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Purposeful scientific research is a basic pillar for the advancement of Iraq

Genetic polymorphism of FTO and MC4R genes in Type 2 Diabetes Mellitus patients with and without obesity

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ABSTRACT
Diabetes can be considered one of the greatest prevalent health problems in the world, and obesity is one of the main causes of diabetes. The main objective of this investigation was to explore the frequency of both alpha-ketoglutarate dependent dioxygenase (FTO) gene_ rs9939609 and melanocortin 4 receptor (MC4R) gene - rs17782313 in diabetes patient with and without obesity.: We enrolled 39 individuals as a control group and 102 as diabetes group (T2DM without obesity (N=52) and T2DM with obesity (N=50)). Genetic analysis was carry out by Tetra Primer of amplification refractory mutation system (ARMS-PCR) for both FTO SNP rs9939609 and MC4R gene rs17782313. FTO rs9939609 polymorphism between control group and diabetes patients was show significant associations. FTO rs9939609 A-A /A-T have high significantly associated between healthy group with both T2DM without obesity , and T2DM with obesity in all inheritances model except in over dominance model. MC4R (rs17782313) gene C-C/C-T have significantly related between healthy group with both T2DM without obesity, and T2DM with obesity in Codominant and Recessive model, while no significantly associated in Dominant and Over dominant model. Our research suggested that rs9939609 in the FTO gene and rs12970134 in the MC4R gene are consider risk candidate factors in patients with diabetes. In diabetic individuals without obesity, the correlation was highly significant.

Keywords: FTO rs9939609, MC4R rs12970134, Polymorphism, Diabetes, obesity.

Introduction
Diabetes can be described as a group of metabolic disorders diagnosed with hyperglycemia due to defects in insulin action, insulin secretion, or both (ADA, 2010). The blood glucose level is keep in normal range through the insulin function, which is done by facilitating the absorption of glucose by cells, regulating the anabolism and catabolism pathways of carbohydrates, lipids and proteins, and also has a role in promoting cell division and growth (Wilcox, 2010). Since 2000, the prevalence of diabetes among adults (20-60 years) has increased more than tripled, which is estimated at 4.6% of the world's population at that time to 10.5 of the world's population today (Sun *et al.*, 2022), and 1.5 million deaths cases are associated to diabetes each year (WHO, 2019).
Previously published studies candidate that obesity is an influence factor for diabetes prevalence, especially raised abdominal obesity is highly associated with increased diabetes prevalence (Lean *et al.*, 2019; Bennett *et al.*, 2019). Obesity has a considerable negative effect on health, therefore it is known as a complex, chronic medical condition (Hu, 2007). In 2016, According to the report approved by the World Health Organization , about 1.9 billion individuals were either overweight or obese, and the number has tripled since 1975 (WHO, 2021). However, it is not necessary that there be a direct relationship between diabetes and obesity. As an illustration, in populations such as Japan, India and China, in which the incidence of Diabetes is high, the incidence of obesity is relatively low (DeFronzo *et al.*, 2015; Wells, 2007). Conversely, in countries such as United Kingdom and Australia, where the obesity prevalence is high, Diabetes prevalence is relatively low (Federation, 2017).
Generally, the diabetes and obesity may vary depending on the genetic factors, sex, ethnic groups and geographical distribution of human populations. In Middle East and North Africa Region, 1 in 6 adults (72.3 million) are living with diabetes and around 796,000 deaths caused by diabetes in 2021 (Ogurtsova *et al.*, 2022). Approximately 9.4 % of Iraqis population have diabetes (IDF, 2020).
Previous research has recognized that hereditary component played a critical role in the advancement

of obesity associated with T2DM (Mahajan *et al.*, 2018). Hundreds of genetic loci associated with diabetes was identified by genetic studies (DeForest & Majithia, 2022). The FTO gene, which is linked with raised Basal metabolic rate (BMR) and likewise appearance powerful relationship with diabetes (Yajnik *et al.*, 2009). The FTO gene is existed in chromosme 16q12.2, and including 9 exons with many single nucleotide polymorphisms (SNPs). At nucleotide position (53786615), the FTO rs9939609 gene contain missense mutation, which exhibits relation with diabetes (Moghanloo *et al.*, 2018). Several previous investigation revealed that an rs17782313 located at 188 kb downstream of MC4R gene, is a prospect as a diabetes risk factor (Qi *et al.*, 2008; Osman *et al.*, 2018) and has also been associated with obesity (Loos *et al.*, 2008; Sull *et al.*, 2013).

