Association Between Type 2 Diabetes Mellitus and TCF7L2 And FTO Gene Variants Among Upper Egyptian Population

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ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) is a metabolic disorder caused by a complex interaction of genetic and environmental variables. T2DM is associated with transcription factor 7-like 2 (TCF7L2) and fat mass and obesity-associated (FTO) genetic polymorphism.

Objectives: The goal of this study was to examine the common genetic risk factors of T2DM and related metabolic traits in Upper Egyptian population, in attempt to understand the genetic structure of T2DM in the Egyptian community.

Methods and Materials: This case control study included 250 participants, 124 T2DM patients and 126 non-diabetics. Using mutagenically separated polymerase chain reaction (MS-PCR), genotyping of single nucleotide polymorphisms (SNP) rs7903146 of TCF7L2 and rs17817449 of FTO genes was carried out.

Results: T allele of TCF7L2 variant rs7903146 confers a risk for T2DM (allelic OR=1.97, 95% CI: (1.34 to 2.88) p =<0.001). The minor G allele of FTO rs17817449 polymorphism was significantly higher in diabetics than controls (allelic OR=1.87, 95% CI=1.30 to 2.68, p<0.001). Genotype risk was evident under both recessive and dominant modes of inheritance (OR=3.18, CI (1.35-7.45), P =0.008, OR= 2.04, CI (1.23-3.38), p=0.006) for TCF7L2 and (OR= 2.55, CI (1.28 -5.09), p=0.008 and OR= 2.14, CI (1.25-3.63), p= 0.005) for FTO respectively

Conclusion: TCF7L2 rs7903146 and FTO rs17817449 variant conferred risk for T2DM in Upper Egyptian population. The study noted the interaction between certain biological and environmental risk factors including BMI, age, and sex and the conferred genetic risk.

Keywords: FTO gene, Obesity, T2DM, TF7L2 gene.

INTRODUCTION

Diabetes is a class of metabolic diseases characterized by hyperglycemia believed to be associated with insulin secretion, insulin action, or both. T2DM persistent hyperglycemia has been related to long-term organ damage and failure [1]. Type 2 diabetes is a common type of diabetes, accounting for over 90% of cases and affecting 10–20% of individuals over 50 years of age in many developed countries [2].

TCF7L2 is a transcription factor with an evolutionarily conserved sequence that is a crucial element of the Wnt signaling cascade [3]. It regulates the expression of genes involved with lipid and glucose metabolism, acting as a main transcription factor of the adipocyte metabolic pathway [4]. The rs7903146 mutation of the TCF7L2 gene is considerably associated with the risk of T2DM, with such a pooled odds ratio of 1.46. TCF7L2 does have an influence on T2D, even though the methods by which it does so are currently unclear [5].

Obesity is aided by fat-mass and obesity-associated gene (FTO) proteins, that also enhance energy intake, gluconeogenesis, adipogenesis, and mitochondrial oxidative phosphorylation [6]. FTO controls energy storage and expenditures by turning on and off different genes (e.g., IRX3 and IRX5) that can lead the cell to store or burn energy [7]. Polymorphisms in the FTO gene have now been caused by obesity in numerous studies, and obesity is a major risk factor for type 2 diabetes (T2DM) [8].

The present study aimed to demonstrate whether T, and C alleles of TCF7L2 SNP rs7903146, and FTO SNP rs17817449 T/G are risk variants for T2DM in population of Upper Egypt and the impact of these SNPs on the glycemic control, HBA1c, fasting blood glucose, insulin level and lipid profile.

SUBJECTS AND METHODS
a. Subjects:

This is a case control study that included a total of 250 subjects, 124 cases and 126 controls. They were recruited from patients attending Assiut University Hospital.

Medical history was taken, and physical examination was carried out by specialized physicians. Demographic, anthropometric and physical characteristics were collected including age, sex, family history, body weight, height, and BMI, systolic and diastolic blood pressure. For diabetics, duration of

diabetes, age of onset, type and duration of treatment and presence or absence of complications were assessed.

