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Original Article

The association of the common fat mass and obesity associated gene polymorphisms with type 2 diabetes in obese Iraqi population

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ABSTRACT

Background & objectives: This study investigates the association of two potential Fat mass and obesity associated gene (FTO) gene polymorphisms (rs9939609 and rs918031) as potential predictors of type 2 diabetes (T2D) in obese Iraqi population and their metabolic effects on hyperglycemia and insulin sensitivity.
Materials & methods: The study included 400 participants with obesity & T2D, with a matching 400 obese non-diabetic cohort. Venous blood samples were collected for DNA extraction. Using specific primers and restriction enzymes, genotyping was performed to identify the various alleles for each gene. The genotype and allele frequencies determined by multinomial logistic regression analysis for FTO single nucleotide polymorphisms (rs9939609) among all the study groups.
Results: There is a two-fold increase in the risk of T2D within the homozygous genotype (TT) group ($OR = 2.43$, CI 95% 3.57–11.2, $P \leq 0.001$) as compared to the wild type (TA). In addition, there was a significantly higher level of the minor allele genotype (T) in T2D patients when compared to the control group, ($P \leq 0.001$).
Conclusion: We conclude that the FTO rs9939609 genotype significantly affect the development of insulin resistance, therefore the future occurrence of T2D, in obese individuals.

1. Introduction

Type 2 diabetes (T2D) and obesity have reached epidemic proportions worldwide and the prevalence is still increasing at critical rates, especially in developing countries [1]. Previous studies revealed that obesity is related to both genetic and environmental factors [2]. Fat mass and obesity associated gene (FTO) is a diabetogenic gene which previously showed to affect the degree of obesity, and is strongly linked to "body mass index" (BMI) [2].

FTO was the first gene found to be linked with common forms of obesity in 2007 [3]. FTO gene spans more than 400 Kb on chromosome 16 and has 9 exons. The first intron is most associated with obesity [4]. This association has been confirmed in several countries with different ethnic background and various age groups [4]. It was showed that the FTO single nucleotide polymorphisms (SNPs) possess similar effects on obesity in Asian countries as well as in European & African countries [5]. However, there is no current evidence to show that the FTO SNPs influence the degree of physical activity [4]. Having said that, studies showed that physical activity may reduce the effects of FTO gene on obesity by up to 30% [4].

FTO protein is a 2-oxyglutarate dependent non-heme dioxygenase family member and localizes in nucleus [6]. Studies suggest that FTO is highly expressed not only in the hypothalamic nuclei involved in energy balance but also in the peripheral tissues [7]. The hypothalamic expression of FTO suggests an increased potential in the control of food intake and whole-body metabolism [7]. A number of SNPs in FTO gene have act as risk factor with obesity in all age groups [6].

In this study, we focused on rs918031 and rs9939609. These are two SNPs, which were showed to be associated with obesity and diabetes [8–10]. However, inconsistent data were previously

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reported about their activities in obesity and diabetes. Therefore, we tested both SNPs individually in this study [8–10].

2. Materials & Methods

2.1. Study protocol

Four-hundred patients with clinically diagnosed T2D [11], aging between 42 and 70 years (mean 54.77 ± 8.84 years), were included in this study. A matching group of 400 obese participants were included as control subjects [12]. With age ranged between 40 and 70 years with mean 53.9 ± 8.98 [12]. The Diabetes Clinic at Al-Sadar Teaching Hospital in Al-Najaf receive patients from all regions of Iraq, therefore, this study should be representative of the Iraqi population. The current study was conducted after it was reviewed and approved by the Faculty of Medicine Human Research Ethics Committee, University of Kufa (KUM434).

2.2. Phenotypic data

The phenotypic criteria included BMI [13], fasting plasma glucose, lipid profile and level of serum insulin.

2.3. Genotype measurements

Before blood collection, participants were asked to be fast 12 h before the blood sampling time. Five milliliters of venous blood samples were collected in Ethylenediaminetetraacetic acid (EDTA) tubes from each participant. Samples were stored in freezers (−20 °C) for DNA extraction.

Genomic DNA was extracted from blood samples using Promega kit (Promega, USA). Restriction enzymes from Promega kit for FTO gene polymorphism (rs9939609and rs918031), as well as the primers and Hot Green mastermix kit were also purchased from the same manufacturer. Then, DNA concentration were assessed using UV absorption technique using wave length 260 nm and 280 nm, (Table 1).

Polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) was used for genotyping to detect the presence of FTO (rs9939609) & (rs918031) SNPs among all participants of the study, using thermo cycler (Biometra, Germany) [14], Table 2.

