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FTO rs9939609 Polymorphism is Associated with Type 2 Diabetes among Jordanians

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Abstract

Introduction: Obesity plays a critical role in the development of type 2 diabetes (T2D). FTO (fat mass and obesity-associated) gene has been associated with energy balance. MC4R (melanocortin 4 receptor) gene was the first loci in which mutations were associated with morbid obesity in humans.
This case control study investigated the associated of *FTO* rs9939609 and *MC4R* rs117782313 and obesity among T2D. Methods: This study includes 420 subjects diagnosed with T2D diabetes and 200 healthy controls. Blood samples were All samples analyzed for Glycosylated hemoglobin, Triglycerides, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol. 25-OH-vitamin D and B12 were determined using the Alinity c analyzer. Genetic analysis was carried out by PCR-RFLP method and restriction digestion. Results: The results of this study showed that *FTO* rs9939609 TT, TA, AA variants were significantly associated with T2D (p=0.001), Odd ratio (95% CI) 3.71 (1.71to 8.05). However, there is no significant association between rs17782313 *MC4R* and T2D. Obesity is significantly associated with *FTO* rs9939609 AA genotype with frequency (71%).
Conclusion: There is a significant association between rs9939609 *FTO* AA genotype with diabetes among Jordanians.

Keywords: *FTO* rs9939609, *MC4R* rs17782313, Polymorphism, Type 2 Diabetes

1. Introduction

Obesity is a complex disease defined by excessive fat deposits that treat health. It is developed from excessive accumulation of body fat, due to greater intake of calories than consumption. Type 2 diabetes (T2D) risk factors include: dysregulation of the leptin hormone, abnormal glucose metabolism, genetic factors, environmental factors, poor exercise, and excessive alcohol consumption. Advanced food manufacturing technology and excessive glucose consumption enhance fat tissue. Obesity results from increased adipose tissue caused by excessive glucose consumption and sophisticated food production methods. Obesity plays a crucial role in the development of metabolic syndrome, T2D, hypertension and cardiovascular disease (Shariq & McKenzie, 2020). In Jordan, Total Diabetes prevalence is 20.5% in adults (https://idf.org/our-network/regions-and-members/middle-east-and-north-africa/members/jordan/ accessed 16/May/2025).

SNP genotyping technologies have made genome-wide association studies easier and have identified many SNPs correlated with obesity in people with T2D like *FTO* rs9939609 and *MC4R* rs117782313. These SNPS never studied in Jordan. *FTO* and *MC4R* genes played a critical part in appetite, energy regulation and obesity(Khan *et al.*, 2020). *FTO* and *MC4R* genes are located on chromosomes 16q12.2 (Fig 2) and 18q21.3 (Fig 3), respectively. *FTO* rs9939609 and *MC4R* rs117782313 were obesity-associated risk alleles among different ethnic groups(Nagy *et al.*, 2017). These SNPs were associated with obesity risk among T2D, in Italy (Sentinelli *et al.*, 2012), Japan (Kamura *et al.*, 2016), and Caucasian and Asian children (Dastgheib *et al.*, 2021).

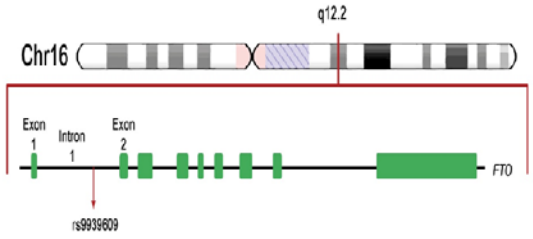

Figure 1. *FTO* rs9939609 are location on chromosomes 16q12.2

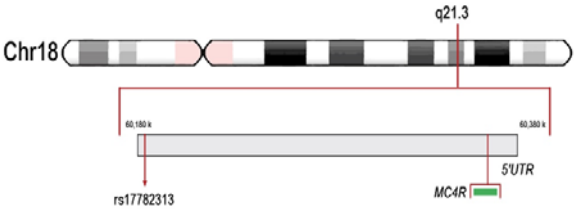

Figure 2. *MC4R* rs117782313 located on chromosomes 18q21.3

FTO rs9939609 and *MC4R* rs117782313 variants are critical for understanding the etiology of obesity among T2D. To date, several epidemiological studies reported that *FTO* rs9939609 and *MC4R* rs117782313 polymorphisms were associated with obesity; however, the

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results were conflicting (Amine Ikhanjal *et al.*, 2023). To our knowledge, *FTO* rs9939609 and *MC4R* rs117782313 variants were never studied among diabetics in Jordan. This case-control study aimed to determine the association between the *FTO* rs9939609A and *MC4R* rs117782313 with obesity among T2D within Jordanians.