Subjects with type 1 diabetes, prediabetics, and other endocrine dysfunctions were excluded. Also, individuals with chronic liver disease, renal impairment unrelated to diabetes, malignancy, autoimmune diseases, or chronic inflammatory disease were excluded from the study.

Biochemical analysis

Standard assays were used to measure fasting blood glucose level, urea, creatinine, calculate creatinine clearance and lipid profile. Fasting insulin was determined by ELISA kit supplied by DRG international instruments Inc., Germany. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated (Fasting insulin (μIU/mL) X fasting glucose (mmol/L)/22.5

b.   Genotyping (Figures 1 and 2)

In this study, genotyping of single nucleotide polymorphisms (SNP) rs7903146 of TCF7L2 and rs17817449 of FTO genes was carried out using mutagenically separated polymerase chain reaction (MS-PCR). Genomic DNA was isolated from peripheral whole blood collected on EDTA using QIAamp DNA mini kit (Qiagen) according to the manufacturer's instructions. The three-primer method was used for genotyping the

TCF7L2- rs7903146, C>T polymorphism. The four-primer method was used for genotyping of the FTO rs T>G polymorphism. Genotyping was done in a 25 ul reaction volume using 12.5 ul of Taq polymerase 2X mater mix (Qiagen), additional 1ul of 50 mM MgCl, 400 pmol of common, 200 pmol of the allele specific primers and 20 ng of genomic DNA.

The primers used for detection of genetic polymorphism included:

TCF7L2 rs7903146 (C>T)
-      **Common forward primer /TCF146F:** 5'-AAAGGGAGAAAGCAGGATTG-3'
-      **C allele reverse primer /TCF146RC:** 5'-GCCTCATACGGCAATTAAATTATCAG-3'
-      **T allele reverse primer/TCF146RT:** 5'-CACTAAGGACGCAATGATTGTGCCTCATA CGGCAATTAAATTATGAA-3'