This study aims to contribute to this growing area of research by exploring association frequency of FTO - rs9939609 and MC4R -rs17782313 in diabetes patients with and without obesity of Tikrit population / Iraq.

Material and methods

Research design and Population Study: In this study, a random sample of patients with diabetes was recruited from the saladin governorate/Iraq. In general, the study samples were divided into three groups: healthy group (39 samples: 14 males and 25 females), diabetes with obesity (50 samples: 25 males and 25 females), and diabetes without obesity (52 samples: 25 males and 27 females). Blood Samples were collected between November 2021 and February 2022. For the current study, ethical approvals were acquired from the local committee in the College of Science / Tikrit University (No. 3/7/ 5334 in 9/11/2021), in agreement with the Declaration of Helsinki Principles. All volunteers signed the written consent form before collecting samples

Genomic DNA Extraction

The DNA of the study samples was extracted according to the previously described method (Gaaib *et al.*, 2011). The DNA extraction result was estimated by electrophoresis, through 1% of agarose gel. While the amount and purity were identified by a Nanodrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA).

Genotyping

The polymorphism of FTO and MC4R genes was carry out by ARMS-PCR. The table below (1) illustrates the primers.

Table 1: Primers sequence, PCR yield and optimal annealing temperature for FTO and MC4R genes.

Primer Sequence for FTO gene- rs9939609 OF GCTGCTATGGTTCTACAGTTCCA OR TGTTCAAGTCACACTCAGCCTC IF CCTTGCGACTGCTGTGAATATA IR CAGAGACTATCCAAGTGCATCTCA	PCR Product	Annealing Temperature	Source
	Product size for A allele: 211 Product size for T allele: 296 Product size of two outer primers:461	58 C	(Mozafarizadeh *et al.*,2019)
Primer Sequence for MC4R gene - rs17782313 OF TCCACATGCTATTGGTTTAAGACAA OR TGCTGAGACAGGTTCATAAAAAGAG IF GAAGTTTAAAGCAGGAGAGATTGTATACC IR GCTTTTCTTGTCATTTCCAGCA	Product size for T allele: 184 Product size for C allele: 218 Product size of two outer primers: 351	59 C	(Mozafarizadeh *et al.*, 2019)

Statistical analysis

The odds ratios (ORs), with 95% confidence intervals (CIs) were computed for the lipid profile, glucose level, and genotyping (FTO gene- rs9939609, MC4R gene - rs17782313) among study groups (MedCalc Software, 2022). Scatter plot was used to represent distribution of lipid profile and glucose level among study groups (GraphPad Prism 9.3.1/2022).

Esults

As can be seen from the table 2, there were 102 T2DM patients (52 T2DM without obesity and 50 T2DM with obesity) and 39 healthy controls in this research. This result recorded significant differences in very-low-density lipoprotein VLDL_C ($p = 0.025$), low-density lipoproteins LDL_C ($p = 0.046$), high-density lipoprotein HDL_C ($p = 0.025$), total cholesterol ($p = 0.025$), fasting blood glucose (FBG) ($p = 0.0008$), Postprandial glucose (PPG) ($p = 0.00002$), Hemoglobin A1C H b A 1 c ($p = 0.00000$), between the patients and healthy group. In addition, distribution of lipid profile and glucose level between control and cases are present in scatter blot figure 1 and 2 resp.

