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Association of FTO Gene Variants with Some Biochemical Markers of Type 2 Diabetes Mellitus Patients in Iraqi Population

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Abstract

Background: The Fat-mass and obesity associated (*FTO*) gene modulates the gene expression through methylation–demethylation modifications since it is part of Fe (II) - and 2-oxoglutarate-dependent dioxygenases superfamily. This study was carried out in the Department of Clinical Laboratories / College of Applied Medical Sciences / University of Kerbala during the period from November 2022 to April 2024. The study aimed to investigate the association between the variation in the *FTO* gene and serum level of some biochemical markers in Type 2 Diabetes Mellitus Patients within the Iraqi Population.

Patients and Methods: One hundred volunteers participated in this study, 50 individuals with Type 2 DM as a patient's group (25 females and 25 males), and 50 apparently healthy individuals as a control group (25 females and 25 males). The ages of all participants were ranged between 25 to 75 years at the time of the investigation. We investigate three sites in the *FTO* gene (*FTO* 1, *FTO* 2, and *FTO* 3). The variation of the *FTO* gene was investigated by the Sanger sequencing method. The levels of biochemical markers were measured in blood serum.

Results: The results of the present study identified the presence of four previously registered variants in *FTO* gene. These variants might be of interest to *FTO* gene studies due to their presence in the coding regions that included in the gene expression**.**

Conclusion: The two variants, 53769662 T/A and 53782363 C/A, may be the most important variables because there are statistical associations with some biochemical markers.

ارتباط متغيرات جين *FTO* مع بعض المعلمات الكيموحيوية عند مرضى السكري من النوع الثاني في المجتمع العراقي

زيد عبد الحسين كاظم، جودت نوري غائب

الخلاصة

المقدمة: يُعد جين FTO (مرتبط بالكتلة الدهنية والسمنة) أحد الأعضاء في عائلة الإنزيمات ثنائية الأكسجين التي تعتمد على الحديد (Fe II) والـ 2-أوكسوجلوتارات، حيث يُعدّل تعبير الجين من خلال تعديلات الميثيل والدي-ميثيل. تم إجراء هذه الدراسة في قسم المختبرات السريرية / كلية العلوم الطبية التطبيقية / جامعة كربلاء خلال الفترة من نوفمبر 2022 إلى أبريل 2024. هدفت الدراسة إلى التحقيق في العلاقة بين التباين في جين FTO ومستوى بعض العلامات البيوكيميائية في مصل الدم لدى مرضى السكري من النوع الثاني داخل المجتمع العراقي.

المرضى وطرق العمل: شارك في هذه الدراسة مئة متطوع، 50 منهم يعانون من مرض السكري من النوع الثاني كمجموعة مرضى (25 أنثى و25 ذكر)، و50 فردًا يتمتعون بصحة جيدة كمجموعة ضابطة (25 أنثى و25 ذكر). تراوحت أعمار جميع المشاركين بين 25 إلى 75 عامًا في وقت إجراء الدراسة. تم التحقيق في ثلاثة مواقع في جين FTO (FTO 1، FTO 2، وFTO 3). تم تحليل التباين في جين FTO باستخدام طريقة تسلسل سانجر. كما تم قياس مستويات العلامات البيوكيميائية في مصل الدم.

النتائج: حددت نتائج هذه الدراسة وجود أربع متغيرات مسجلة سابقًا في جين FTO. قد تكون هذه المتغيرات ذات أهمية في دراسات جين FTO نظرًا لوجودها في المناطق المرمزة التي تشارك في تعبير الجين.

الاستنتاج: قد يكون المتغيران T/A 53769662 وC/A 53782363 هما الأكثر أهمية، نظرًا لوجود ارتباطات إحصائية مع بعض العلامات البيوكيميائية.

1. Introduction

The FTO also known as alpha-ketoglutarate-dependent dioxygenase FTO is an enzyme that in humans is encoded by the *FTO* gene that is located on chromosome 16. As one homolog in the Alkylatin B family proteins, it is the first mRNA demethylase that has been identified(Jawiarczyk-Przybyłowska et al., 2023; Jia et al., 2012). Human obesity appears to be associated with specific *FTO* gene variations(Popović et al., 2023; R.J.F. and G.S.H., 2014). The transcribed FTO protein's amino acid sequence bears a strong resemblance to that of the oxidatively demethylating enzyme AlkB. *FTO* belongs to the superfamily of non-heme iron-containing proteins called alpha-ketoglutarate-dependent hydroxylases. It was initially found that recombinant FTO protein could, albeit inefficiently, catalyze the demethylation of 3-methylthymine in single-stranded DNA and 3-methyluridine in single-stranded RNA(Gerken et al., 2007; Xu et al., 2023). N6-methyladenosine (m6A), a nucleoside that is often modified in RNA, was later discovered to be a significant substrate of *FTO*. Obesity raises the risk of several common diseases, making it an important global health concern. It's unclear whether hereditary factors contribute to obesity. A common mutation in the *FTO* gene, which predisposes to diabetes through an influence on body mass index, was found during a genome-wide search for genes linked to type 2 diabetes susceptibility(Frayling et al., 2007; Tian et al., 2023). A typically elevated triglyceride deposition and the generation of hepatic glucose can result from enhanced *FTO* expression, which can also promote de novo lipogenesis, decrease lipolysis and fatty acid oxidation, and boost gluconeogenesis(Witka et al., 2019).