FTO rs17817449 (T/G)
-      **Common forward primer/ FTO449F:** 5'- TACATTTACTCAAGAGTTTGTCTTTTCT- 3'
-      **Common reverse primer/FTO449R:** 5'- TATTCAGATGAGTTACACTAAAAGCTG -3'
-      **T allele reverse primer/FTO449TR:** 5'-GAGCTGGACTGTTAAATTAAATCA -3'
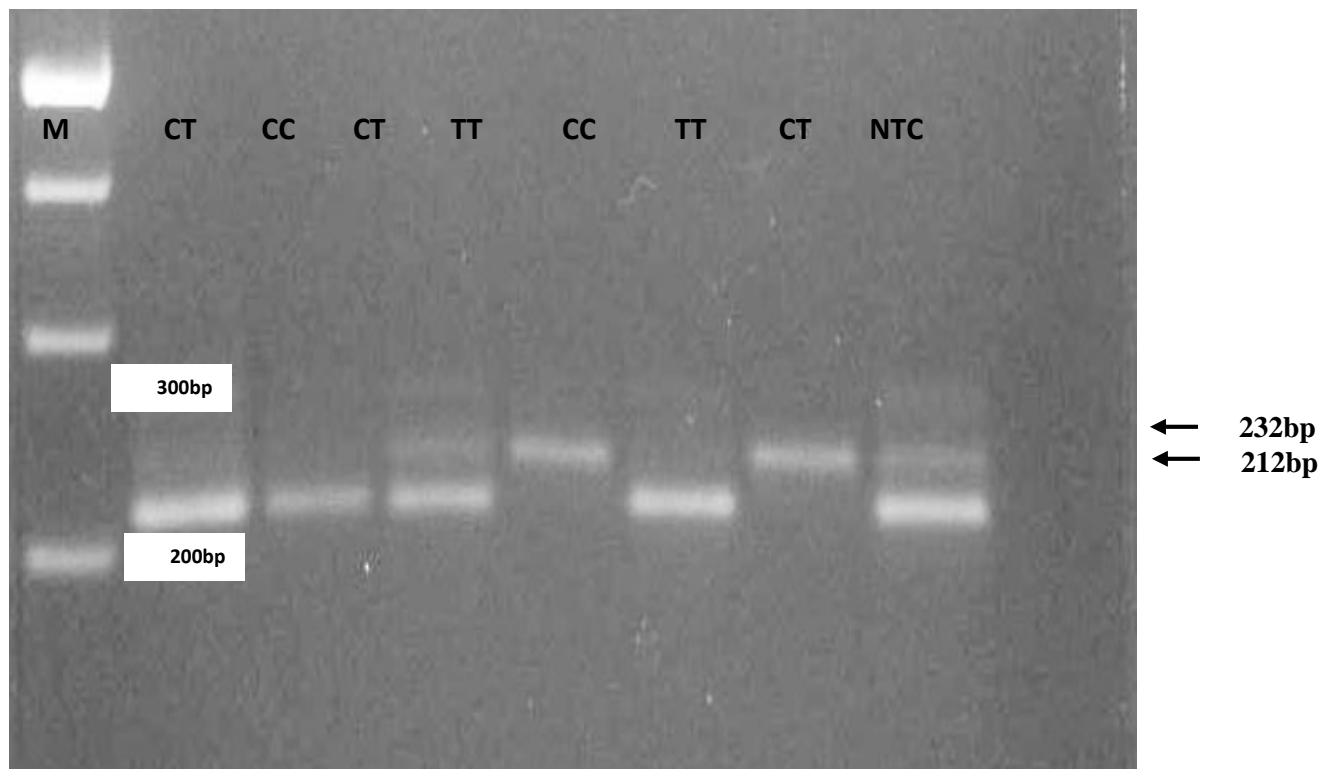-   **G allele Forward primer/FTO449GF:** 5'- AGCTTGGCACACAGAAATG-3'

Figure (1): Electophoresis banding pattern for SNP rs7903146: MS-PCR for rs79023146 C>T polymorphism, showing the T allele at 232-bp and the C allele at 212-bp.

Figure 2: Tetra-MS PCR for the detection of FTO rs17817449 T>G genotypes: MS-PCR for **rs17817449 T>G** polymorphism. M: 100 bp DNA marker, 180 bp T allele, 280 bp for G allele, and 420 bp for control. NTC is the no template control.

Ethical Approval:

The study was approved by the Ethics Board of Assiut University and an informed written consent was taken from each participant in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

c.   Statistical analysis

For statistical analysis, SPSS (version 20, SPSS, Inc., Chicago, IL, USA) was used. Continuous quantitative variables were expressed as the mean ± SD and median (range) and categorical qualitative variables were expressed as absolute frequencies (number) and relative frequencies (percentage). The chi-square (X^2) test was used to examine the genotypes and allele distributions of diabetics and controls, as well as the binary logistic regression for calculation of odds ratio (OR) and 95% confidence intervals (CI).

For two group comparison, Student t test; Mann Whitney test were used for parametric and non-parametric data respectively. Also, ANOVA, and Kruskal Wallis were applied for three group comparisons for parametric and non-parametric data respectively. To see if the observed genotype frequencies for the examined SNP are consistent with Hardy-Weinberg equilibrium, a Hardy-Weinberg equation was utilized. $P<0.05$ was considered significant.

RESULTS

1. Baseline characteristic

The present study recruited 250 subjects included 124 diabetic patients and 126 controls. The demographic, anthropometric, biochemical and clinical characteristics of patients and controls are presented in table 1, Fasting insulin and HOMA-IR were measured in controls only to avoid the effect of disease and treatment on their values.