2.4. Statistical analysis

Statistical package for social sciences (SPSS) version 23 (SPSS Inc., Chicago, IL), was used for data analysis. Numeric variables were presented as mean ± standard deviation. Categorical variables were expressed as number and percentage. Student t-test (one-side) was performed to test for significance between the two study groups. Odds ratio (OR) and 95% confidence interval (CI) was used to evaluate the risk using $P \leq 0.05$ as the significance level.

3. Results

Table 3 shows the characteristics of all participants in this study,

Table 2
The restriction enzymes were used in PCR -RFLP assay with their company and country of origin.

Restriction enzymes	SNP	Polymorphism	Company/Country
ScaI	*Rs9939609*	C/T	Promega, USA
HaeIII	*rs16947*	A/G	Promega, USA

Table 3
Clinical and biochemical characteristics of study subjects.

Parameters	Group 1	Group 2	P value
Male/Female	400 (190/210)	400 (195/205)	1
Age (y)	54.77 ± 8.84	53.9 ± 8.98	0.7
BMI (kg/m$^{2)}$)	32.14 ± 1.82	31.9 ± 2.01	0.09
FBS (mmol/L)	13.3 ± 0.4	4.8 ± 0.4	0.0001
Cholesterol (mmol/L)	5.98 ± 0.14	5.97 ± 0.15	0.1
Triglycerides (mmol/L)	2.82 ± 0.07	2.81 ± 0.08	0.3
VLDL-C (mmol/L)	1.29 ± 0.03	1.25 ± 0.02	0.3
LDL-C (mmol/L)	3.65 ± 0.17	3.6 ± 0.18	0.7
HDL-C (mmol/L)	1.04 ± 0.089	1.02 ± 0.09	0.1
Fasting plasma insulin (μU/ml)	33.16 ± 2.34	29.6 ± 5.65	0.0001
HOMA-IR	19.59 ± 1.55	6.38 ± 1.05	0.0001
HBA1c	7.71 ± 0.73	4.56 ± 0.49	0.0001

Group 1: Obese type 2 diabetic participants; Group 2: Obese non-diabetic participants; BMI: Body mass index; FBS: Fasting blood sugar; VLDL-C: Very low density lipoproteins-Cholesterol; LDL-C: Low density lipoproteins-Cholesterol; HDL-C: High density lipoproteins-Cholesterol; HOMA-IR: Homeostatic model assessment of insulin resistance; HBA1c: Glycated hemoglobin.

Table 3.

The FTO (rs9939609) genotype was digested using ScaI restriction enzyme, giving rise to 1 (182 bp) band for AA wild type; 3 (182, 154, 28 bp) bands for heterozygous (TA) and 2 (154, 28 bp) bands for homozygous (TT) genotypes, Fig. 1.

The FTO (rs918031) genotype was digested by HaeIII restriction enzyme, giving rise to 1 (206 pb) for (CC) wildtype, 2 (196, 10bp) for (TT) homozygous, and 3 (206, 196, 10 bp) bands for (CT) heterozygous genotype, Fig. 2.

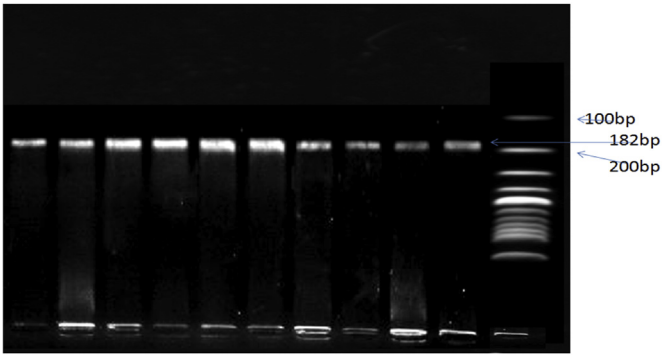

Fig. 1. Agarose gel electrophoresis showing the product analysis of (rs9939609) SNP by using ScaI restriction enzyme.
The PCR product was electrophoresed on 2% agarose (75Vand 120min) and directly visualized with ethidium bromide under UV light. Line 1-10: PCR product (amplicon size 182bp) Line 11: DNA marker (100bp-1.3 kb).

Table 1
The PCR primers with their sequence and amplicon size.