Diabetes Mellitus term describes a metabolic disorder of multiple an etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both(Ahmed, 2024; AL-Sahi et al., 2024) . DM is characterized by immune-mediated (Type 1 diabetes), insulin resistance (Type 2 diabetes), gestational hyperglycemia, or other chronic hyperglycemia; genetic, environmental, infectious, or medication-induced problems; or affects the beta cells of the islets of Langerhans(Abdulhakeem et al., 2023; Azeez et al., 2024). Type 2 diabetes mellitus (T2DM) is characterized by hyperglycemia, insulin resistance and relative insulin deficiency. Over 23 million Americans live with diabetes mellitus, out of the 366 million individuals who have the condition worldwide. By 2030, this figure will increase to 552 million.(Damanik and Yunir, 2021; Qalaf et al., 2024) The causes of T2DM are not completely understood but there is a strong association between overweight, obesity, family history, and ethnicity(Aschner, 2017; Basu et al., 2013). The aim of the study: Investigate the Association of FTO gene variants with some biochemical markers in Type 2 Diabetes Mellitus patients of Iraqi Population.

2. Materials and Methods

2.1. Blood Sample Collection

One hundred volunteers participated in this study, 50 individuals with Type 2 Diabetes Mellitus as a patient's group (25 females and 25 males) and 50 apparently healthy individuals as a control group (25 females and 25 males). The ages of all participants were ranged between 25 to 75 years at the time of the investigation. The blood samples were collected from the individuals in Al-Imam Al-Hassan Center for Endocrinology and Diabetes in Karbala city / Iraq. An ethical consent form was signed by each volunteer. Six milliliters of the venous blood sample were obtained from each participant using gel tubes, and the blood was drawn using disposable syringes under sterile condition. The collected blood was centrifuged to separate serum to be used later. The levels of biochemical markers HbA1c, FBS, Cholesterol, TG, LDL, HDL, VLDL, FIB and CRP were measured in blood serum using the ARCHITECT c4000 clinical chemistry instrument from Abbott Diagnostics

2.2. Molecular Detection

A total volume of 25 µl was used in the PCR reaction (5 µl DNA, 2 µl from each primer Table 1, 8 µl master mix, and 8 µl nuclease free water). The PCR program that was used to amplify the target sequence of *FTO* 1, *FTO* 2, and *FTO* 3 region consisted of 35 cycles, each cycle included denaturation for 30 seconds at 94ºC, annealing for 45 seconds at 57ºC and extension for 45 seconds at 72ºC. Agarose gel electrophoresis was used to separate PCR product bands on 1% agarose gel stained by fluorescent Redsafe dye. The gel electrophoresis system was set at 70 volts for 60 minutes, and then the gel was displayed under UV transilluminator to check the PCR products(see Fig.1, Fig.2, and Fig.3).

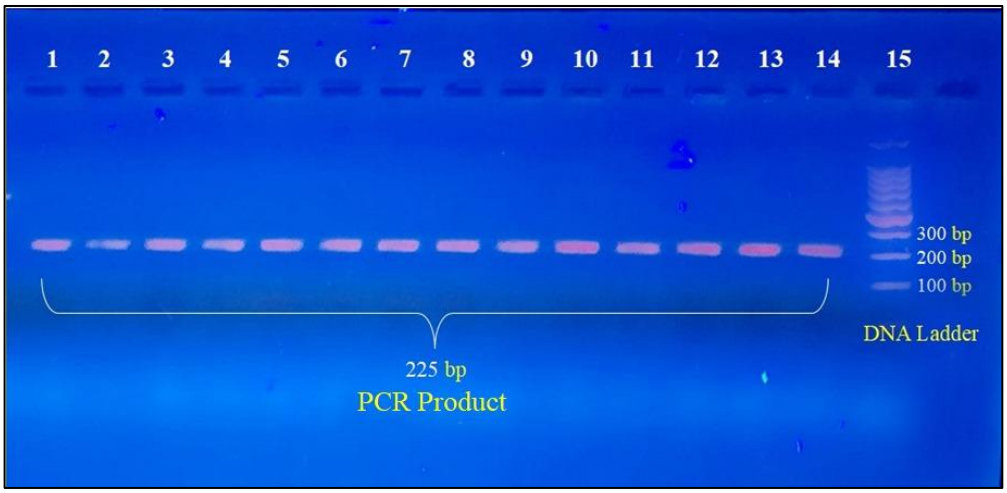

Figure 1: PCR Products (225 Bp) Found in Study Samples That Demonstrates the FTO Gene's FTO 1 Target Region's Presence. Lanes 1-14, PCR Products. Lane 15, DNA Ladder.

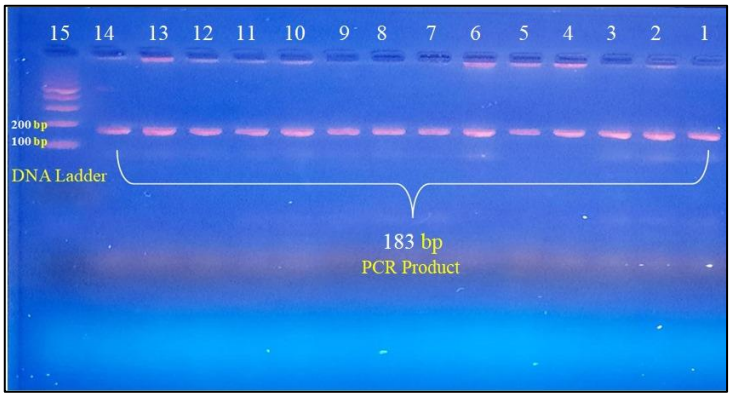

Figure 2: PCR Products (183 Bp) Found in Study Samples That Demonstrates the *FTO* Gene's *FTO* 2 Target Region's Presence. Lanes 1-14, PCR Products. Lane 15, DNA Ladder.