Table (1): Demographic, anthropometric, biochemical and clinical characteristics of diabetic subjects and controls

Variables	Controls (n=126)	Diabetic patients (n=124)	P value
Age (years) Mean ± SD	53.15 ± 8.45	57.37 ± 6.70	<0.001
Sex Number(percentage)	F = 42 (33.3%) M= 84 (66.7%)	F = 49 (39.5%) M = 75 (60.5%)	0.310
Family history +ve -ve Number(percentage)	NA	71 (57.3%) 53 (42.7%)	
Body weight (kg) Mean ± SD	69.6 ± 16.7	76.6 ± 16.6	0.001
Height (cm) Mean ± SD	168.80 ± 8.9	167.90 ± 9. 6	0.443
BMI (kg/m²) Mean ± SD	24.40 ± 5.9	27.24 ± 6.29	<0.001
Obesity state Non obese Obese Number(percentage)	101 (80.2%) 25 (19.8%)	81 (65.3%) 43 (34.7%)	0.008
Blood pressure (mmHg) Systolic Diastolic Mean ± SD	119.92 ± 12.09 74.36 ± 9.67	124.51 ± 17.95 76.61 ± 10.96	0.02 0.09
Fasting blood sugar (mg/dl) Median(range)	90 (66-110)	154 (56-270)	<0.001
Fasting insulin (m IU/L) Median(range)	8.40 (3-33)	-	
HOMA-IR (normal range 0.5-1.4) Median(range)	2.28 (1-12)	-	
Triglycerides (mg/dl) Median(range)	91.5 (54-275)	101 (40-817)	0.005
Cholesterol (mg/dl) Mean ± SD	145.94 ± 37.31	168.67 ± 38.17	<0.001
HDL- cholesterol (mg/dl) Median(range)	35 (10-77)	33.5 (7-65)	0.13
LDL- cholesterol (mg/dl) Median(range)	83 (29-205)	109.4 (50-181)	<0.001
UREA (mmol/L) Median(range)	5.6 (1.40-77)	6.9 (1.20-112)	0.03
CREA (umol/L) Median(range)	81.3 (7.60-915)	84.9 (8.8-1426)	0.06

Data are presented as mean ± SD for parametric tests, median (range) for non-parametric tests, or number and percentage for qualitative data, **BMI** for body mass index, **HDL** for high density lipoprotein, **LDL** for low density lipoprotein, **CREA** for creatinine,

2. Association of TCF7L2 and FTO genes polymorphism and type 2 diabetes:

Genotype frequencies were in Hardy Weinberg equilibrium in the control group (p>0.05).

The genotype and allelic distribution of TCF7L2 rs7903146 and FTO SNP rs17817449 variants are shown in table 2. In the current study sample, the TT genotype of TCF7L2 variant rs7903146 was strongly related with T2DM. After adjusting for BMI alone or age, sex, and BMI together, the association remained significant. While a single copy of the T allele raises the risk of diabetes in the dominant

model of inheritance, comparing CT + TT genotypes to CC genotypes TT genotype tripled the risk of diabetes in the recessive model of inheritance.

In the mean-time, the minor G allele of FTO rs17817449 polymorphism was significantly higher in diabetics than controls. The association with T2DM was observed under additive, and dominant models of inheritance but the highest was observed with the recessive mode of inheritance. Interestingly the association was markedly attenuated after adjustment for BMI alone or BMI, age and sex together.

Table (2): Genotyping and alleles frequencies distribution of TCF7L2 SNP rs7903146 C>T and FTO SNP rs17817449 T/G in controls and diabetic patients