2. Material and Methods

This cross-sectional study was conducted at the Diabetes Center in Amman, Jordan (between January and December 2023). It enrolled 420 Type 2 diabetic patients and 200 healthy controls. This study was approved by The Hashemite University Institutional Review Board (IRB) Committee (Reference Number: 12/01/2021/2023). All patients signed an informed consent form to participate in the study following the Helsinki Declaration. Patients and healthy control were interviewed to obtain demographic information and Body mass index (BMI). BMI was calculated as weight (kg) divided by height squared m^2: normal BMI 18.5-24.99 kg/m^2, overweight BMI 25-29.9 kg/m^2, and obese BMI 30 kg/m^2. Waist circumference (WC) was measured and waist-to-height ratio (WHtR) was calculated; a WHtR ≥0.5 is a common cutoff value for obesity.

Cases were aged 33-66 who were diagnosed with T2D according to the American Diabetes Association (ADA) diagnostic guidelines. This study included confirmed diabetes patients with previous records of FBG ≥126 mg/dL and HbA1c ≥ 6.5. All controls were Jordanians aged 33 to 66 years with no history of diabetes (type 1 or type 2), confirmed by having HbA1c<5.7% and FBS<100 mg/dL. All participants under 33 years old or with autoimmune diseases or other chronic illnesses were excluded.

Glycosylated hemoglobin (HbA1c), Triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), 25-OH-vitamin D and B12 were carried out using the Alinity c analyzer (Abbott, USA).

Genomic DNA was extracted from whole blood by DNA extraction kit (Qiagen, USA). DNA concentration was determined at 260nm by a Nano drop, then stored at -80˚C for genotyping. PCR amplification was carried out in a volume of 25 µL reaction the mixture containing 9 µL Master Mix (QIAGEN, USA), 2 µL of the genomic DNA, 1 µL for each 10 pmol/ul primer. MC4R gene was amplified with following primers: MC4R; Forward primer: 5′-AAGTTCTACCTACCATGTTCTTGG-3′ Reverse primer: 5′-TTCCCCCTGAAGCTTTTCTTGTCATT-3′ (Amplicon size: 137 bp). PCR cycle included an initial DNA denaturation for MC4R at 95 °C for 5 min, followed DNA denaturation by 30 cycles at 95 °C for 30 s, annealing 52°C temperature for 30 s, extension at 72 °C for 10 min, and a final extension period of 5 min at 70 °C. PCR products were visualized on 2% agarose gel electrophoresis using Trans illuminator.

To determine rs17782313 MC4R polymorphism, restriction endonuclease digestion was carried out in a 10 µL reaction mixture containing: 4 µL of PCR products, 2.5 µL of nuclease free water, 1 µL of the 10X Buffer, and 2.5 µL of BclI restriction enzyme (New England Biolabs, USA). The product was incubated at 57 °C for 3h. CC, CT, and TT genotypes produced fragments 137 bp, (137,107 bp), and 107 bp; respectively.

PCR amplification for *FTO* rs9939699 T/A SNP determination carried out by forward: 5′-GGCTCTTGAATGAAATAGGA-3′ and reverse: 5′AGAGACTATCCAAGTGCAGTAC-3′ primers then digested by *ScaI* restriction enzyme. PCR reaction involves denaturation at 94^{o}C for 10 min, followed denaturation at 94^{o}C for 30s for 30 cycles, annealing at 61^{o}C for 30s and elongation at 72^{o}C for 30s for 30 cycles. PCR product was then digested at room temperature with *ScaI* (New England Biolabs, USA). The A allele produces 150 bp and 20bp fragments, and the T allele produces 70bp fragment. Fragments then electrophoresed on agarose gel.

The IBM SPSS Statistics program was used to analyze the data. (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp). Genotype frequencies between cases and control were estimated using the *chi-square* test. Analysis of variance (ANOVA) were used to compare Means ± standard deviation (SD) and percentage. The body weight distribution of patients with *FTO* rs9939609 and *MC4R* rs17782313 genotypes were calculated using the *chi*-square test.

3. Results

Table 1. Clinical parameters comparison between T2D cases and control

Clinical parameter	Diabetic Mean±SD (n= 420)	control Mean±SD (n=200)	*p-value*
HbA1c (%)	8.4 ± 1.8	5.6 ± 1.6	< 0.0001
Triglycerides (mg/dL)	224 ±97.3	132.7 ± 8.7	< 0.0001
Total cholesterol (mg/dL)	207.7 ± 32.1	157.4 ± 32.2	< 0.0001
LDL-cholesterol (mg/dL)	106.1 ± 43.8	111.0 ± 30.7	0.09
HDL-cholesterol (mg/dL)	41.9 ± 13.1	41.7 ± 14.8	0.38
25-OH-vitamin D (ng/mL)	12.7 ± 8.1	11.9 ± 6.6	0.28
Vitamin B12 (pg/mL)	257.3 ± 112.7	299.8 ± 105.2	0.04

Data presented as mean ± SD for biochemical parameters. Significant differences were found in HbA1c, Triglycerides, Total cholesterol, and vitamin B12 among diabetic compared to control (p< 0.05).

