Original Article

Lack of association between fat mass and obesity-associated genetic variant (rs8050136) and type 2 diabetes mellitus

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ABSTRACT

الأهداف: تقييم التركيب الجيني وتكرار الأليلات لمتغير FTO rs8050136 A>C والتحقيق في ارتباط المتغير الجيني FTO rs8050136 A>C مع علامات مرض السكري النوع الثاني في المدينة المنورة خلال الفترة من أغسطس 2018م إلى مارس 2021م.

المنهجية: تم عمل هذه الدراسة على 118 مريضًا بالسكري النوع الثاني و106 فردا سليمًا (مجموعة ضابطة) من مراجعي مستشفى الأمير محمد بن عبدالعزيز بالمدينة المنورة في المملكة العربية السعودية. تم تحديد النمط الجيني باستخدام تحليل TaqMan SNP للتنميط الجيني للمتغير rs8050136.

النتائج: وجد أن نسبة المتغير الجيني AA في مجموعة مرضى السكري النوع الثاني مساوية لما هو موجود في المجموعة الضابطة (21%). بينما كانت نسبة المتغير الجيني CC في مجموعة مرضى السكري النوع الثاني 21% و 24.5% في المجموعة الضابطة. لم يكن هناك ارتباط كبير بين المتغير الجيني FTO SNP rs8050136 وزيادة خطر الإصابة بمرض السكري من النوع الثاني. علاوة على ذلك، لم يكن هناك ارتباط بين المتغير الجيني AA ومعدل سكر دم الصائم أو الهيموجلوبين السكري أو كتلة الجسم (المعدل الإحصائي: 0.561 and، 0.177، p=0.092 على التوالي).

الخلاصة: تظهر نتائجنا أن متغير FTO rs8050136 A>C لايرتبط بمرض السكري من النوع الثاني في السكان السعوديين.

Objectives: To study the genotype and allele frequency of the fat mass and obesity-associated (FTO) rs8050136 A>C genetic variant and investigate its association with type 2 diabetes mekkitus (T2DM) parameters.

Methods: This study was carried out on 118 diabetic patients and 106 healthy individuals (control) from Prince Mohammed bin Abdulaziz Hospital, Al Madinah Al Munawarah, Saudi Arabia. The TaqMan single-nucleotide polymorphism (SNP) genotyping assay was used for rs8050136 genotyping.

Results: The frequency of the genotype AA was the same among T2DM and healthy control groups (21%). However, the frequency of genotype CC was 19.5% in T2DM patients and 24.5% in control individuals. There was no significant association between FTO SNP rs8050136 and an increased risk of T2DM. Furthermore, there was no association between the risk AA genotype and fasting blood glucose (p=0.092), glycated hemoglobin (p=0.177), or body mass index (p=0.561).

Conclusion: Our findings show that the FTO rs8050136 A>C variant is not associated with T2DM in the Saudi population.

Keywords: GWAS, type 2 diabetes, allele frequency, FTO gene, gene variant

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Diabetes mellitus (DM) is an epidemic disease with significant global medical and economic burden. The International Diabetes Federation estimated that approximately 463 million people were afflicted with DM in 2019, and that this number would reach 700 million by 2045.[1] In addition, the global estimated cost of DM is expected to increase from $1.3 trillion in 2015 to $2.2 trillion by 2030, which is 2.2% of global gross domestic product.[2] Diabetes mellitus is a chronic multifactorial metabolic disease caused by elevated blood glucose levels, which can eventually result in serious complications for the cardiovascular system, nervous system, and kidneys. Of the 2 types of DM, type 2 (T2DM) is much more common (~90% of DM cases).[1] Type 2 DM manifests when body target cells become insulin-resistant and is strongly associated with obesity and physical inactivity.[3]