	Controls (n=126)	Diabetics (n=124)	OR (95%CI) P value	OR (95%CI)[1] P value	OR (95%CI)[2] P value
TCF7L2 SNP rs7903146 C>T					
CC **CT** **TT**	72 (57.1%) 46 (36.5%) 8 (6.3%)	49 (39.5%) 53 (42.7%) 22 (17.7%)	1.89 (1.29-2.76) 0.001	1.88 (1.27-2.76) 0.002	1.94 (1.29-2.89) 0.001
Recessive **CC+CT** **TT**	118 (93.7%) 8 (6.3%)	102 (82.3%) 22 (17.7%)	3.18 (1.35-7.45) 0.008	3.25 (1.36-7.75) 0.008	3.60 (1.46-8.87) 0.005
Dominant **CC** **CT+TT**	72 (57.1%) 54 (42.9%)	49 (39.5%) 75 (60.5%)	2.04 (1.23-3.38) 0.006	1.99 (1.19-3.35) 0.009	2.03 (1.19-3.48) 0.010
Allelic **C** **T**	190 (75%) 62 (25%)	151 (61%) 97 (39%)	1.97 (1.34 to 2.88) <0.001		
FTO gene SNP rs17817449 T/G					
TT **TG** **GG**	55 (43.7%) 57 (45.2%) 14 (11.1%)	33 (26.6%) 61 (49.2%) 30 (24.2%)	1.87 (1.29 -2.70) 0.001	1.67 (1.14 -2.44) 0.008	1.66 (1.12-2.47) 0.012
Recessive **TT+TG** **GG**	112 (88.9%) 14 (11.1%)	94 (75.8%) 30 (24.2%)	2.55 (1.28 -5.09) 0.008	2.14 (1.05 -4.36) 0.036	2.11 (1.01 -4.39) 0.047
Dominant **TT** **TG+GG**	55 (43.7%) 71 (56.3%)	33 (26.6%) 91 (73.4%)	2.14 (1.25-3.63) 0.005	1.84 (1.07 - 3.19) 0.028	1.84 (1.04 -3.25) 0.037
Allelic **T** **G**	167 (66%) 85 (34%)	127 (51%) 121 (49%)	1.87 1.30 to 2.68 <0.001		

OR= odds ratio, CI = confidence interval, OR (95%CI)[1] adjusted for BMI. OR (95%CI)[2] adjusted for BMI, age, sex.

3. Association of TCF7L2 and FTO genes polymorphism with diabetic traits and complication in diabetic subjects.

In the diabetic group, the age at onset tend to be lowered from the homozygous genotype of the common allele (CC), over the heterozygous genotype (CT), to the homozygous genotype (TT). Although carrier of the T allele showed high frequency of diabetic complications in CC, CT, TT genotypes respectively, this does not attain statistical significance. TT variants of TCF7L2 SNP rs7903146 showed higher levels of triglycerides and LDL. There were no significant differences between the three genotypes regarding family history, blood pressure measures, BMI, FBS, and HBA1c. As regard FTO, the minor genotype GG was associated with family history, and lower levels of HDL.

Both fasting blood sugar, and HBA1c tended to be higher in TG and GG genotypes compared to TT genotype. None of the other investigated traits including body weight, BMI age at onset, frequency of complication, total cholesterol, triglycerides, or LDL were associated with the FTO rs17817449 genotype distribution among diabetic subjects. Data are shown in table 3

Table (3): The relationship between different genotype variants of TCF7L2 SNP rs7903146 C>T and FTO SNP s17817449 T/G and the anthropometric, biochemical and clinical data in diabetic subjects

