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# Evaluation of the relationship between intronic polymorphism TPO gene and risk of type 2 diabetes in a Tehran population

**Massoud Houshmand[1*], Mehrshad Seresht-Ahmadi[2], Mahsa Alikarami[3]**

[1] National Institute of Genetic Engineering and Biotechnology, Tehran, Iran.

[2] Department of Genetics, Reproductive Biomedicine research center, Royan Institute of Reproductive Biomedicine, ACECR, Tehran, Iran.

Department of Basic Science and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran.

[3] Personalized Medicine Research Center of AmitisGen, Tehran, Iran.

*Corresponding author: Massoud Houshmand, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran. E-mail: housh62@yahoo.com*

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**Abstract**

Obesity and type 2 diabetes are two complex disorders with strong genetic backgrounds, and both disorders cause other diseases and increase mortality. FTO is a protein-coding gene located on the long arm of chromosome 16 and linked to type 2 diabetes and obesity. The current study investigated the relationship between the RS16953002 polymorphism gene and type 2 diabetes and obesity in a population from Tehran. The study population included 150 people with type 2 diabetes and 150 healthy individuals. Genotyping was performed by RFLP-PCR. The results indicated the presence of the GG allele in 81% of diabetic patients but in only64% of non-diabetic participants, which shows a statistically significant difference in this regard. Thus, the current study has shown the role of FTO gene polymorphisms in the pathogenesis of people with type 2 diabetes and obesity.

## INTRODUCTION

Obesity and type 2 diabetes are two complex disorders with strong genetic backgrounds, and both disorders cause other diseases and increased mortality (1).Genome-wide association studies have recently discovered a new gene linked to type 2 diabetes and obesity (2). Known as FTO and located on the long arm of chromosome 16, this gene was first detected in mice (1) and is associated with energy balance in the body. It is strongly expressed in the hypothalamus and the pituitary and adrenal glands, which are involved in controlling energy homeostasis in the brain (3),indicating that it is also involved in regulating body weight and can affect people's susceptibility to diabetes through obesity, which is measured by an index called BMI (4). Obesity increases the risk of developing type 2 diabetes by up to 10 times. The function of this gene is still unknown, but it is predicted that its structure will encode a non-hemi oxygenation protein dependent on 2-oxoglutarate which has nucleic acid demethylation activity. Several genome-wide association studies have shown that the gene's polymorphisms are associated with BMI and the risk of overweight in children and adults in European and American populations (5). These results have been confirmed in studies with fewer patients in Germany and Belgium (6,7). A number of polymorphisms in this gene, which are within intron 1, have been linked to obesity. In India, the gene's polymorphisms have been associated to type 2 diabetes more than the BMI index (8). Racial differences between populations, which are more genetic, are due to obesity and obesity-related diseases such as type 2 diabetes (9). In Asian populations, contradictory results have been obtained from the association of polymorphisms of this gene with obesity and type 2 diabetes. In the current study, the relationship between the RS16953002 polymorphism gene and type 2 diabetes and obesity in a population of Tehran was evaluated.

## METHODS AND MATERIALS

The study population included 150 people with type 2 diabetes who were given 5 ml of peripheral blood during a monthly checkup with personal consent. The control group consisted of 150 healthy individuals who, similar to the first group, received 5 ml of blood after giving consent. Data on the weight, height, gender, and body mass index of participants in both groups was also obtained to be compared with the corresponding polymorphism. DNA extraction was performed by the phenol chloroform method. The quantity and quality of the extracted DNA were evaluated by nanodrop and electrophoresis on agarose gel, respectively. To study the relevant polymorphism, the RFLP-PCR method

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was used. First the relevant genetic area, which included polymorphism, was amplified by specific primer (Table 1). The PCR product was treated with a restrictive enzyme Nla III to perform an enzymatic reaction if the target sequence was present. Finally, to evaluate the results, the enzymatic process was run on 2% agarose gel and the results were examined. All statistical analyses in this study were performed by SPSS v.16 software, and a *p*-value ≤0.05 was considered significant.

**Table 1.** primer sequencing and PCR product size

| Primer | Sequence | Product Size |
|---|---|---|
| Forward-fto | GAGTTTGTCGTTACTGTTGTCCT | 420bp |
| Reverse-fto | TCTGTCAGTTACCTCTTCTCTCT | |

## RESULTS

It is expected that if there is AA genotype, the enzyme will cut the PCR product and generate two pieces with lengths of 300 and 120 nucleotides. If there is a GG genotype, no incisions will be made and only a 420 nucleotide piece, which is the same as the PCR product, will be seen on the gel. If there is a heterozygous genotype, there will be three bands (420, 120, and 300) on the gel. Evaluation of the demographic characteristics in the two groups showed that despite the lack of a significant difference between the two groups, the difference in BMI was significant ($p = 0.001$). The results also showed no significant relationship between gender and diabetes or between alcohol consumption and smoking and diabetes. The results of the study of different alleles indicated the presence of a GG allele in 81% of diabetic patients and in 64% of non-diabetic participants, which showed a statistically significant difference. Similarly, a significant difference was observed between the prevalence of GA alleles in diabetic (14%) and non-diabetic (27%) participants. AA alleles were observed in only 5% of diabetic and 9% of non-diabetic participants (Table 2).

**Table 2.** Genotype frequency in diabetic participants and non-diabetic participants

| Genotype | Diabetic Participants | Non-diabetic Participants | *p*-value |
|---|---|---|---|
| AA | 5% | 9% | 0.122 |
| GA | 14% | 27% | 0.004 |
| GG | 81% | 64% | 0.001 |

## DISCUSSION

The FTO gene was first discovered in a GW study for type 2 diabetes in European populations (10). This gene is located on chromosome 6. Its exact function and how its polymorphisms affect the risk of type 2 diabetes are not clear; however, this gene may be involved in the epigenetic regulation of the progression of type 2 diabetes (11). Protein and mRNA levels of the FTO gene in muscle cells are much higher in people with type 2 diabetes than in non-diabetic, non-obese or control individuals. Therefore, the overproduction of FTO in muscle myotobols reduces oxidative metabolism, fat metabolism, and oxidative stress in muscles, which in turn leads to complications, a common characteristic of people with type 2 diabetes (13). Several recent large-scale genomic studies have shown an association between FTO gene polymorphisms and diabetes and obesity in various Asian and Caucasian populations (14). In the current study, the dependence of polymorphism rs16953002 on the FTO gene with obesity and type 2 diabetes in a Tehran population was investigated. Other studies of the genes in other polymorphisms in different populations have achieved conflicting results regarding the association of these polymorphisms with type 2 diabetes and obesity. Scuteri et al. studied G/A rs9940128 FTO gene polymorphism and reported a significant association between it and type 2 diabetes (15). The GG genotype of this polymorphism had a higher risk of developing type 2 diabetes, which is similar to the findings of studies in Chinese populations (16). Polymorphism A/T rs9939609 in the intron 1 FTO gene was shown to have a very strong association with type 2 diabetes, but this relationship was independent of the BMI index (17). Further studies on the relationship between the polymorphism rs9939609 FTO gene and type 2 diabetes have shown that this relationship changes under the influence of diet. In the current study, the association between RS16953002 gene polymorphism and type 2 diabetes and obesity was evaluated in a case-control study. The results confirmed that the homozygous genotype GG and heterozygous GA are associated with type 2 diabetes. Homozygous genotypes GG and heterozygous GA are also associated with obesity. Thus, this study showed the role of FTO gene polymorphisms in the pathogenesis of people with type 2 diabetes and obesity.

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