Diabetes mellitus results in approximately 11.3% of annual mortality, and approximately half of these deaths occur in people <60 years of age.[1] In 2019, the global prevalence of DM was 9.3%. The prevalence was higher (10.8%) in residents aged 20-79 years in the Middle East which includes the Gulf Cooperation Council (GCC) countries and North Africa. Furthermore, 44.7% of those with DM in the region are believed to be undiagnosed.[1] The prevalence of DM in GCC countries has markedly increased during the past 20 years and is likely to double by 2035.[4,5] In Saudi Arabia, DM prevalence was 18.3% in 2019, only second in the region to Kuwait (22%), making this disease one of the leading health problems for the Saudi population.[1]

It is believed that T2DM results from an intricate interaction between genetic and environmental factors.[6] Obesity is one well-known factor associated with a higher risk of T2DM development.[7] It is a major global health concern, as more than 1.9 billion people aged 18 and older worldwide are overweight and 650 million are obese.[8]

Initial candidate gene association and linkage studies have investigated potential genetic components involved in T2DM development. However, since 2007, genome-wide association studies (GWASs) have become the leading method for identifying T2DM susceptibility loci as a result of the availability of the HapMap data and the advances in genotyping methods such as high-throughput single nucleotide polymorphism (SNP) genotyping methods.[9] Genome-wide association studies conducted on different ethnic populations have confirmed previously identified variants and identified many new susceptible variants likely associated with DM.[10] Furthermore, GWASs have linked more than 250 susceptible loci to a high risk of T2DM development.[11]

One of the major genes studied for its potential association of its SNPs with T2DM development is the fat mass and obesity-associated (FTO) gene.[12] The FTO gene is composed of 9 exons and 8 introns, and encodes a 2-oxoglutarate (2-OG) Fe(II)-dependent demethylase.[13] It is expressed in various body tissues and is suggested to be active in DNA repair and modification.[13] The rs8050136 SNP located in the FTO gene was reported to be significantly associated with a high risk of T2DM in studies conducted in Asian and European populations.[14-20] In addition, several meta-analyses revealed significant association of the rs8050136 SNP with a high risk of developing obesity and T2DM.[21-25] However, studies of European, Mexican, and Middle Eastern populations found no association between rs8050136 SNP and T2DM.[26-32] This study investigates the link between rs8050136 A>C variant and T2DM in the Saudi population.

Methods. This case-control study was carried out between August 2019 and March 2021 in Saudi populations (118 patients diagnosed with T2DM and 106 healthy individuals serving as a control group). Type 2 DM group consisted of patients undergoing treatment at a diabetes clinic in Prince Mohammed bin Abdulaziz Hospital, National Guard Ministry, Al Madinah Al Munawarah, Saudi Arabia. Participants of control group were outpatients who were following up at other clinics in the same hospital as well as hospital visitors. To be included in the case group, a patient was required to be: i) diagnosed with diabetes as defined by the American Diabetes Association, ii) of Saudi ancestry, iii) >30 years of age, iv) have a body mass index (BMI) of <40 kg/m^2, and v) have stable glycemic control (HbA1c [hemoglobin A1c] <11%). Subjects with metabolic syndrome, type 1 DM, a history of gestational diabetes, or of non-Saudi origin were excluded. Control group individuals were required to be healthy, free of diabetes and kidney diseases, and have normal blood glucose and HbA1c levels. A written consent form was used to collect personal details and the family and clinical history of all participants. The study was performed following the guidelines of the

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Anthropometric measurements. Weight and height were measured for each participant, and calculation of BMI was carried out by following this formula: BMI = weight (kg)/height^2 (m^2).

Blood specimens and biochemical measurements. Approximately 5 mL of whole blood from each participant was drawn into tubes with and without ethylenediamine tetraacetic acid (EDTA), an anticoagulant. Sera used for fasting blood glucose (FBG) measurements were collected from the tubes without EDTA by centrifugation. Hemoglobin A1cc level was measured using. In addition, genomic DNA was extracted from blood samples in EDTA, as described below. Fasting blood glucose and HbA1c levels were measured at the Clinical Chemistry Laboratory at Prince Mohammed bin Abdulaziz Hospital, National Guard Ministry, Al Madinah Al Munawarah, Saudi Arabia.