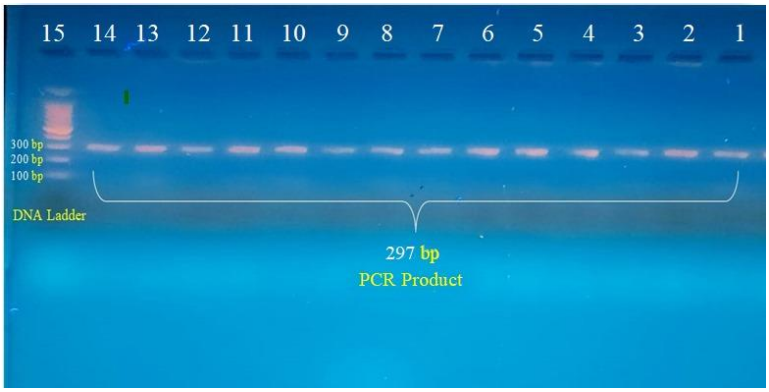

Figure 3: PCR Products (297 Bp) Found in Study Samples That Demonstrates the *FTO* Gene's *FTO* 3 Target Region's Presence. Lanes 1-14, PCR Products. Lane 15, DNA Ladder.

Table 1: Primers Designed for Amplification of *FTO* Gene.

Primer name		**Primer sequence**	**PCR product size**
***FTO* 1**	*FTO* -Forward	5'- TCTAAATTATTAATCAGGGCCATTT-3'	225 base pair
	FTO -Reverse	5'- TGTCCTACCACCCTGTTTACC-3'	
***FTO* 2**	*FTO* -Forward	5'- ACAGTGCCAGCTTCATAGCC -3'	183 base pair
	FTO -Reverse	5'- TTGAGGTGCCATTCCTCAAT -3'	
***FTO* 3**	*FTO* -Forward	5'- TTGAATGAAATAGGATTCAGAAGAGA -3'	297 base pair
	FTO -Reverse	5'- TGTCCAAACAGTAGGTCAGGAA -3'	

2.3. Nucleotides Sequencing and Analysis

In the present study, three regions of the *FTO* gene were selected, and we named them *FTO* 1, *FTO* 2, and *FTO* 3. (see Fig.4, Fig.5, and Fig.6). PCR products from 24 patients, along with 12 PCR products from control group (total= 36) for each target region of the *FTO* gene were sent to the Alpha DNA (S.E.N.C.) Corporation in (Montreal, Quebec, Canada) to perform nucleotide sequencing. Sanger sequencing method was applied using an automated DNA sequencer (see Fig.7). The results of sequencing were manually examined using bioinformatics tools, and they were compared to human reference gene sequences that already uploaded to the National Center for Biotechnology Information (NCBI). The NCBI's Basic Local Alignment Search Tool (BLAST) was used to complete the alignments. The target gene's sequenced area was examined by using Molecular Evolutionary Genetics Analysis X (MEGAX), where the CLUSTALW program carried out several sequence alignments to validate the existence of variants found by the BLAST tool. The locations of every variant found in the current study were reported and examined using Ensembl Genome Browser's tools to determine the type of variant and forecast its functional implications (see Fig.8, Fig.9, Fig.10 and Fig.11).

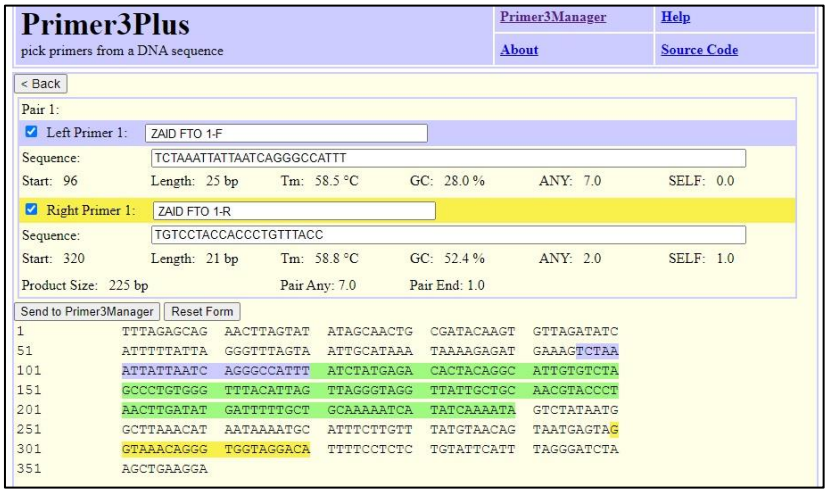

Figure 4: Forward and Reverse Primer Design for *FTO* 1 Gene in Primer 3 Plus Program.

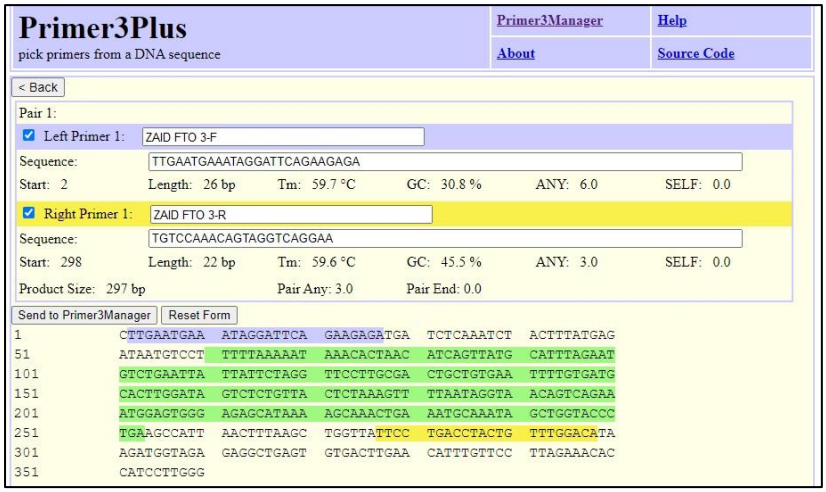

Figure 5: Forward and Reverse Primer Design for *FTO* 2 Gene in Primer 3 Plus Program.