TCF7L2 SNP rs7903146 C>T				
Variables	CC (N=49)	CT (N =53)	TT (N=22)	P value
Family history **+ ve** **- ve** Number (percentage)	31 (63%) 18 (37%)	29 (54%) 24 (46 %)	11 (50%) 11 (50%)	0.513
Age of onset (Mean ± SD)	52.224 ± 7.837	50.83 ± 7.757	47.454 ± 9.184	0.074
Complications **Non complicated** **Complicated** Number (percentage)	39 (79%) 10 (21%)	35 (66%) 18 (34%)	15 (68%) 7 (32%)	0.289
Body weight (kg) Mean ± SD	77.53 ± 17.32	75.94 ± 15.55	75.86 ± 17.85	0.87
Height (cm) Mean ± SD	168.61 ± 9.55	167.28 ± 9.82	167.81 ± 9.29	0.78
BMI (kg/m²) Mean ± SD	27.31 ± 6.10	27.28 ± 6.32	27.03 ± 6.92	0.98
Systolic Blood pressure (mm Hg) Mean ± SD	127.14 ± 18.14	123.01 ± 16.002	122.27 ± 21.80	0.42
Diastolic Blood pressure (mm Hg) Mean ± SD	78.77 ± 11.11	74.71 ± 10.115	76.36 ± 12.16	0.17
Fasting blood sugar (mg/dl) Median(range)	146 (67-265)	167 (56-270)	154 (87-256)	0.286
HbA1C (Mean ± SD)	7.051 ± 1.203	7.407 ± 1.377	7.275 ± 1.351	0.38
Cholesterol (mg/dl) Mean ± SD	163.10 ± 39.23	175.754 ± 35.397	164.045 ± 41.179	0.20
Triglycerides (mg/dl) Median(range)	96 (40-344)	100 (43-466)	119 (47-817)	0.19
HDL-Cholesterol (mg/dl) Median(range)	34 (9-65)	34 (18-49)	30 (7-65)	0.56
LDL-Cholesterol (mg/dl) Median(range)	106.6 (50-180)	118.8 (56-181)	87.8 (60-147)	0.01 [$]
FTO SNP s17817449 T/G				
Variables	TT (N=33)	TG (N=61)	GG (N=30)	P value
Family history **+ ve** **- ve** Number(percentage)	21 (63.6%) 12 (36.4%)	40 (65.6%) 21 (34.4%)	10 (33.3%) 20 (66.7%)	0.01
Age at onset Mean ± SD	51.81 ±8.22	50.09 ± 8.13	51.03 ±8.28	0.61
Complications Number(percentage)	+ ve = 10 (8.06%) - ve = 23 (18.54%)	+ ve = 15 (12.09%) - ve = 46 (37.09%)	+ ve = 10 (8.06%) - ve = 20 (16.12%)	0.652
Body weight (kg) Mean ± SD	74.33 ± 18.85	76.21 ± 15.70	79.70 ± 15.66	0.43
Height (cm) Mean ± SD	167.51 ± 10.80	168.00 ±9.54	168.13 ±8.40	0.96
BMI (kg/m²) Mean ± SD	26.43 ± 6.56	27.12 ± 6.08	28.39 ±6.47	0.46
Systolic Blood pressure (mm Hg) Mean ± SD	126.36 ± 20.73	123.27 ± 17.19	125.00 ±16.55	0.72
Diastolic Blood pressure (mm Hg) Mean ± SD	77.87 ± 12.18	76.39 ± 10.95	75.66 ± 9.71	0.71
Fasting blood sugar (mg/dl) Median(range)	148.5 (67-235)	151 (56-260)	170 (90-270)	0.172
HbA1c (%) Mean ± SD	7.17 ± 1.118	7.13 ± 1.41	7.53 ± 1.26	0.38
Cholesterol (mg/dl) Mean ± SD	169.36 ± 40.17	168.47 ± 39.18	168.33 ± 34.98	0.99
Triglycerides (mg/dl) Median(range)	102 (64-308)	102 (40-817)	99.5 (47-472)	0.87
HDL-Cholesterol (mg/dl) Median(range)	38 (9-65)	33 (18-49)	30.5 (7-59)	0.007 [$$]
LDL-Cholesterol (mg/dl) Median(range)	98 (65-180)	107.8 (57-181)	112.7 (50-166)	0.54

Data are presented as mean ± SD for parametric tests, median (range) for non-parametric tests, or number and percentage for qualitative data.