Nucleic acid extraction and genotyping. Two hundred microliter of whole blood was subjected for genomic DNA extraction using the Magnetic Beads gDNA Kit for blood (Geneaid, Taipei, Taiwan) following the manufacturer's protocol. The NanoDrop 1000 UV-Vis Spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) was used to determine DNA purity and concentration. Extracted DNA was stored at -20°C until genotyping analysis. Genotyping of FTO SNP rs8050136 was carried out using a customized TaqMan SNP Genotyping Assay (Assay ID: C_2031259_10) (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Briefly, polymerase chain reaction (PCR) was carried in 25 µl reaction mixture containing 5 µL of 2X TaqMan Master Mix, 0.5 µL 20X Assay Working Stock, 0.2 ng/µl of DNA, and RNase-free water. The PCR reaction conditions were as follows: 1 cycle of AmpliTaq enzyme Activation for 10 minutes at 95°C, 35 cycles of denaturation for 15 seconds at 95°C, and annealing/extension for 1 minute at 60°C. Amplification was carried out on the StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA).

Statistical analysis. Measured parameters were analyzed using IBM SPSS Statistics for Windows, version 27.0 (IBM Corp., Armonk, N.Y., USA). The student's t-test was applied to compare age, biochemical parameters (blood glucose and HbA1c), and BMI between the case and control groups. The SNPStats online analysis software (https://www.snpstats.net/start.htm)[33] was used to compare the frequencies of genotypes and alleles of the rs8050136 SNP between the T2DM and control groups to assess the association of different genotypes and the risk for T2DM. A one-way analysis of variance (ANOVA) was used to assess the effect of the different rs8050136 genotypes on FBG, HbA1c, and BMI in all participants. A p-value of <0.005 was considered statistically significant.

Results. Anthropometric and biochemical results of T2DM patients and healthy controls are presented in Table 1. The T2DM patient group was significantly older than the control group (p<0.005). More than 50% of the participants were male: 54.7% in the control and 53.39% patient groups. There was a significant increase in the levels of FBG, HbA1c, and BMI in the group of T2DM patients than in the control group (p<0.001).

The allele and genotype frequencies of rs8050136 SNP for patient and control groups are presented in Table 2. The rs8050136 SNP allele frequencies were in Hardy–Weinberg equilibrium (p=0.234) in patient and control groups. There was an insignificant difference in the frequencies of rs8050136 genotypes between

Table 1 - Anthropometric and biochemical characteristics of study groups.

Parameter	Patient group	Control group	*P*-value
Mean age: year	53.19 (10.96)	51.34 (13.78)	0.002
Gender n(%)			
Female	55 (46.6)	48 (45.3)	
Male	63 (53.4)	58 (54.7)	
FBG	8.4 (3.5)	5.41 (1.1)	<0.001
HbA1c	8.69 (3.0)	5.32 (0.6)	<0.001
BMI	30.68 (6.3)	25.90 (3.4)	<0.001

Values are presented as numbers and percentages (%). FBG: fasting blood glucose, HbA1c: hemoglobin A1c, BMI: body mass index

Table 2 - Fat mass and obesity-associated rs8050136 single nucleotide polymorphism genotypes and variant allele frequencies among study groups.

Genotype	Patients	Controls	Odd ratio (95% CI)	*P*-value
CC	23 (19.5)	26 (24.5)	1	0.54
CA	74 (62.7)	59 (55.7)	1.42 (0.73-2.74)	
AA	21 (17.8)	21 (19.8)	1.13 (0.50-2.58)	
Allele C	120 (50.8)	111 (52.4)		0.75
Allele A	116 (49.2)	101 (47.6)		

T2DM patient and control groups (p=0.54), indicating that FTO SNP rs8050136 A>C is not associated with the risk of T2DM in our Saudi cohort.