Table 2: Biochemical data between control and cases

Factor	Control (N=39)	T2DM without obesity (N=52)	T2DM with obesity (N=50)	p-value
VLDL	29.53 b	39.49 a	36.36 ab	0.024
LDL	112.76	99.81 b	117.29 a	0.046
HDL	37.003 b	41.880 a	37.772 b	0.025
Chol.	180.76 ab	167.55 b	192.88 a	0.025
FBG	85.2 c	176.4 a	132.2 b	0.0008
PPG	93.56 c	227.80 a	195.73 b	0.00002
H b A 1 c	5.128 c	7.845 a	6.934 b	0.00009

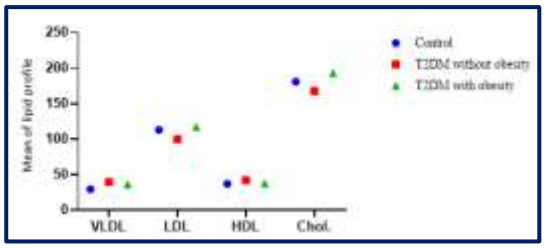

Fig. 1: Scatter plot for lipid profile distribution among study groups.

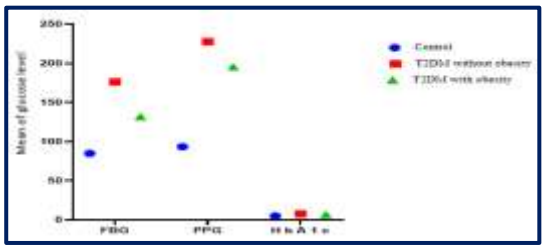

Fig. 2: Scatter plot for glucose level distribution among study groups.

One hundred and two volunteer were genotyped. The PCR products for FTO (rs9939609) and MC4R (rs17782313) are represented in Fig. 3 and Fig. 4 resp. In the FTO gene- rs9939609, the PCR fragment size was 201 bp for allele-specific wild type (A) and 296 bp for polymorphic-allele type (T). For MC4R gene - rs17782313, the PCR product sizes of two alleles were 218 bp (C, polymorphic allele) and 184 bp (T, Wild allele), respectively.

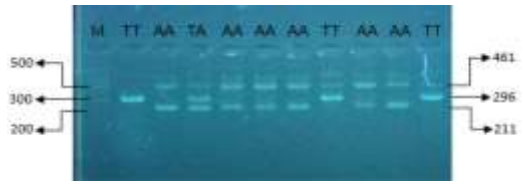

Fig. 3: Agarose gel electrophoresis (1.5%) of polymerase chain reaction (PCR) product of tetra-primer Amplification refractory mutation system-PCR (T-ARMS-PCR) for FTO gene- rs9939609. M=DNA ladder (100 bp).

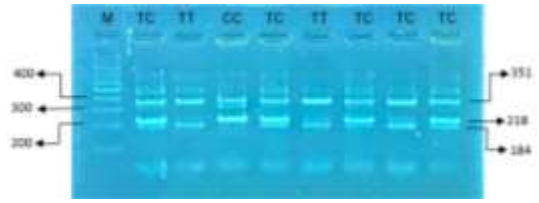

Fig. 4: Agarose gel electrophoresis (1.5%) of polymerase chain reaction (PCR) product of tetra-primer Amplification refractory mutation system-PCR (T-ARMS-PCR) for MC4R gene - rs17782313, M=DNA ladder (100 bp).

One hundred and forty-one adults were genotyped. Homozygous and heterozygous distribution among study groups for both FTO SNP rs9939609 and MC4R gene - rs17782313 are shown in Figures 4 and 5. Also, The analysis of the alleles and genotypes distributions according difference inheritance models was carry out on cases patients (T2DM without obesity and T2DM with obesity) and healthy group (Table 3). For FTO rs9939609 in Codominant models, the genotype AA in T2DM patients (with and without obesity) was significantly different when compared with healthy individuals group (without obesity P = 0.0018 and with obesity P = 0.0022). Both TT and TA genotypes demonstrated no significant difference in distribution (p>0.05). The Dominant model (TA+AA) revealed a significant difference (without obesity P = 0.0190 and with obesity P = 0.0271). The recessive model (TA+AA) exhibited a significant difference (without obesity P = 0.0034 and with obesity P = 0.0031), while the Over dominant (TT+TA) did not record any significant difference between T2DM and controls group (p>0.05). Additionally, a significant difference of the alleles was evident in T2DM patients compared with healthy controls (without obesity P = 0.0006 and with obesity P = 0.0007), candidate that the A allele is a risk factor for T2DM susceptibility.

