 | Genotypic | TT | 38(50.6) | 14(22.6) | 0.060 | – | – |
| | | AT | 20(26.7) | 32(51.6) | | | |
| | | AA | 17(22.7) | 16(25.8) | | | |
| | | A | 54(36) | 64(51.6) | 0.097 | | |
| | | T | 96(64) | 60(48.4) | | | |
| | Recessive | TT+TA | 58(77.3) | 46(74.2) | 0.823 | – | – |
| | | AA | 17(22.7) | 16(25.8) | | | |
| | Dominant | TT | 38(50.6) | 14(22.6) | 0.**026** | **3.429** | 1.14-10.35 |
| | | TA+AA | 37(49.4) | 48(77.4) | | | |
| ***FTO*** rs9926289 | Genotypic | GG | 30(40.0) | 14(22.6) | 0.123 | – | – |
| | | GA | 20(26.7) | 32(51.6) | | | |
| | | AA | 25(33.3) | 16(25.8) | | | |
| | | G | 80(53.3) | 60(48.4) | 0.585 | | |
| | | A | 70(46.7) | 40(51.6) | | | |
| | Recessive | GG+GA | 50(66.7) | 46(74.2) | 0.519 | – | – |
| | | AA | 25(33.3) | 16(25.8) | | | |
| | Dominant | GG | 30(40.0) | 14(22.6) | 0.142 | – | – |
| | | GA+AA | 45(60.0) | 48(77.4) | | | |

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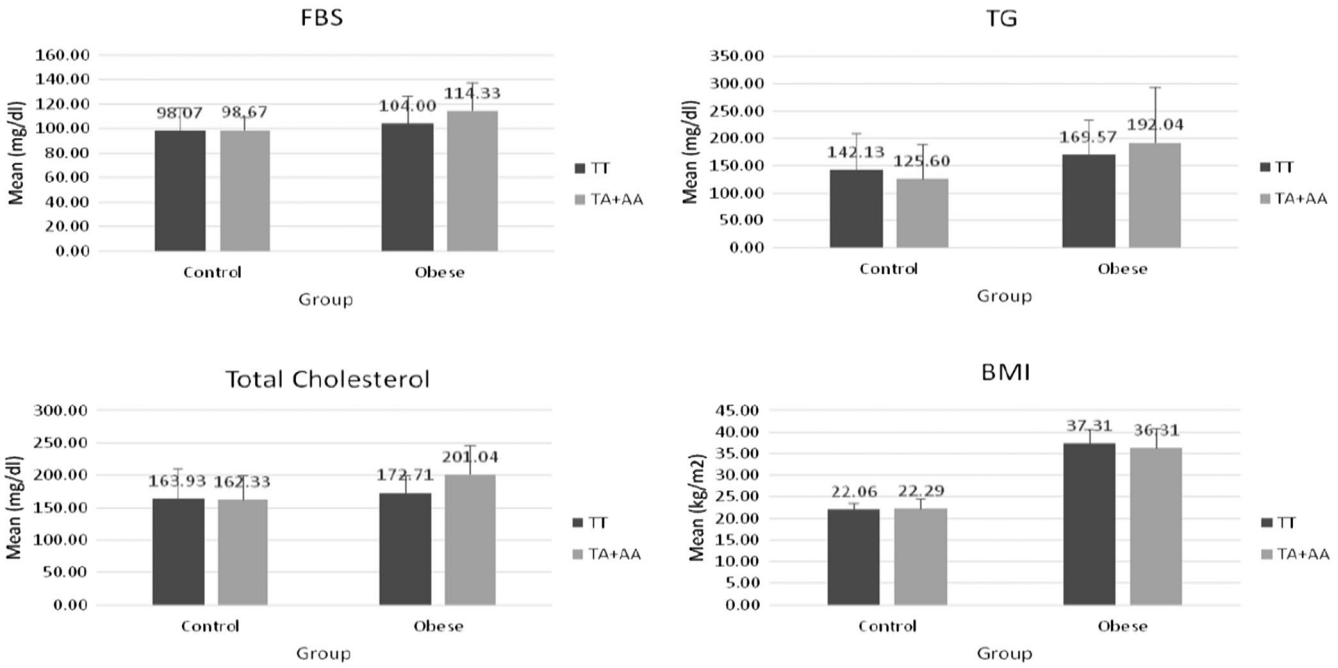
**Figure 2: Association of *FTO* rs9939609 dominant model genotypes with biochemical parameters (FBS, cholesterol, and triglycerides) and BMI.**




FBS: fasting blood sugar; TG: triglyceride; BMI: body mass index.

we investigated the association of polymorphism with the clinical variables. Interestingly, no difference was observed between the two groups of genotypes and all parameters of obesity.

