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Study the Association of Fat Mass and Obesity-associated (*FTO*) Gene Polymorphisms (SNP rs9939609) with Biochemical Markers in Obese Iraqi Patients

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Abstract

This study aimed to evaluate the association of single nucleotide polymorphisms (SNP rs-9939609) of the fat mass and obesity-associated (*FTO*) gene with body mass index (BMI) and some biochemical markers in obese patients. The fat and mass obesity (*FTO*) gene variant specific single nucleotide polymorphism (SNP rs-9939609) with the T to A missense mutation may have a powerful association with obesity. This case-control study included 106 obese patients (57 males and 49 females) and 80 healthy control (40 males and 40 females). DNA was extracted from whole blood, and then the tetra-primer amplification refractory mutation system - polymerase chain reactions (ARMS.PCR) technique was used to limit the single nucleotide gene polymorphisms (rs9939609) of the *FTO* gene. Lipid profile, glycated hemoglobin (HbA1C), fasting blood sugar, insulin and *FTO* concentrations were measured by standard methods. In this study, the A allele in the rs9939609 was at a higher frequency of 35 (33.1%) in the obese patients and at a significant *p*-value = 0.039 compared with control 15 (18.7%). It significantly increased in the additive model and allele frequency (p= 0.003, 0.002 respectively). The rs9939608 SNP showed a significant association with increased BMI, insulin and homeostatic model assessment-insulin resistance (HOMO-IR) with *p*-values of 0.001, 0.001 and 0.028 respectively. After making adjustments for age and sex, lower levels of high-density lipoprotein (HDL) were observed in the AA and TA genotypes compared to the TT genotype (p = 0.004). While, no significant differences were recorded between the rs9939608 SNP and HbA1C, total cholesterol (TC), triglyceride (TG), very low-density lipoprotein (VLDL), and low-density lipoprotein (LDL) in obese patients

Keywords: Fat mass and obesity-associated (*FTO*), SNP (rs9939609), Gene polymorphism.

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(609 لجين (*FTO*) Fat mass and obesity–associated مع بعض المتغيرات الحيوية لدى الاشخاص المصابين بالسمنة في العراق

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الخلاصة

هدفت هذه الدراسة الى تقييم ارتباط تعدد اشكال النيوكليوتيدات المنفردة (SNP rs9939609) في جين (*FTO*) Fat mass and obesity–associated مع مؤشر كتلة الجسم (BMI) وبعض المتغيرات البايوكيميائية في مرضى السمنة.بالاضافة الى الطفرات الخاطئة من Tالى A لتعدد الشكال الجيني (rs9939609) في جين *FTO* التي قد يكون لها ارتباط قوي بالسمنة. الدراسة تضمنت 106 مريض بالسمنة (57 رجل و 49 امرأة) و 80 شخص سليم كمجموعة سيطرة (40 رجل و 40 امراة). تم استخلاص الحمض النووي من الدم الكلي للانسان، و استخدمت تقنية Tetra–primer amplification refractory mutation system– polymerase chain reactions (ARMS.PCR) لتحديد (SNP) . مجموعة الدهون، السكر التراكمي (HbA1C) ، السكر اليومي، هرمون الانسولين، و تركيز انزيم FTO. حيث تم قياسهم بواسطة طرق قياسية. في هذة الدراسة، كانت نسبة الاليل A (33.1%) 35 في (rs9939609) عند مرضى السمنة و ذات فرق معنوي (p=0.039) عند المقارنة مع مجموعة السيطرة (18.7%) 15. وزيادة معنوية في اليل A عند additive model و allele frequency عند قيمة معنوية ,0.003 =p) (0.002 علي التوالي. بين تعدد اشكال النيوكليوتيدات المفردة SNP rs9939609) علاقة معنوية مع BMI، Insulin، و HOMO–IR عند (p= 0.001, 0.001, 0.028) على التوالي. لوحظ انخفاض في مستوى الدهون عالية الكثافة HDL في الانماط الجينية AA و TA مقارنة مع النمط الجيني TT عند = p) (0.004. بينما لم تظهر فروق ذات دلالة احصائية بين (rs9939609 SNP) وA1C ، TG،TC ، VLDL و LDL في مرضى السمنة.