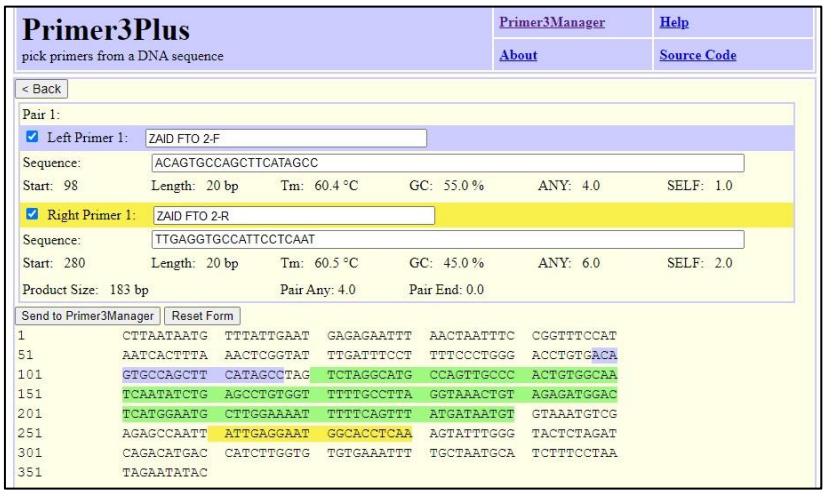

Figure 6: Forward and Reverse Primer Design for FTO 3 Gene in Primer 3 Plus Program.

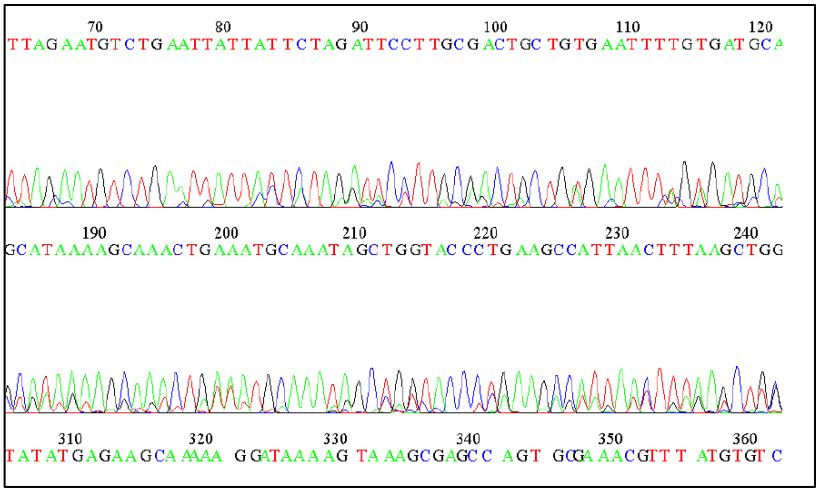

Figure 7: An Automated Sanger DNA Sequencing Method Shows the Electropherogram with Peaks of The Forward Strand of the Sample Sequence