## 4. DISCUSSION

The results showed that FBS, triglycerides, total cholesterol, and BMI were significantly higher in obese patients. According to the dominant model, our results show that carriers of the AA+TA genotype in *FTO* rs9939609 were characterized by a higher risk of obesity. Moreover, there is no significant association between allelic distribution and genotypes with obesity in *FTO* rs9939609 and *FTO* rs9926289.

Numerous studies in various populations and age groups have demonstrated that *FTO* variants were significantly associated with obesity. The relationship between the *FTO* rs9930506 polymorphism with higher BMI was confirmed in the study on the obese Italian subjects [14]. Wu *et al*. indicated that BMI of subjects with A allele of *FTO* rs9939609 is higher than with T allele [15]. In addition, the research of Ursu *et al*. revealed that *FTO* rs9939609 polymorphism has been identified as a common gene variant in the Romanian Caucasian cohort, proving a high association with all the parameters of obesity and obesity comorbidities [16]. Research on obesity in the Mexican population revealed that *FTO* is a major risk factor for obesity and is upregulated in the subcutaneous of obese individuals [17]. Another study from Spain confirmed the association of *FTO* gene variants with obesity, including parameters of visceral (abdominal) obesity, in an adult general population [18]. In the French MINICA study, it was confirmed that A allele of the *FTO* rs9939609 polymorphism on the risk of obesity and type 2 diabetes mellitus plays a role [19]. Among the United Arab Emirates population, rs7903146 (*TCF7L2*) is a risk for T2DM susceptibility while rs5219 (*KCNJ11*), rs10946398 (*CDKAL1*), and rs9939609 (*FTO*) variants are not directly related to T2DM development but to some of its risk factors and related traits [20]. In a study by Albuquerque *et al*., It was found that there is a nominal significant associations of the rs17817449 *FTO* polymorphism with BMI, weight waist circumference in Portuguese children, and a nominal association was also observed with the risk of obesity ($p = 0.027$). The same polymorphism was found significantly associated with obesity ($p = 0.0005$) in Spanish adults [21].

Our results show that, according to the dominant model (TA+AA/TT), individuals with TA+AA genotypes in *FTO* rs9939609 had a significantly higher risk of obesity (OR = 3.429). In other populations, codominant models will be better than recessive models [22].

On the other hand, *FTO* rs9939609 and rs9926289 exhibited a strong codominant obesity-predisposing effect of genotypes homozygous for minor alleles in Polish children aged 6-16 [23].

Interestingly, our results revealed that the distribution of genotypes AA, TA, and TT in rs9939609 was the same in the obese group with AA, GA, and GG genotypes of rs9926289. These results are also confirmed in the other study [22]. Of course, a larger sample will allow us to verify our very preliminary hypothesis of the co-occurrence of the AA, TA, and TT genotypes in rs9939609 with AA, GA, and GG genotypes of rs9926289 in the obese group.

As most studies show that the role of *FTO* polymorphisms in increased risk of obesity is significantly intensified by reduced physical activity and a high-calorie diet [24], the next part of our study will be the role of different gene polymorphisms in lifestyle and physical activity.

## 5. CONCLUSION

Our results suggest that the dominant model of rs9926289 is associated with obesity. However, further examination should be carried out on large populations and more polymorphisms of risk factor genes.

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**Author Contributions**
**Conceptualization:** MO. **Methodology:** YBE. **Formal analysis:** FRK. **Data collection:** MO, YBE. **Validation:** MO, SE. **Investigation:** YBE, MO. **Writing - original draft preparation:** YBE. **Writing - review and editing:** MO. **Approval of final manuscript:** all authors.

**Conflict of Interest**
None.

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