Introduction

Being overweight increases the likelihood of developing numerous chronic illnesses, including type II diabetes mellitus, cardiovascular disease, arthritis and even cancer, making obesity a serious public health concern.. The prevalence of obesity has increased worldwide, particularly in recent years. For instance, the prevalence of obesity in the United States is about 20–25%, while 10%–25% in Europe. According to some researchers in Iraq the prevalence of overweight and obesity in Basra and Baghdad was 55.1% and 43.7% respectively. The physiological mechanisms of obesity include nutritional status, environmental conditions and genetic makeup [1, 2]. Various types of genes such as fat mass and obesity-associated (*FTO*) genes, leptin gene and adiponectin gene, are strongly associated with overweight and obesity [3].

A common single nucleotide polymorphism (SNP) in a recently described fat mass and obesity gene (*FTO*), is related to human body weight and fat. The expression of FTO is highly expressed in the human hypothalamus, pituitary and adrenal glands, indicating a potential role for this gene in weight regulation [4]. In humans the fat mass and obesity-associated polymorphic gene are located on chromosome 16 q12.2. The gene is known to be one of the most effective in the metabolic pathway of the human body [5]. The relationship between

FTO SNPs and body mass index (BMI) was first discovered in diabetic people in Europe. The classic *FTO* SNPs associated with BMI are rs9939609 (T/A), and compared to those not carrying the risk allele, 16% of adults carried the homozygous risk allele [6]. Genome-wide association studies (GWAS) on obesity traits and the important role of the *FTO* locuswere established, several *FTO* SNPs in the intronic region such as rs9930506, rs1421085, rs8050136 and rs1121980 have been reported, these SNPS likely play a role in genetic predisposition to obesity [7]. The risk allele A *FTO* SNP rs9939609 has been strongly associated with obesity and BMI in Chinese, South Asian, Malaysian Singaporean, and East Asian populations[8]. The *FTO* gene encodes a fat mass and obesity protein that is involved in deoxyribonucleic acid DNA repair and fatty acid metabolism as well as catalyzing the demethylation of single-stranded nucleic acids RNA [9]. The biological function of *FTO* is not fully known yet. It is suggested that the catalytic activity of *FTO* may regulate the transcription of genes involved in fatty acids and glucose metabolism [10].

Materials and Methods

This case-control study included 186 participants, 106 patients diagnosed with obesity (57 males and 49 females), and 80 normal-weight individuals as control (40 males and 40 females)**.** The ages of the participants ranged between 18-45 years. In regard with BMI, the patient groups was greater than 30 Kg\m^2 and the control group's BMI ranged between 18.5-24.9 Kg\m^2. Chronic diseases, obesity due to secondary causes, pregnant women and patients who took any drug for obesity were excluded from the study.

Fasting blood sugar (FBS), total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), and high- desnsity lipoprotein (HDL) were determined with an automatic biochemical analyzer, the Cobas E311 device (Roche / Germany). Fasting plasma insulin and FTO were measured by the enzyme-linked immunosorbent assay (ELIZA) and ELK Biotechnology (China). Glycated hemoglobin (HbA1c) reading was done using a fully automated instrument (D-10 Hemoglobin A1c program). The concentration and purity of genomic DNA were measured by UV absorption (Nano-drop, USA) at 260 and 280 nm after genomic DNA was extracted from peripheral blood using the Genomic DNA Mini Kit (Geneaid, Korea). The tetra-primer amplification refractory mutation system—polymerase chain reaction (ARMS-PCR) was used to determine the genotyping for the *FTO* gene by using specific primers. The sequences of primers and PCR program in this study for the SNP (rs9939609) of the *FTO* gene were obtained from [11] as shown below.

Font .5′-TGGCTCTTGAATGAAATAGGATTCAGAA-3
Rout: 5′-AGCCTCTCTACCATCTTATGTCCAAACA-3
Fin: 5′ -TAGGTTCCTTGCGACTGCTGTGAATATA-´3
Rin: 5′-GAGTAACAGAGACTATCCAAGTGCATCTCA-3

Statistical Analysis:

MedCla-version 20.115 was used to analyze the genotypes and allele frequencies in the control and patients. SPSS for Windows (version 26, USA) was used to analyze the relationship between rs9939609 SNP in the *FTO* genotype group and biochemical markers.