Table 2. Genotype distribution for *FTO* rs9939609 and *MC4R* rs17782313 gene polymorphism among T2D cases and control

FTO	TT (%)	TA (%)	AA(%)	Odd ratio (95% CI)	*chi* square	*p*-value
Diabetic	135(32%)	64(15%)	221(53%)	3.71 (1.71to 8.05	14.14	0.001
control	54(27%)	94(47%)	52(26%)			
MR4C	GG (%)	GA (%)	AA(%)			
Diabetic	179(43%)	59(14%)	182(44%)	1.82 (0.76 to 4.31)	5.46	
control	54((27%)	33(16%)	113(57%)		0.17	

The association between rs17782313 *MC4R* and rs9939609 *FTO* genotypes with diabetes are shown in Table 2. This study showed that *FTO* TT, TA, AA variants were significantly associated with type 2 diabetes (*p*= 0.001).

Table 3. Genotype association for *FTO* rs9939609 and *MC4R* rs17782313 gene polymorphisms among T2D cases with biochemical tests

parameter	*FTO*(n=420)			*MC4R*(n=420)		
	Genotype	Mean ±SD	*p*-value	Genotype	Mean ±SD	*p*-value
HbA1c (%)	TT	8.12 ± 1.73	0.148	GG	8.39 ± 1.78	0.422
	TA/AA	8.39± 1.81		GA/AA	8.42 ± 1.83	
Triglycerides (mg/dL)	TT	231.20 ±93.29	0.351	GG	219.41 ±96.7	0.0734
	TA/AA	221.91 ±96.29		GA/AA	231.44 ±97.29	
Total cholesterol (mg/dL)	TT	203.7 ± 30.23	0.128	GG	212.82 ± 57.33	0.092
	TA/AA	208.67 ± 31.13		GA/AA	207.89 ± 41.23	
LDL-cholesterol (mg/dL)	TT	105.43 ± 40.81	0.725	GG	106.1 ± 43.8	0.233
	TA/AA	106.73 ± 41.83		GA/AA	103.11 ± 23.89	
HDL-cholesterol (mg/dL)	TT	40.78 ± 14.1	0.441	GG	42.90 ± 14.13	0.040
	TA/AA	39.78 ± 12.17		GA/AA	40.92 ± 14.12	
25-OH-vitamin D (ng/mL)	TT	11.75 ± 6.54	0.0002	GG	12.9 ± 9.12	0.0001
	TA/AA	13.56 ± 7.19		GA/AA	15.91± 7.90	
Vitamin B12 (pg/mL)		265.23 ± 102.98	0.038		250.32± 122.07	0.158
	TA/AA	249.32 ± 122.67			261.31 ± 102.34	

The results of this study showed a significant association between *FTO* rs9939609 and 25-OH-vitamin D (*p*= 0.0002) and vitamin B12 (*p*=0.038). *MC4R* rs17782313 was significantly associated with HDL-cholesterol (*p*= 0.040) and OH-vitamin D (*p*= 0.0001).

Table 4. Association between body weight and genotypes for *FTO* rs9939609and *MC4R* rs17782313 among diabetics

FTO					
Body weight / genotype	TT	TA	AA	*chi*-square	*p*-value
Normal	36(27%)	9(14%)	27(12%)	26.12	.00003
Overweight	48(36%)	23(36%)	37(17%)		
Obese	51(38%)	32(50%)	157(71%)		
MC4R					
Body weight / genotype	GG	GA	AA	9.28	.054
Normal	47(26%)	8(14%)	55(30%)		
Overweight	69(39%)	26(44%)	54(30%)		
Obese	63(35%)	25(42%)	73(40%)		

The result of this study showed a significant association between body weight and *FTO* rs9939609 genotypes (*p*= .00003), while no significant association was found between *MC4R* rs17782313 genotypes and body weight.

4. Discussion

Polymorphism studies about the association between *FTO* and *MC4R* genotypes among T2D and obesity were controversial, according to differences in lifestyle and genetic backgrounds. Previous studies showed that *FTO* gene affects insulin resistance and T2D development (Masoud Abd El Gayed *et al.*, 2021) (Nasser *et al.*, 2019). This study showed a significant association between *FTO* rs9939609 TT, TA, AA genotypes among diabetic patients. Similar results were reported in population-based prospective study that showed *FTO* rs9939609 polymorphism A allele was associated with 1.35-fold increased risk for T2D (Binh *et al.*, 2022). Another meta-analysis reveals that *FTO* rs9939609 SNP increase the risk of both obesity and T2D within the Asian population (Yanasegaran *et al.*, 2024). A significant association between *FTO* rs9939609 polymorphism and T2DM risk was also reported among Kuwaitis (Chaudhary *et al.*, 2024)(Chaudhary et al., 2024), Egyptians (Barseem *et al.*, 2019), Vietnamese (Binh *et al.*, 2022), Indians (Irgam *et al.*, 2021), and Pakistanis(Shaikh *et al.*, 2021).