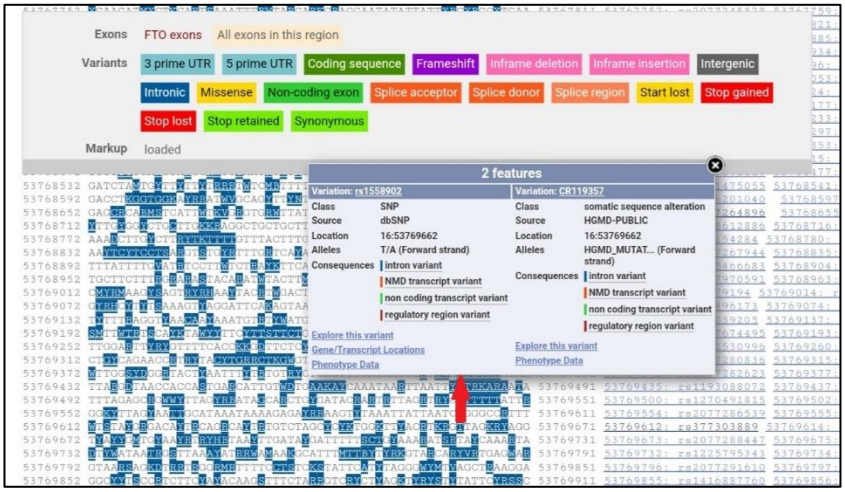

Figure 8: Detect (53769662 T/A) Variant in Study Sample

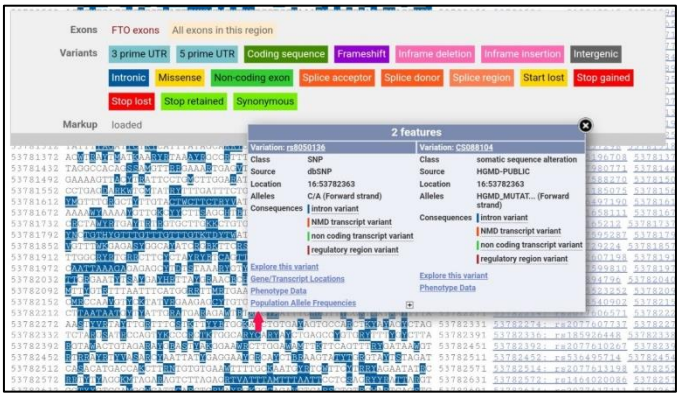

Figure 9: Detect (53782363 C/A) Variant in Study Sample

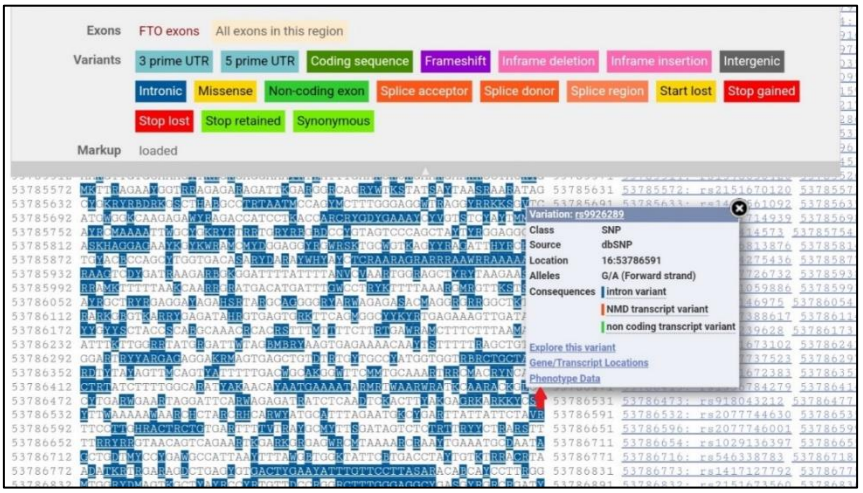

Figure 10: Detect (53786591 G/A) Variant in Study Sample

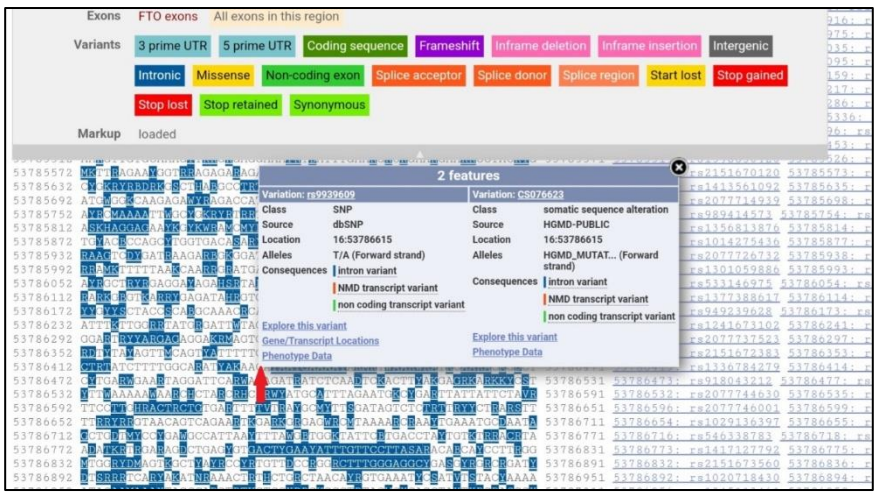

Figure 11: Detect (53786615 T/A) Variant in Study Sample

2.4. Statistical Analysis

Statistical analysis was carried out using SPSS version 22.0 (SPSS, IBM Company, Chicago, IL 60606, USA). Data were expressed as means ± standard deviation if the data were normally distributed. Data were expressed as median±IQR if the data were non-normal distributed. $P \leq 0.05$ was statistically significant.

3. Results

The results of the statistical analysis showed the mean value, standard deviation, standard error, minimum and maximum value for the parameters included in the study (BMI, HbA1c, FBS, Cholesterol, TG, LDL, HDL, VLDL, FIB and CRP) for the patient's group and the control group. This information is presented in Table 2 and Table 3.

Table 2: Mean Values of the Biochemical Markers in The Patient Group.

Variable	Description				
	Normal Value	**Mean ±SD**	**Min.**	**Max.**	**SE**
BMI	18.5-24.9	31.36±4.67	25.7	43.0	0.66
HbA1c	Below 5.7 %	8.42±1.72	6.3	11.4	0.24
FBS	Below 100 mg/dl	194.38±49.14	134	280	6.95
Cho	120-200 mg/dl	185.16±42.63	122	242	6.03
TG	35-160 mg/dl	148.82±56.16	68	271	7.94
HDL	30-70 mg/dl	43.0±14.88	25	65	2.1
LDL	30-130 mg/dl	112.36±33.81	54	154	4.78
VLDL	13-60 mg/dl	30.61±11.02	14	54	1.55
FIB	200-400 mg/dl	307.91±52.27	182	392	7.39
CRP	0-6 mg/l	4.16±3.09	0.7	11.1	0.43

The data presented in the table as means: ±SD standard deviation of mean, Min minimum, Max maximum, SE standard error. BMI refers to body mass index, HbA1c hemoglobin A1c, FBS fasting blood sugar, Cho cholesterol, TG triglycerides, HDL high-density lipoprotein, LDL low-density, lipoproteins, VLDL very-low-density lipoprotein, FIB fibrinogen, CRP c-reactive protein

Table 3: Mean Values of The Biochemical Markers in The Control Group.