Results

The biochemical and clinical characteristics of the study participants can be seen in Table 1 below. There were significant differences in BMI, fasting plasma, insulin, and HOMO-IR levels in the obese patients compared with the healthy group; *p*-value = 0.02, 0.031, 0.013, and 0.001 respectively. Whereas, highly significant differences in TC, TG, LDL, and very low density lipoprotein (VLDL) levels were noticed in obese patients compared with the control group, *p*-value= 0.001 for all. On the other hand, the study showed a decrease in high-density

lipoprotein (HDL-c), and FTO levels, *p*-value= 0.001 in the obese patients compared to the control. No significant differences (*p*-value > 0.05) were recorded in the level of HbA1c, sex and age in the patient's group compared with the control group.

Table 1: Anthropometric and biochemical characteristics of study participants.

Variables	Control (N=80) Mean± SD	Patients (N=106) Mean ± SD	*p*. Value
Age (years)	31.28 ± 5.87	31.62 ± 6.01	0.329
BMI (Kg/m^2)	21.68 ± 2.14	38.54 ± 5.31	0.02
FBS (mg/dl)	99.6 ± 14.6	109.8 ± 22.1	0.031
HbA1C (%)	5.10 ± 0.66	5.05 ± 0.57	0.161
TC (mg/dl)	137.1 ± 19.7	183.4 ±38.6	0.001
TG (mg/dl)	109.7 ± 24.4	175.4 ±75.7	0.001
HDL (mg/dl)	21.95 ± 4.88	31.9 5± 9.04	0.001
LDL (mg/dl)	77.3 ± 19.03	111.3 ±32.9	0.001
VLDL (mg/dl)	73.83 ± 8.33	34.71±15.45	0.001
Insulin pmol/L	10.65 ± 5.38	58.6 ± 42.2	0.013
HOMA IR	2.48 ± 1.33	15.9 ± 12.8	0.001
FTO ng/ml	1.89 ± 0.836	1.46 ± 0.49	0.001

Significant; *p*-value <0.05

Genotyping

As shown in Figure 1 below, the analysis of PCR genotypes of fat mass and obesity-associated SNP (rs9939609) by agarose gel electrophoresis discovered two bands (321, 178 bp) for TT wild-type, three bands (321, 210, 178 bp) for TA heterozygous, and two bands (321, 201 bp) for AA homozygous genotypes [12].

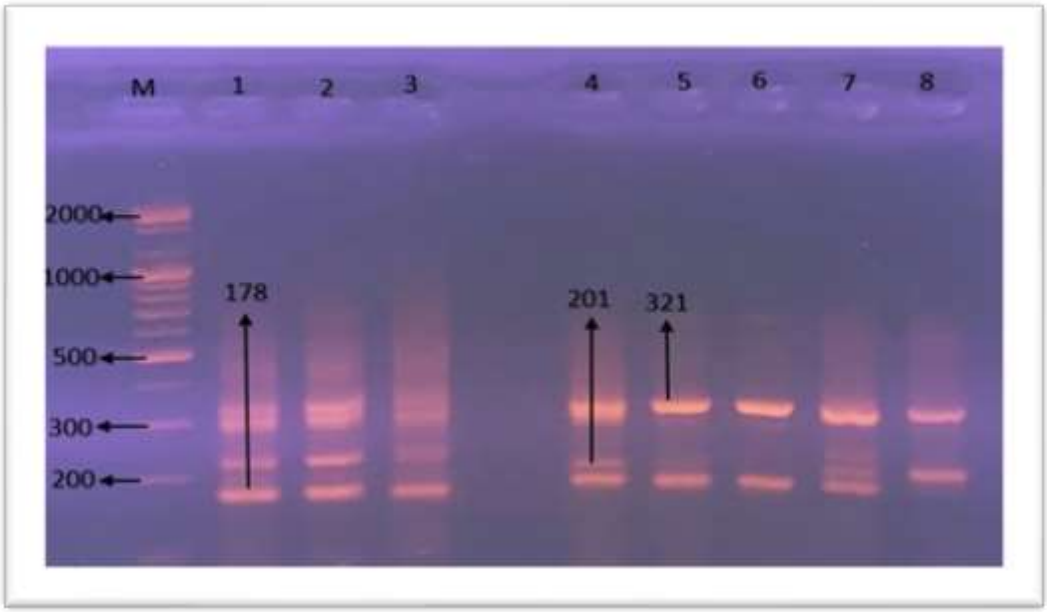

Figure 1: ARMS-PCR product of fat mass and obesity-associated *(FTO)* SNP (rs9939609) on agarose gel electrophoresis (2%, 90V for 60 minutes), M: Marker lane (100bp).: Lanes 1, 2, 3, 4, 5, 6, 7, 8: *FTO* genotypes

Table 2 shows the genotypes' compatibility with Hardy–Weinberg equilibrium in the obese patients and the control group (p=0.068 and 0.094 respectively).