For MC4R gene - rs17782313 in Codominant models, the genotype CC in T2DM patients (with and without obesity) was significantly different when compared with their respective controls (without obesity P = 0.0091 and with obesity P = 0.0265). Both TT and TC genotypes showed no significant difference in distribution (p>0.05). The Dominant model (TC+CC) revealed no a significant difference (without obesity P = 0.0604 and with obesity P = 0.0814). The recessive model (TC+CC) revealed a significant difference (without obesity P = 0.0089 and with obesity P = 0.0475), while the Over dominant (TT+TC) did not appear any significant difference between T2DM and healthy group (p>0.05). Furthermore, a significant difference of the alleles was evident in T2DM compared with healthy group (without obesity P = 0.0031 and with obesity P = 0.0169), candidate the C allele is a risk factor for T2DM susceptibility.

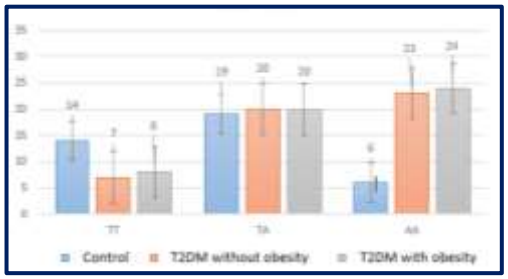

Fig. 5: Histogram represent genotyping distribution among study group for FTO gene - rs9939609

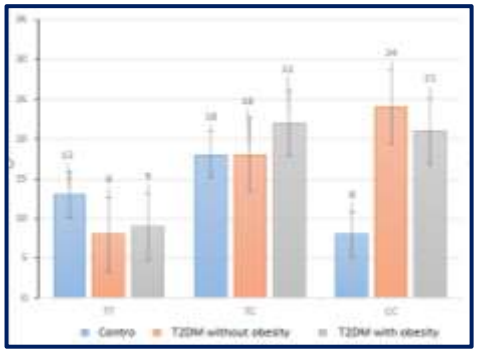

Fig. 6: Histogram represent genotyping distribution among study group for MC4R gene - rs17782313

Table 3: Multivariate analysis of risk factors for diabetes with FTO gene-rs9939609 and MC4R gene-rs17782313genotypes

FTO gene (rs9939609)	Control	T2DM without obesity	T2DM with obesity	OR (95% CI)	p-Value
Codominant					
TT	14	7	-	1 (ref.)	
		-	8		
TA	19	20	-	2.1053(0.6984 - 6.3460)	P = 0.1860
		--	20	1.8421(0.6307 - 5.3804)	P = 0.2639
AA	6	23	-	**7.6667(2.1382 - 27.4891)**	**P = 0.0018**
		-	24	**7.0000(2.0116 - 24.3585)**	**P = 0.0022**
Dominant					
TT	14	7	-	1 (ref.)	
		-	8		
TA+AA	25	43	-	**3.4400(1.2250 - 9.6605)**	**P = 0.0190**
		-	44	**3.0800(1.1356 - 8.3538)**	**P = 0.0271**
Recessive					
TT+TA	33	27	-	1 (ref.)	
		-	28		
AA	6	23	-	**4.6852(1.6685 - 13.1559)**	**P = 0.0034**
		-	24	**4.7143 (1.6887 -13.1604)**	**P = 0.0031**
Allele					
T	47	34	-	1 (ref.)	
		-	36		
A	31	66	-	**2.9431(1.5929 -5.4376)**	**P = 0.0006**
		-	68	**2.8638(1.5602 - 5.2566)**	**P = 0.0007**
Over dominant					
TT+AA	20	30	-	1 (ref.)	
		-	32		
AT	19	20	-	0.7018(0.3014 -1.6340)	P = 0.4115
		-	20	0.6579(0.2839 -1.5247)	P = 0.3288
MC4R gene (rs17782313)					
Codominant					
TT	13	8	-	1 (ref.)	
		-	9		
TC	18	18	-	1.6250 (0.5428 - 4.8652)	P = 0.3855
		-	22	1.7654 (0.6155 -5.0640)	P = 0.2904
CC	8	24	-	**4.8750 (1.4832 - 16.0237)**	**P = 0.0091**
		-	21	**3.7917 (1.1685 - 12.3034)**	**P = 0.0265**
Dominant					
TT	13	8	-	1 (ref.)	
			9		
TC+CC	26	42	-	2.6250 (0.9585 - 7.1888)	P = 0.0604
			43	2.3889 (0.8970 - 6.3620)	P = 0.0814
Recessive					
TT+TC	31	26	-	1 (ref.)	
		-	31		
CC	8	24	-	**3.5769 (1.3765 -9.2948)**	**P = 0.0089**
		-	21	**2.6250 (1.0107 - 6.8178)**	**P = 0.0475**
Allele					
T	44	34	-	1 (ref.)	
		-	40		
C	34	66	-	**2.5121 (1.3656 - 4.6211)**	**P = 0.0031**
			64	**2.0706 (1.1398 - 3.7614)**	**P = 0.0169**
Over dominant					
TT+CC	21	32	-	1 (ref.)	
		-	30		
TC	18	18	-	0.6563 (0.2793 -1.5420)	P = 0.3339
		-	22	0.8556 (0.3708 -1.9740)	P = 0.7146