Variable	Description				
	Normal Value	**Mean ±SD**	**Min.**	**Max.**	**SE**
BMI	18.5-24.9	32.16±4.58	26.4	46.5	0.64
HbA1c	Below 5.7 %	5.59±0.41	5.0	6.0	0.55
FBS	Below 100 mg/dl	113.73±11.96	97.0	126.0	1.69
Cho	120-200 mg/dl	195.24±34.04	135.0	242.0	4.81
TG	35-160 mg/dl	124.06±48.2	57.0	158.0	6.81
HDL	30-70 mg/dl	44.44±10.85	21.0	69.0	1.53
LDL	30-130 mg/dl	132.04±37.87	81.0	192.0	5.35
VLDL	13-60 mg/dl	24.71±9.63	11.0	32.0	1.36
FIB	200-400 mg/dl	274.62±25.83	203.0	297.0	3.65
CRP	0-6 mg/l	3.03±1.92	1.2	4.6	0.27

The data presented in the table as means: ±SD standard deviation of the mean, Min minimum, Max maximum, SE standard error. BMI refers to body mass index, HbA1c hemoglobin A1c, FBS fasting blood sugar, Cho cholesterol, TG triglycerides, HDL high-density lipoprotein, LDL low-density lipoproteins, VLDL very-low-density lipoprotein, FIB fibrinogen, CRP c-reactive protein.

The results of this work indicated that there was no significant difference when the mean height, weight, and BMI of the patients and controls were compared ($p = 0.33$, $p = 0.09$, $p = 0.19$, respectively). The comparisons of the mean FBS and HbA1C between the patients and control groups revealed a very highly statistically significant difference ($p<0.001$) with effect sizes of 2.23 and 2.26, respectively. The lipid profile analysis showed inconsistent findings, with statistically insignificant differences observed when comparing the mean serum cholesterol and HDL levels of the patients and controls ($p=0.09$, $p=0.29$). However, TG, LDL, and VLDL levels exhibited statistically significant differences, with highly significant disparities noted ($p=0.01$, 0.004, 0.003) when comparing the same two groups. The effect sizes of the comparisons mentioned later were as follows: 0.47, -0.55, and 0.57. Finally, the comparison of FIB revealed a highly statistically significant difference between the mean concentration levels of the patients and controls ($p<0.001$), with an effect size of 0.8. Additionally, CRP levels showed a statistically significant difference ($p=0.016$) between the two groups, with an effect size of 0.44 see Table 4.

Table 4: Description of Biochemical Marker P-Values (n=100)

Variable	Category	Mean	Standard Deviation	Standard Error	P-value	Cohen sd
Weight	Patients	85.344	11.5001	1.6264	0.09	-0.27
	Controls	88.920	14.9933	2.1204		
Height	Patients	165.320	9.4484	1.3362	0.33	-0.09
	Controls	166.180	9.8077	1.3870		
BMI	Patients	31.362	4.6729	0.6608	0.19	-0.17
	Controls	32.166	4.5818	0.6480		
HbA1c	Patients	8.428	1.7272	0.2443	<0.001	2.26
	Controls	5.590	0.4171	0.0590		
FBS	Patients	194.38	49.148	6.951	<0.001	2.23
	Controls	113.73	11.967	1.692		
Cho	Patients	185.16	42.638	6.030	0.09	-0.26
	Controls	195.24	34.043	4.814		
TG	Patients	148.82	56.165	7.943	0.01	0.47
	Controls	124.06	48.206	6.817		
HDL	Patients	43.00	14.885	2.105	0.29	-0.11
	Controls	44.44	10.857	1.535		
LDL	Patients	112.36	33.819	4.783	0.04	-0.55
	Controls	132.04	37.871	5.356		
VLDL	Patients	30.61	11.023	1.559	0.003	0.57
	Controls	24.71	9.630	1.362		
FIB	Patients	307.92	52.272	7.392	<0.001	0.8
	Controls	274.62	25.839	3.654		
CRP	Patients	4.162	3.0936	0.4375	0.016	0.44
	Controls	3.038	1.9232	0.2720		

The data presented in the table as means: $p\leq0.05$ is considered statistically significant, BMI refers to body mass index, HbA1c hemoglobin A1c, FBS fasting blood sugar, Cho cholesterol, TG triglycerides, HDL high density lipoprotein, LDL low-density lipoproteins, VLDL very-low-density lipoprotein, FIB fibrinogen, CRP c-reactive protein

Conventional PCR was employed to amplify the DNA target regions within the *FTO* gene. The PCR products (225 base pairs) of the *FTO* 1 region, (183 base pairs) of the *FTO* 2 region and (297 base pairs) of the *FTO* 3 region were detected in all of the study samples. This indicates the presence of the target regions in the *FTO* gene. Genetic analysis of the results detected four registered variants: [5376966 T/A; rs1558902] were detected in the *FTO* 1 region [53782363 C/A; rs8050136] were detected in the *FTO* 2 region and [53786591 G/A; rs996289, 53786615 T/A; rs9939609] were detected in the *FTO* 3 region see Table 5.

Table 5: Previously Registered Variants that are Detected in Study Samples.