The data were stratified based on genotypes among the study groups to test the association of FTO rs80501363 genotypes with FBG, HbA1C, and BMI. An increasing trend of BMI was found in the 3 genotypes of T2DM patients, and the risk genotype (AA) carriers showed a higher BMI (31.3 ± 1.26) than AC (30.7 ± 0.75) and CC carriers (29.4 ± 1.28), although there were no significant differences (p=0.561). Similarly, this (non-significant) trend of increased BMI was observed in the control group (p=0.457), as presented in **Table 3**. Moreover, no association was detected between the FTO rs80501363 genotypes and FBG and HbA1c among the T2DM patients and control groups.

Discussion. Type 2 DM is a global, epidemic, long-term illness with a strong hereditary component. It is a complex multifactorial condition with unknown contributing genetic factors that enhance the risk of T2DM development in susceptible individuals. It is extremely important to identify those at risk for T2DM, as this would help in the appropriate implementation of measures that could reduce the burden of T2DM and its serious complications.

Although numerous risk factors for T2DM have already been identified, finding new genetic markers will enhance the detection of individuals at higher risk of developing the disease. This research could assist in reducing the socioeconomic burden of T2DM by implementing screening programs that might result in appropriate interventions in high-risk populations. Many GWASs have been carried out on different ethnic populations, and more than 250 gene variants and susceptible loci have been associated with T2DM development.[10,11] However, all the identified variants explain approximately 10-15% of T2DM heritability.[34] As large genetic heterogeneity has been shown between the different populations, exploring the susceptibility of T2DM in other populations has been suggested to contribute more to our knowledge of disease pathophysiology.

Fat mass and obesity-associated gene variants have been shown by GWASs to be associated with the risk of obesity and T2DM.[35] The association of the rs8050136 variant of FTO gene with obesity and T2DM has been studied among different populations with conflicting and inconsistent results reported between different populations or among the same population, as shown in **Table 4**.[14-20,26-32] To our knowledge, this study is considered to be the first to investigate the link between FTO gene polymorphisms (rs8050136) and T2DM in the Saudi population.

Our findings revealed insignificant differences in genotype frequencies between T2DM patient and control groups. Due to the small number of participants in our study, the analysis could not demonstrate a significant association between the rs8050136 A>C SNP and T2DM risk. Although several previous studies of different populations reported the association of rs8050136 variant with a higher risk of T2DM,[14-20] study within Arab populations (Omanis and Lebanese), Iranian, Dutch, West Balkan, Russian, and Mexican Mestizo populations have shown no such association.[26-32] A larger sample size is necessary to determine the implications of this variant on T2DM risk in the Saudi population.

Furthermore, obesity is known to be one of the main risk factors for developing T2DM. Therefore, it was

Table 3 - The relationships between the FTO rs8050136 SNPs and FBG, HbA1c, and BMI among study groups.

Parameter/ genotype	AA	AC	CC	F value	*P*-value
Control group	21	59	26		
FBG	5.38 ± 1.25	5.37 ± 1.28	5.51± 0.63	0.15	0.864
HbA1c	5.17 ± 0.62	5.3 ± 0.56	5.49 ± 0.72	1.63	0.201
BMI	26.54 ± 3.06	25.94 ± 3.81	25.29 ± 2.66	0.79	0.457
Diabetic group	20	71	23		
FBG	9.9 ± 1.3	8.1 ± 0.35	7.9 ± 0.35	2.40	0.092
HbA1c	8.7 ± 0.54	8.39 ± 0.25	9.7 ± 1.01	1.70	0.177
BMI	31.3 ± 1.26	30.7 ± 0.75	29.4 ± 1.28	0.58	0.561

BMI: body mass index, FBG: fasting blood glucose, HbA1c: hemoglobin A1c, BMI: body mass index, SNP: single nucleotide polymorphism FTO: fat mass and obesity-associated

Table 4 - Summary of studies on the association of the rs8050136 variant of FTO gene with obesity and T2DM.