Discussion

Generally, the current study showed significant differences between individuals with diabetes compared with the control group with regard to the level of lipid profile, noting that there are some differences in the level of lipids between the two groups of patients on the one hand and with the control group on the other hand. The reason may be due to variation in diet, age, sex, and genetic variation between individuals. In many previous studies, it is commonly confirmed that a clear association was found between BMI and the profile (Roland, *et al.*, 2020; Raja *et al.*, 2020). On other hand there are previous studies that revealed a negative correlation between the BMI and the profile (Telles *et al.*, 2018). Regarding the level of blood glucose, significant differences can be observed between the study groups. It is also worth observation the high level of glucose in diabetes patients without obesity compared with obese individuals with diabetes, the reason is that diabetes patients without obesity have an advanced diabetes. However, hyperglycemia is closely associated with lipid metabolism in individuals with diabetes (Silitonga *et al.*, 2019).

In present research, the FTO gene- rs9939609 A > T gene polymorphism was observed statistically significant between Diabetes cases and control group. But can also differ in p value and volume odd ratio among different inheritance models. The MC4R gene- rs17782313 C > T gene polymorphism observed between Diabetes cases and control group was statistically significant. Also differ in p value and volume odd ratio among different inheritance models. These results are in accord with recent studies indicating that the FTO rs9939609 (AA+AT) and The MC4R gene- rs17782313 (CC+CT) genotypes were significantly in the diabetes population (with and without obesity) compared to healthy normal-weight individuals (Younus *et al.*, 2017; Ghafarian *et al.*, 2018; Sull et al., 2020; Szywacz *et al.*, 2021). It is noteworthy that, despite previous studies indicating a link between the FTO-rs9939609 and the MC4R-rs17782313 polymorphisms with T2DM was significant but be dependent on diet and that a high Commitment to the MedDiet Withstands the genetic predisposition (Ortega *et al.*, 2012). However, the results of the current research do not support previous research indicating that there is no association between FTO and MC4R with type 2 diabetes (Huang et al., 2011; Bazzi *et al.*, 2014).

In addition, recent research has indicated that there are 700 genetic loci diagnosed as a general risk for diabetes to today (DeForest & Majithia, 2022). Also, through previous studies, it can be observed that the association between diabetes and the genetic factor may differ depending on the diet, race, gender and age. The findings of this study have a number of important implications for future studies.

Conclusion

One of the more significant findings to emerge from this study is a significant association between diabetes and both the FTO-rs9939609 gene and MC4R-rs17782313. Where the association in diabetic individuals without obesity was slightly more than in those with diabetes with obesity. These findings are important for individuals who have a genetic predisposition to diabetes or obesity to pay more attention to lifestyle and diet. Through this study, the FTO-rs9939609/A allele and the MC4R-rs17782313/C allele can be considered as a risk factors for diabetes.

Author Contributions: Asmaa Sultan Mohammed: Funding Acquisition and Execution of the experiment. Maan Hasan Salih: Supervision, Conceptualization, Execution of the experiment, analyzed the data, and Writing -Review and the drafting of the manuscript. Maksood Adil Mahmoud Al-Doori: Supervision, conceived, collected the data, and Investigation. All authors have read and approved the original manuscript.

Conflicts of Interest: no conflict of interest.

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