Primer	Sequence	Amplicon
FTO (rs9939609)	5′-AGA GTA ACA GAG ACT ATC CAA GTG CAG TAC-3′ 5′-AAC TGG CTC TTG AAT GAA ATA GGA TTC AGA-3′	182bp
FTO (rs918031)	5-GGA GGG CTG CTG AGA GGG GG-3 5-CTG CCA AGG GCC CAA GAG GC-3	206bp

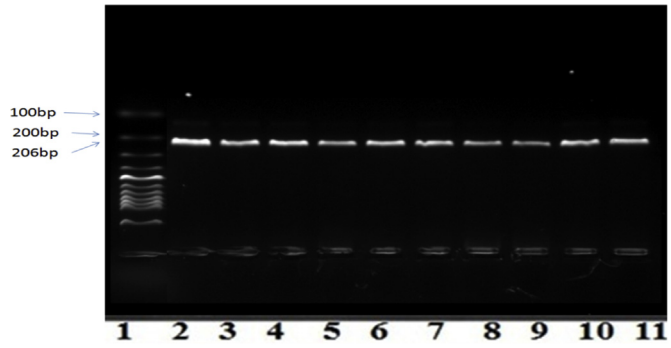

Fig. 2. Agarose gel electrophoresis showing the product analysis of (rs918031) SNP by using HaeIII restriction enzyme.
The PCR product was electrophoresed on 2% agarose (75Vand 120min) and directly visualized with ethidium bromide under UV light. Line 1: DNA marker (100bp-1.3 kb) Line 2–11: PCR product (amplicon size 206bp).

Table 4 and Table 5 shows the genotypes/allele frequencies of the two FTO (rs9939609 and rs918031) SNPs. The genetic power of (rs9939609) was (92.1%) when compared to the control cohort, Table 4. This was much higher than that of the (rs918031) which was (29.3%) when compared to the control cohort, Table 5.

Table 6 shows the clinical criteria of the study group according to their FTO gene rs9939609 genotype. There were significant impacts on the degree of BMI, LDL-C, HDL-C, FBS and fasting plasma insulin levels, Table 6.

Table 7 shows the clinical criteria of study subjects according to FTO gene rs918031 genotype. Unlike rs9939609, this genotype possesses weak effects on most of the clinical biomarkers of diabetes, Table 7.

4. Discussion

Obesity and its related cardiovascular diseases account for a large-scale of morbidity and mortality all over the world [15], which possess significant health burdens. Obesity is the main risk factor for T2D [12], both of which, currently affecting millions of individuals worldwide [15]. Both of these conditions share common risk factors, which predispose the development of obesity and T2D [1], such as genetic predisposition [16], composition of the bowel microbiota [17], as well as the modifiable environmental factors (include sedentary lifestyle and lack of physical exercise) [18].

Studies also revealed the association of an increased weight gain with T2D [19]. FTO protein act as body weight regulation [4]. One of our previous studies have highlighted the association between the FTO (rs9939609 and rs17817449) gene polymorphisms with T2D [14]. In this study, we look for a different Iraq cohort using both rs9939609 and rs918031 SNPs. Hardy–Weinberg equilibrium (HWE) was calculated to check the relationship of SNPs with diabetes in obesity (study group 1). The FTO rs9939609 SNP HWE value was significant (0.069), however The FTO rs918031 SNP value was much lower (0.032). Hence, the rs9939609 SNP is a better predictor for diabetes development in obese subjects. This can also be confirmed when looking into the percentage of the genetic power for FTO rs9939609 SNP which was 92.1%, while it was only 29.3% for the FTO rs918031 SNP, (Tables 4 and 5). The low prediction value of the FTO rs918031 SNP can be attributed to either no sufficient sample size or due to the fact that the rs918031 SNP is uncommon in Iraqi population.

The statistical power, which is applied in this study to reveal the true effect of SNPs, is associated with disease as a risk factor and is dependent on several parameters such as study design, sample size, and the frequency of the allele among the Iraqi cohort [20]. The FTO rs9939609 SNP was significantly correlated with both obesity and T2D (i.e. in the study group 1). Allele T of the FTO rs9939609 SNP may predict the higher incidence of diabetes development among obese individuals by about two folds in both TT and TA when compared with AA genotype. This is in accordance with previous studies done in Asian countries the results are in agreement with the previous results in Asian populations [21,22].

Few researches have been done about the SNP rs918031, which is found in non-translated region, in the current study the results show no association between T2D and obesity so the findings are consistent with results of study in Indian population, that show no effect of this SNP on T2D regardless the degree of obesity [9].