Author	Year	Region	Risk allele frequency		*P*-value
			T2DM	Control	
Xiao et al[14]	2016	China	0.31	0.28	0.014
Votsi et al[15]	2017	Cyprus	0.41	-	0.006
Rong et al[16]	2009	USA	0.15	0.14	0.800
Hotta et al[18]	2011	Japan	0.40	0.45	0.000
Ramya et al[19]	2012	India	0.14	0.11	<0.0001
Chauhan et al[20]	2011	India	0.35	0.34	0.36
Vatankhah et al[26]	2020	Iran	0.76	0.74	0.132
Bego et al[27]	2019	West Balkan	0.50	0.46	0.659
Almawi et al[28]	2013	Lebanon	0.49	0.55	-
Al-Sinani et al[29]	2015	Oman	0.46	0.43	0.770
Nikitin et al[30]	2017	Russia	067	0.67	0.100
Gamboa et al[31]	2012	Mexico	0.19	0.2	0.278
van Herpt et al[32]	2017	Netherlands	0.41	0.38	0.45
Xiao et al[37]	2015	China	0.31	0.27	0.027

FTO: fat mass and obesity-associated, T2DM:n type 2 diabetes mellitus

logical to test if the rs8050136 A>C SNP risk A allele is associated with an increased risk of obesity. Our results were consistent with previous studies' findings that showed an insignificant association between the FTO polymorphism rs8050136 A>C and BMI, one of the most important markers of obesity, as it affects the body's response to insulin.[27,36] In contrast, the results of this study did not support the findings of numerous studies that reported association between the risk A allele of rs8050136 A>C variant and BMI. A strong relationship between rs8050136 A>C and higher BMI was demonstrated in the Indian population.[20] Similar association results were obtained in 2 studies of Chinese populations.[37,38] Additionally, a large study conducted in an African American population found a significant association between the incidence of the rs8050136 A>C SNP risk A allele and increased BMI.[39]

In addition, our results found an insignificant association of the rs8050136 A>C SNP risk A allele with high levels of fasting blood glucose and glycated hemoglobin in T2DM patients. These results contrast with data reported by Bego et al[27] that HbA1c was significantly associated with the rs8050136 A>C SNP risk A allele.

Study limitation. This study has a relatively small number of study participants. However, this study included many newly diagnosed T2DM patients who are essential in analyzing the associations of genetic variants with T2DM. They may provide new paths for studying its complex pathogenesis, but a greater sample size is required to test for significant polymorphisms that may substantially impact the disease. Additionally, as the average age of our patient group was near late middle age, any association of genetic variants with T2DM could be made more detectable by limiting the study population to middle-aged individuals.

While numerous GWASs and meta-analyses have shown the association of rs8050136 A>C SNP of FTO gene with T2DM, our study did not exhibit the same trend. It is not uncommon for GWASs carried out on different populations to have variable or contradictory results. Different ethnicities, study subject characteristics (such as, undiagnosed chronic diseases), sample sizes (such as, statistical power), epigenetic changes, and predominant environmental conditions may affect study results. The frequency of T2DM risk alleles or the strength of their effect size may be ethnicity-specific, and susceptibility variants associated with T2DM may be classified as either common or ethnicity-specific, requiring identification within each population.[40]

In conclusion, this study examined the association between T2DM and the rs8050136 variant in the FTO gene in Saudi Arabia. We found no association between the FTO genotype (rs8050136) and T2DM, and the risk A allele of rs8050136 did not correspond with T2DM risk. However, there is a possibility that the studied variant only minimally impacted T2DM. As a result, more research with larger sample sizes is needed to understand the role of rs8050136 variants in the predisposition to T2DM in Saudi people.

Furthermore, future work should focus on examining additional SNPs that could help identify other genetic components of T2DM.

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