Table 2: Results of Hardy-Weinberg equilibrium for fat mass and obesity-associated (*FTO*) SNP (rs9939609) in patients and control

Subjects	X^2	*p*. Value
Patients	5.354	**0.068**
Control	4.713	**0.094**

X^2= Chi-Square

The allele frequencies and genotypes of the *FTO* gene variants are laid down in Table 3. No statistically significant association was observed between the TT and TA genotypes of the *FTO* gene variant rs9939609 in obese patients and control group subjects. While the AA genotype showed a statistically significant difference.

The frequencies of TA in the control were 28 (35.0%) and 41 (38.6%) in the patients in the co-dominant model, and the frequencies of TT were 37 (46.2%) in the control and 30 (28.1%) in the patients (OR = 1.239 and 0.697 respectively). No significant differences were noticed between TA and TT genotype frequencies among control and patient populations (*p*-values = 0.485 and 0.247 respectively). The AA genotypes were significantly higher (*p*-value = 0.039) in the patients' 35 (33.1%) compared with the controls 15 (18.7%), OR = 2.70.

There were no statistical differences in AA+TA genotype frequencies between 76 (71.6%) patients and 43 (53.7%) controls 43 (53.7%) in the dominant model, OR = 1.433, and *p*-value 0.247. In the recessive model, the TT+TA genotypes were found at 65 (81.2%) and the AA genotypes at 15 (18.7%) in the control group, which was significant (*p*-value = 0.046) compared with obese at patients 71 (66.95%), 35 (33.1%) for TA+TT and AA respectively.

In the additive model, allele A frequency showed a highly significant difference (*p*-value = 0.003 and 0.002 respectively). The 2AA+TA genotypes and A allele frequencies were found in 58 (72.5%) of the control, lower than patients. The odd ratio for 2AA+TA in the additive model was 2.364, and that for the A allele, it was 1.923.

Table 3: Results of genotype and allele frequency of fat mass and obesity-associated (*FTO*) SNP (rs9939609) in patients and control group.

Rs9939609	Control N=80	Patients N= 106	OR (95% CI)	Adjusted OR (95% CI)	*p*. Value
Co-dominant					
TT	37(46.2%)	30(28.3%)	0.697	0.3793 to 1.2839	**0.247**
TA	28(35.0%)	41(38.6%)	1.239	0.680 to 1.289	**0.485**
AA	15(18.7%)	35(33.1%)	2.070	1.030 to 4.142	**0.039**
Dominant					
AA+TA	43(53.7%)	76(71.6%)	1.4331	0.778 to 2.639	**0.247**
Recessive					
TT+TA	65(81.2%)	71(66.95)			
AA	15(18.7%)	35(33.1%)	0.588	0.251 to 0.998	**0.046**
Additive					
2(AA)+TA	58(72.5%)	111	2.364	1.325 to 4.203	**0.003**
Frequency of A allele	58(72.5%)	111	1.923	1.269 to 2.942	**0.002**

Table 4 shows the BMI, FBS, HbA1C, cholesterol, TG, VLDL, HDL, LDL, fasting insulin, and HOMA-IR of patients with the *FTO* SNP (rs9939609) in the co-dominant model

as analyzed by an ANOVA test. There were no significant differences between the AA, TT, and TA genotypes of the *FTO* rs9939609 SNP; FBS, TC, TG, VLDL, LDL, HbA1C, and *FTO* genotypes (*p*-value > 0.05). There were highly significant differences in BMI, insulin, and the three genotypes (AA, TA, TT) (*p*-values = 0.014 and 0.001 respectively). This study showed significant differences in the serum levels of HDL and HOMO-IR (*p*-values = 0.023 and 0.019 respectively).

Table 4: Clinical characteristics of patients according to *FTO* SNP (rs9939609) genotypes (Co-dominate model).