The mechanism by which the SNP rs9939609 develop obesity is still hypothetical and not fully interpreted until now. However, a study explained this by the position of this SNP in the intron 1, that is the most common region correlated to the obesity at the FTO gene, this region is characterized by a highly DNAase sensitivity, and many transcriptional factors are attach to the obesity associated region in which the receptor of glucocorticoid has the strongest signal. In obesity, there is beta cells insufficiency and a tendency to insulin resistance. This will produce persistent hyperglycemia. Many diabetic individuals suffer from high body weight, the fatty acid supply in to the circulation stimulate a positive energy that increase triglyceride storage and induce adipocyte hypertrophy leading to decrease in adipocyte sensitivity to anti-lipolysis action of insulin and this will leads to increase liberation of fatty acid, increase its oxidation, increase the energy produced by liver that will be used later in gluconeogenesis and this cause an impaired glycolytic pathway of insulin and reduce the utilization of glucose then insulin resistance and hyperglycemia [23].

Table 4
The genotype and allele frequency of FTO gene polymorphism of (rs9939609) SNP in both the Study and control groups.

SNP rs9939609 (A/T)	Group 1 n = 400	Group 2 n = 400	unadjusted OR (95% CI)	*P*	Adjusted OR (95% CI)	*P*
Codominant						
AA (Reference)	80	130				
TA	185	180	1.67 (1.18–2.36)	0.004	2.74 (1.81–4.16)	0.000
TT	135	90	2.43 (1.65–3.58)	0.000	6.33 (3.57–11.2)	0.000
Dominant						
TT + TA	320	270	1.75 (1.28–2.4)	0.000	2.3 (1.55–3.41)	0.000
Recessive						
AA + TA (Reference)	265	310				
TT	135	90	1.76 (1.28–2.4)	0.000	2.33 (1.55–3.41)	0.000
Additive						
2(TT) + TA	455	360	2.02 (1.5–2.8)	0.000		
Frequency of T allele	227 (56.88%)	180 (45%)	2.04 (1.45–2.88)	0.000		

Group 1: Obese type 2 diabetic participants; Group 2: Obese non-diabetic participants; OR: Odd ratio; CI: Confidence interval.

Table 5
The genotype and allele frequency of FTO gene polymorphism of (rs918031) SNP in both the Study and control groups.

SNP rs918031 (C/T)	Group 1 n = 400	Group 2 n = 400	Unadjusted OR (95% CI)	*P*	Adjusted OR (95% CI)	*P*
Codominant						
CC (Reference)	176	182				
CT	194	189	1.061 (0.79–1.41)	0.68	1.11 (0.51–2.44)	0.78
TT	30	29	1.07 (0.61–1.85)	0.81	1.12 (0.43–2.94)	1.12
Dominant						
CT + TT	224	218	1.037 (0.61–1.76)	0.892	1.01 (0.58–1.75)	0.966
Recessive						
CC + CT (Reference)	370	371				
TT	30	29	1.037 (0.61–1.76)	0.892	1.01 (0.58–1.75)	0.966
Additive						
2(TT) + CT	254	227	2.06 (1.59–2.68)	0.000		
Frequency of T allele	127	123	1.06 (0.77–1.47)	0.69		
	31.75%	30.8%				

Group 1: Obese type 2 diabetic participants; Group 2: Obese non-diabetic participants; OR: Odd ratio; CI: Confidence interval.

Table 6
The clinical characteristics of the obese T2D subjects according to the FTO gene polymorphism (rs9939609) genotype (codominant model).

Clinical characteristics	AA (n = 80)	TA (n = 185)	TT (n = 135)	*P*
Age (years)	54.27 ± 9.025	53.98 ± 8.79	54.25 ± 8.83	0.95
BMI (kg/m^2)	30.15 ± 1.82	33.04 ± 1.82	33.25 ± 1.83	0.000
Cholesterol (mmol/L)	5.97 ± 0.15	5.99 ± 0.15	5.99 ± 0.15	0.29
Triglycerides (mmol/L)	2.8 ± 0.07	2.8 ± 0.069	2.7 ± 0.07	0.978
VLDL-C (mmol/L)	1.29 ± 0.03	1.29 ± 0.03	1.26 ± 0.03	0.98
LDL-C (mmol/L)	3.58 ± 0.19	3.66 ± 0.16	3.7 ± 0.18	0.000
HDL-C (mmol/L)	1.12 ± 0.09	1.04 ± 0.09	0.96 ± 0.09	0.000
Fasting plasma insulin (μU/ml)	31.9 ± 2.27	33.85 ± 2.26	33.73 ± 2.41	0.000
HOMA-IR	18.68 ± 1.28	19.5 ± 1.43	20.24 ± 1.57	0.000
HbA1c	7.69 ± 0.72	7.71 ± 0.73	7.72 ± 0.74	0.95
FBS (mmol/L)	13.4 ± 0.5	13.4 ± 0.3	13.1 ± 0.4	0.000

BMI: Body mass index; FBS: Fasting blood sugar; VLDL-C: Very low density lipoproteins-Cholesterol; LDL-C: Low density lipoproteins-Cholesterol; HDL-C: High density lipoproteins-Cholesterol; HOMA-IR: Homeostatic model assessment of insulin resistance; HBA1c: Glycated hemoglobin.