No.	Region	Variant Location	Allele	Consequence	Sample No.	Total samples
1	Intron	53769662	T/A	Intron Variant	1,3,4,5,6,7,9,11,12,13,14 15,16,17,19,21,22,23,24,25 27,28,29,31,33,34,35	28
2	Intron	53782363	C/A	Intron Variant	3,6,7,11,15	5
3	Intron	53786591	G/A	Intron Variant	1,3,4,5,6,9,10,11,12,14,15 16,17,19,22,23,24,25,27,28 29,32,33,34,36	25
4	Intron	53786615	T/A	Intron Variant	3,6,11,15,22	5

The effects of *FTO* gene variations on the study parameters (BMI, HbA1c, FBS, Cho, TG, HDL, LDL, VLDL, FIB, and CRP) were investigated by comparing the level of each parameter in the samples sharing the same variation. It was found that the variant 53769662 T/A of the *FTO* 1 region is statistically significantly associations with cholesterol serum levels (p=0.03). The results revealed that there were no other significant associations with the rest of the parameters see Table 6 and Table 7.

Table 6: Effects Of 53769662 T/A Variants on Study Parameters (Normally Distributed)

Parameters	53769662 T/A mutation	Mean±SD	Frequency	P value
Cho	Mutant	178.5±27.5	28	0.03*
	Non-mutant	205.3±36.1	8	
TG	Mutant	143.6±58.4	28	0.16
	Non-mutant	122.8±25.9	8	
HDL	Mutant	41.3±10.7	28	0.92
	Non-mutant	40.9±19.0	8	
LDL	Mutant	109.8±25.3	28	0.48
	Non-mutant	118.1±42.5	8	
VLDL	Mutant	28.7±11.7	28	0.17
	Non-mutant	24.6±5.4	8	
FIB	Mutant	288.9±54.2	28	0.58
	Non-mutant	277.4±35.7	8	

The data presented in the table as means: ±SD standard deviation of the mean $p \leq 0.05$ is considered statistically significant Cho refers to cholesterol, TG triglycerides, HDL high-density lipoprotein, LDL low-density lipoproteins, VLDL very-low-density lipoprotein, FIB fibrinogen

Table 7: Effects of 53769662 T/A Variants on Study Parameters (Non-normally Distributed)

Parameters	53769662 T/A mutation	Median±IQR	Frequency	P value
BMI	Mutant	31.6±5.8	28	0.27
	Non-mutant	34.1±6.4	8	
HbA1c	Mutant	7.1±3.6	28	0.99
	Non-mutant	7.1±5.5	8	
FBS	Mutant	155.6±103.0	28	0.99
	Non-mutant	155.6±158.0	8	
CRP	Mutant	2.7±2.9	28	0.72
	Non-mutant	3.5±2.6	8	
The data presented in the table as means: ±IQR the interquartile, p≤0.05 is considered statistically significant**,** BMI, refers to body mass index, HbA1c hemoglobin A1c, FBS fasting blood sugar**,** CRP c-reactive protein				

It was found that the variant 53782363 C/A of the *FTO* 2 region is statistically significantly associations with the FIB and CRP serum levels (p=0.04 respectively). The results revealed that there were no other significant associations with the rest of the parameters (see Table 8 and Table 9).

Table 8: Effects Of 53782363 C/A Variants on the Study Parameters (Normally Distributed)

Parameters	53782363 C/A mutation	Mean±SD	Frequency	P value
Cho	Mutant	181.0±35.4	5	0.79
	Non-mutant	185.0±31.0	31	
TG	Mutant	156.0±48.1	5	0.45
	Non-mutant	136.3±54.3	31	
HDL	Mutant	39.2±9.3	5	0.66
	Non-mutant	41.5±10.7	31	
LDL	Mutant	104.4±31.5	5	0.56
	Non-mutant	112.8±29.5	31	
VLDL	Mutant	31.3±9.6	5	0.44
	Non-mutant	27.3±10.9	31	
FIB	Mutant	328.8±57.4	5	0.04*
	Non-mutant	279.5±46.7	31	
The data presented in the table as means: ±SD standard deviation of the mean**,** p≤0.05 is considered statistically significant**,** Cho refers to cholesterol, TG triglycerides, HDL high-density lipoprotein, LDL low-density lipoproteins, VLDL very-low-density lipoprotein, FIB fibrinogen				

Table 9: Effects of 53782363 C/A Variants on the Study Parameters (Non-normally Distributed).

Parameters	53782363 C/A mutation	Median±IQR	Frequency	P value
BMI	Mutant	31.0±10.5	5	0.86
	Non-mutant	32.7±6.9	31	
HbA1c	Mutant	7.1±4.0	5	0.89
	Non-mutant	6.7±2.3	31	
FBS	Mutant	157.1±115.0	5	0.89
	Non-mutant	145.6±66.0	31	
CRP	Mutant	3.6±2.9	5	0.04*
	Non-mutant	1.4±1.9	31	
The data presented in the table as means: ±IQR the interquartile, p≤0.05 is considered statistically significant**,** BMI refers to body mass index, HbA1c hemoglobin A1c, FBS fasting blood sugar**,** CRP c-reactive protein				

There were no statistically significantly associations between the 53786591 G/A of the *FTO* 3 region and all the parameters see Table 10 and Table 11.

Table 10: Effects Of 53786591 G/A Variants on Study Parameters (Normally Distributed).