Variables	TT (n=30)	TA (n=41)	AA (n=35)	*p*. Value
BMI (kg/m$^{2)}$)	35.21 ± 2.47	36.31 ± 2.99	42.56 ± 5.16	**0.001**
FBS (mg/dl)	101.30±17.95	105.26±15.21	109.89 ± 17.95	**0.293**
HbA1C %	4.98 ± 0.531	4.99 ± 0.43	5.12 ± 0.66	**0.842**
Insulin (µU/ml)	34.31 ± 26.66	47.02 ± 32.68	91.52 ± 43.29	**0.001**
HOMA-IR	13.23 ± 10.42	13.25 ± 11.83	21.24 ± 14.45	**0.019**
Cholesterol (mg/dl)	180.5 ± 42.15	180.8 ± 36.84	190.4 ± 37.07	**0.321**
Triglycerides (mg/dl)	175.3 ± 61.66	163.3 ± 76.51	192.64±82.11	**0.278**
VLDL (mg/dl)	31.26 ± 14.13	36.07 ± 13.30	37.09 ± 17.82	**0.157**
LDL (mg/dl)	106.1± 30.94	112.3 ± 32.3	114.2 ± 31.07	**0.416**
HDL (mg/dl)	35.51 ± 9.12	31.19 ± 10.14	29.51 ± 6.31	**0.023**
FTO (ng/dl)	1.46 ± 0.410	1.43 ± 0.564	1.47 ± 0.381	**0.762**

Discussion

Various types of genes are associated with obesity that are a risk factor for type 2 diabetes such as the *FTO* gene (rs9939609) SNP [13, 14]. In this study, the *FTO* gene of the SNP rs9939609 was analyzed in the normal-weight group and obese Iraqi patients, and the relationship between *FTO* gene polymorphism with some biochemical markers. The role of the *FTO* gene in obesity and predicting type II diabetes mellitus was also investigated [15].

The risk allele A of the *FTO* SNP rs9939609 showed to be significantly associated with higher BMI, HOMO-IR, and insulin in the current study which agrees with others results of some other studies [16, 17].

The study also showed a relationship between the *FTO* rs9939609 polymorphism and HDL-C levels. The carriers of the A-allele of the rs9939609 polymorphism had lower levels of HDL-C compared with carriers of the T allele. Furthermore, the current study found that carriers of the A-allele of rs9939609 had lower HDL-C than healthy people [18,19] Obese patients with the homozygous AA genotype had highly significant serum levels of plasma insulin and HOMO-IR compared with the TT and TA genotypes. This result agrees with those achieved by Mozafarizadeh *et al*.[20]. While the low level of HDL in the homozygous AA genotype was noticed compared with the TT and TA genotypes. The absence of one functional copy of the *FTO* gene mutations corresponded to lean and obese phenotypes. The FTO had high levels of expression in the hypothalamus and brain to regulate appetite [21, 22].

The biochemical markers in this study showed that obesity had an effect on lipid and lipoprotein metabolism. This proved to be a risk factor for cardiovascular diseases by increasing total cholesterol, triglycerides and low-density lipoprotein levels, while high-density lipoprotein (good lipoprotein) level decreased in obese patients. These results agree with those obtained by references [23-25]. An increase in TG caused a decrease in lipoprotein

metabolism, particularly HDL-C which worked to protect blood vessel walls and lower total cholesterol [27, 28].

The biochemical analysis of obese patients revealed increased levels of insulin hormone, fasting glucose, and HOMO-IR which led to insulin resistance (the lack of tissue response to insulin action). It is the major cause of T2DM and increased triglycerides in the liver [29]. Insulin resistance led to impaired activity of endothelial-bound lipoprotein lipase (LPL), which was involved in reduced triglyceride hydrolysis and uptake of VLDL and chylomicron by adipose tissue and muscle [30].

Conclusions:

FTO is one of the important genes that lead to obesity. The existing study showed that carriers of the genotype AA homozygous were more at risk for developing obesity compared to carriers of the TA and TT genotypes. This study also showed that the A allele had significantly lower serum HDL-C and higher serum levels of insulin. It can therefore be concluded that people who have a genetic factor for obesity should regulate their diet and lifestyle.

Conflicts of Interest

The authors have no conflict of interest.

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