Table 7
The clinical characteristics of the obese T2D subjects according to the FTO gene polymorphism (rs918031) genotype (codominant model).

Clinical characteristics	CC (n = 176)	CT (n = 194)	TT (n = 30)	*P*
Age (years)	54.25 ± 8.92	53.98 ± 8.81	54.23 ± 8.8	0.95
BMI (kg/m^2)	32.05 ± 1.97	32.24 ± 1.94	32.33 ± 1.71	0.56
Cholesterol (mmol/L)	5.97 ± 0.15	5.98 ± 0.14	5.97 ± 0.15	0.40
Triglycerides (mmol/L)	2.79 ± 0.06	2.8 ± 0.07	2.7 ± 0.07	0.89
VLDL-C (mmol/L)	1.3 ± 0.02	1.27 ± 0.03	1.26 ± 0.03	0.89
LDL-C (mmol/L)	3.65 ± 0.17	3.65 ± 0.16	3.61 ± 0.19	0.4
HDL-C (mmol/L)	1.04 ± 0.09	1.03 ± 0.03	1.04 ± 0.04	0.79
Fasting plasma insulin (μU/ml)	32.86 ± 2.26	33.09 ± 2.36	35.3 ± 1.44	0.000
HOMA-IR	19.03 ± 1.41	19.78 ± 1.43	21.79 ± 0.89	0.000
HcA1C	7.7 ± 0.7	7.73 ± 0.75	7.6 ± 0.71	0.64
FBS (mmol/L)	13 ± 0.4	13.5 ± 0.8	13.8 ± 0.3	0.000

BMI: Body mass index; FBS: Fasting blood sugar; VLDL-C: Very low density lipoproteins-Cholesterol; LDL-C: Low density lipoproteins-Cholesterol; HDL-C: High density lipoproteins-Cholesterol; HOMA-IR: Homeostatic model assessment of insulin resistance; HBA1c: Glycated hemoglobin.

The FTO rs9939609 SNP significantly affects the levels of (BMI, LDL-C, and insulin levels), being more significant among the TT genotype when compared to the AA wild type. This was associated with a significant negative correlation when compared with HDL-C level that appears much lower in TT genotype. Similar data were provided by Osman et al., in 2014 [24], Table 6. The correlations above indicate that Group 1 (obese diabetic individuals) who have TT/TA rs9939609 genotype are more prone for the development of dyslipidemia, one of the main complications of both obesity and diabetes, via stimulation of adipocytokines pathway (like adiponectin) to stimulate peroxisome proliferation, increasing the hepatic apoproteins A-I and A-II expression, and less HDL-C synthesis by the liver [7]. Therefore, more fatty acid accumulation in muscle and liver which predispose insulin resistance [7].

The 2nd mechanism for development of T2D by the effect of FTO risk alleles carrier through rising body weight and increase in energy intake related to controls, possibly due to an increase in fat consumption this is may be due to the role of FTO in leptin receptor at the ciliary pole, through increasing FTO expression, leading to increase in leptin receptor transferring to the ciliary pole and increase leptin sensitivity also FTO may change food intake by affecting leptin sensitivity leading to increase body weight and insulin resistance then development of T2D [25].

The genetic analysis of FTO gene polymorphism SNP rs918031 on phenotypic data in this study shows a significant difference in (insulin, FBS and HOMA-IR concentrations) between the three alleles of this SNP (Table 7). this result is in agreement with Chang et al., who provided similar results in Chinese population, Table 7, [26].

The relationship of the codominant and dominant models of this SNP to the serum lipid levels and obesity parameters may be independent on modulation of insulin level, and do not rely on insulin secretion and action. Hence, the gene polymorphism has no effect on serum lipid levels, while the SNP rs9939609 shows a direct effect on the atherogenic lipid levels, Table 6.

The SNP rs9939609 of FTO gene can be used as a predictor for T2D development in obese subjects through its effects on insulin and FPG levels. However, the SNP rs918031 showed no significant correlation with diabetes in obese individuals. Carriers of the SNPs rs9939609 of FTO gene were more prone to changes in serum lipids and BMI, while carriers of SNP rs918031 reveal to similar changes.

Conflicts of interest

There is no conflict of interest associated with this manuscript.

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Nothing to declare.

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