This study showed a significant association between *FTO* rs9939609 variants, 25-OH-vitamin D and vitamin B12 levels. In a previous study by Mehrdad et al.

(Mehrdad *et al.*, 2022), *FTO* rs9939609 A allele was associated with bad eating behaviors and higher risk of eating disorders among people with insufficient vitamin D intake.

This study showed a significant association between body weight and *FTO* rs9939609 genotypes, but no significant association was found between *MC4R* rs17782313 genotypes and body weight. In this study,71% of rs9939609 *FTO* AA diabetic genotypes were obese. rs9939609 *FTO* is associated with obesity development through appetite regulation mechanisms in the hypothalamus (da Fonseca *et al.*, 2019). *FTO* rs9939609 variant is a genetic risk factor in the etiology of obesity among females, rs9939609 *FTO* A allele increase abdominal fat mass (Park & Choi, 2023). rs9939609 *FTO* T/A SNP is also associated with obesity among children, and adults (Jiang *et al.*, 2019) (Eghbali *et al.*, 2024). rs9939609 *FTO* A allele is linked to higher appetite and energy intake (Ponce-Gonzalez *et al.*, 2023). People with AA genotype at higher risk to have T2D than TT genotype patients at different genetic models (Amine Ikhanjal *et al.*, 2023). Genetic correlation between *FTO* and obesity acts through the disturbance of AT-Rich Interaction Domain 5B repressor by *FTO* alleles Fig 3. This is due to disturbance in Homeobox 3 during adipocyte differentiation (Claussnitzer *et al.*, 2015). Like our results, a systematic study showed that the *FTO* gene polymorphism is common in older age (Trevisano *et al.*, 2022). A parallel study also demonstrated that *FTO* rs9939609 AA risk genotype is associated with risk for class III obesity (BMI ≥40 kg/m^2) (Martínez-López *et al.*, 2024). Similar results also demonstrated that *FTO* rs99399609 polymorphism increases body fat percentage(Gholamalizadeh *et al.*, 2022). *FTO* rs9939609 was strongly associated with obesity risk factors like increased BMI, cholesterol, triglycerides, and low-density lipoprotein. This association with overweight and obesity was previously reported in codominant AA versus TT among Bangladeshi population (Shill & Alam, 2023) and Kuwaitis (Jamali et al., 2025).

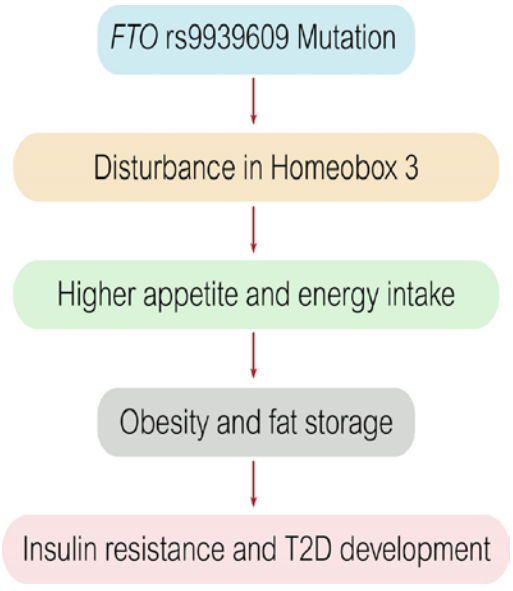

Figure 3. rs9939609 *FTO* is associated with Type 2 diabetes development through appetite regulation mechanisms in the hypothalamus and insulin resistance

This study had some limitations. First, as the study was carried out among Jordanians, the result must be applied for another ethnic groups. Second, we studied gene *FTO* rs9939609 polymorphism and *MC4R* rs17782313, so it is necessary to build genetic models involving many other polymorphisms among different populations. Lastly, obesity is a multifactorial disease involving variable socioeconomic, dietary variables and their interactions that are associated with obesity etiology. This complex relationship may involve interactions between genetic predisposition, lifestyle factors, and other environmental influences.

In summary, polymorphisms in the *FTO* rs9939609 polymorphisms were associated with increased risk for obesity, which is a major risk factor for T2D. This polymorphism should be used to develop predictive models for future diabetes among populations. Conversely, there is no significant association between rs17782313 *MC4R* and T2D.

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Ethics Committee

Not applicable (ref number12/01/2021/2023).

Conflict of Interest

No conflict of interests.

Authors Contribution

Manar Atoum drafted the manuscript and revised/edited the article.

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