Parameters	53786591 G/A mutation	Mean±SD	Frequency	P value
BMI	Mutant	32.9±5.7	25	0.49
	Non-mutant	31.6±2.8	11	
Chol	Mutant	182.1±31.4	25	0.51
	Non-mutant	189.7±31.4	11	
TG	Mutant	142.6±56.2	25	0.55
	Non-mutant	130.8±47.6	11	
HDL	Mutant	42.9±11.4	25	0.14
	Non-mutant	37.3±7.4	11	
LDL	Mutant	112.6±30.5	25	0.76
	Non-mutant	109.3±28.2	11	
VLDL	Mutant	28.5±11.2	25	0.55
	Non-mutant	26.2±9.6	11	
FIB	Mutant	286.7±51.5	25	0.95
	Non-mutant	285.5±50.4	11	
The data presented in the table as means: ±SD standard deviation of the mean, p≤0.05 is considered statistically significant, BMI refers to body mass index, Cho cholesterol, TG triglycerides, HDL high-density lipoprotein, LDL low-density lipoproteins, VLDL very-low-density lipoprotein, FIB fibrinogen				

Table 11: Effects Of53786591 G/A Variants on Study Parameters (Non-Normally Distributed).

Parameters	53786591 G/A mutation	Median±IQR	Frequency	P value
HbA1c	Mutant	7.3±3.4	25	**0.54**
	Non-mutant	8.1±3.6	11	
FBS	Mutant	145.6±96.0	25	**0.54**
	Non-mutant	185.7±103	11	
CRP	Mutant	2.4±2.8	25	**0.17**
	Non-mutant	4.0±2.3	11	
The data presented in the table as means: ±IQR the interquartile range, p≤0.05 is considered statistically significant, HbA1c refers to hemoglobin A1c, FBS fasting blood sugar, CRP c-reactive protein				

There were no statistically significantly associations between the 53786615 T/A of the *FTO* 3 region and all the parameters see Table 12 and Table 13.

Table 12: Effects Of 53786615 T/A Variants on Study Parameters (Normally Distributed).

Parameters	**53786615 T/A mutation**	**Mean±SD**	**Frequency**	**P value**
Cho	Mutant	189.6±35.2	5	**0.70**
	Non-mutant	183.6±31.0	31	
TG	Mutant	164.6±42.0	5	**0.25**
	Non-mutant	134.9±54.3	31	
HDL	Mutant	43.2±6.7	5	**0.65**
	Non-mutant	40.9±11.1	31	
LDL	Mutant	118.4±32.9	5	**0.59**
	Non-mutant	110.5±29.3	31	
VLDL	Mutant	33.0±8.4	5	**0.25**
	Non-mutant	27.0±10.9	31	
FIB	Mutant	323.2±50.7	5	**0.08**
	Non-mutant	280.4±48.6	31	
The data presented in the table as means: ±SD standard deviation of the mean, p≤0.05 is considered statistically significant, Cho cholesterol, TG triglycerides, HDL high-density lipoprotein, LDL low-density lipoproteins, VLDL very-low-density lipoprotein, FIB fibrinogen				

Table 13: Effects of 53786615 T/A Variants on Study Parameters (Non-Normally Distributed).

Parameters	**53786615 T/A mutation**	**Median±IQR**	**Frequency**	**P value**
BMI	Mutant	29.6±10.1	5	**0.30**
	Non-mutant	32.7±5.7	31	
HbA1c	Mutant	6.7±2.6	5	**0.79**
	Non-mutant	7.1±3.9	31	
FBS	Mutant	145.6±76.0	5	**0.79**
	Non-mutant	157.1±112.0	31	
CRP	Mutant	1.4±4.5	5	**0.16**
	Non-mutant	3.0±2.7	31	
The data presented in the table as means: ±IQR the interquartile range, p≤0.05 is considered statistically significant, BMI refers to body mass index, HbA1c hemoglobin A1c, FBS fasting blood sugar, CRP c-reactive protein				

Discussion

Obesity raises the risk of several common diseases, making it an important global health concern. It's unclear whether hereditary factors contribute to obesity. A common mutation in the *FTO* (fat mass and obesity associated) gene, which predisposes to diabetes through an influence on body mass index, was found during a genome-wide search for genes linked to type 2 diabetes susceptibility(Frayling et al., 2007). A typically elevated triglyceride deposition and the generation of hepatic glucose can result from enhanced *FTO* expression, which can also promote de novo lipogenesis, decrease lipolysis and fatty acid oxidation, and boost gluconeogenesis(Witka et al., 2019). These results imply that *FTO* is connected to the regulation of both body weight and glucose metabolism. While there is no doubt that variations in the *FTO* gene are linked to type 2 diabetes and obesity, the biological role of *FTO* remains unclear(Gerken et al., 2007; Han et al., 2010). Finally, we believe that the results of this study, particularly the four variants in the *FTO* gene's *FTO* 1 regions (53769662 T/A), *FTO* 2 regions (53782363 C/A) and *FTO* 3 regions (53786591 G/A and 53786615 T/A) could be significant in the field of *FTO* gene studies. The presence of these variants in important coding regions suggests their potential importance.

Furthermore, among four variants, two variants (53769662 T/A and 53782363 C/A) might be the most significant, as they exhibited a significant effect on certain study parameters. Where the variant 53769662 T/A showed a significant effect on the level of cholesterol in the blood, the variant 53782363 C/A showed a significant effect on the serum levels of FIB and CRP. To precisely identify their role in Type 2 Diabetes Mellitus patients, further studies are needed in future research. It must be noted that the study results were limited by the relatively small sample size of the patients and controls, suggesting the need for large-scale studies to corroborate the results and validate the findings.

Ethics approval and consent to participate

The Institutional Ethics Committee in the Department of Clinical Laboratories / College of Applied Medical Sciences/ University of Kerbala approved this study (IQ.UOK.CAMS.DCL.REC.2). Informed consent was taken from every patient in their language regarding willingness to participate in the study. Patient confidentiality was maintained during all research procedures.

Author contributions

Both authors have contributed to the writing and approved the manuscript before submission.

Conflicts of interest

There are no conflicts of interest.

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