BMI for body mass index, **HA1c** for glycosylated hemoglobin, **HDL** for high density lipoprotein, **LDL** for low density lipoprotein,

[$] p value for post hoc test CT vs TT groups,

[$$] P value for post hoc test TT vs GG groups,

4. **Association of TCF7L2 and FTO genes polymorphism anthropometric, biochemical and clinical risk factors for type 2 diabetes in controls.**

As shown in table 4, in the control groups, the risk T allele of the TCF7L2 rs7903146 was associated with higher fasting blood sugar, fasting insulin, and higher HOMA-IR indices but did not attain statistical significance. In contrast to diabetic group, body weight, BMI of the control subjects was found to be higher in FTO rs1781744 GG, and GT genotypes compared to TT genotype. Also, FBS and insulin sensitivity parameters (fasting insulin, HOMA-IR) tended to be higher in GG genotype compared to GT and TT genotypes, yet statistically not significant. None of the other variables including blood pressure, lipid profile was defined by the genotype frequency in the control group.

Table (4): The relationship between different genotype variants of TCF7L2 SNP rs7903146 C>T and FTO SNP rs17817449 T/G and the anthropometric, biochemical and clinical data in control subjects

TCF7L2 SNP rs7903146 C>T				
Variables	CC (N=72)	CT (N=46)	TT (N=8)	P value
Body weight (kg) Mean ± SD	68.43 ± 17.154	71.826 ± 16.186	67 ± 15.306	0.51
Height (cm) Mean ± SD	168.319 ± 8.929	169.934 ± 9.136	166.625 ±7.170	0.49
BMI Mean ± SD	24.099 ± 5.842	24.922 ± 6.029	24.172 ± 5.790	0.76
Systolic Blood pressure (mm Hg) Mean ± SD	119.722 ± 11.129	119.347 ± 12.184	125 ± 19.272	0.46
Diastolic Blood pressure (mm Hg) Mean ± SD	74.722 ± 9.782	74.347 ± 10.033	71.25 ± 6.408	0.63
Fasting blood sugar (mg/dl) Median(range)	90 (72-110)	93 (66-110)	89 (78-110)	0.586
Fasting insulin (m IU/L) Median(range)	7.20 (3-33)	9.53 (3-33)	16 (10-20)	0.07
HOMA-IR Median(range)	1.57 (1-12)	2.54 (1-8)	3.85 (3-8)	0.04 [#]
Cholesterol (mg/dl) Median(range)	138 (66-269)	139.5 (97-254)	138 (110-244)	0.74
Triglycerides (mg/dl) Median(range)	90.5 (65-275)	93 (54-185)	90.5 (83-193)	0.72
HDL-Cholesterol (mg/dl) Median(range)	34.5 (10-50)	36.5 (20-77)	35 (25-40)	0.58
LDL-Cholesterol (mg/dl) Median(range)	82.4 (29-205)	86.6 (46-193)	86 (69-180)	0.60
FTO SNP s17817449 T/G				
Variables	TT (N=55)	GT (N=57)	GG (N=14)	P value
Body weight (kg) Mean ± SD	65.01 ± 15.097	71.64 ± 17.04	79.071 ±16.37	0.008 [$]
Height (cm)	168.12 ± 8.19	169.52 ± 8.98	168.50 ±11.40	0.70
BMI (kg/m²) Mean ± SD	22.86 ± 4.53	24.97 ± 6.30	28.12± 6.95	0.006 0.009 [$$]
Systolic Blood pressure (mm Hg) Mean ± SD	117.63 ± 11.04	122.10 ± 12.78	120.00 ± 12.40	0.15
Diastolic Blood pressure (mm Hg) Mean ± SD	73.63 ± 8.01	74.73 ± 10.708	75.71 ± 11.578	0.72
Fasting blood sugar Median(range)	87 (72-110)	95 (66-110)	95 (78-110)	0.07
Fasting insulin (m IU/L) Median(range)	8.7 (3-25)	7.45 (3-30)	13 (3-33)	0.47
HOMA-IR Median(range)	2.26 (1-12)	1.83 (1-8)	3.4 (1-7)	0.18
Cholesterol (mg/dl) Median(range)	133 (66-269)	140 (78-250)	150 (100-218)	0.296
Triglycerides (mg/dl) Median(range)	91 (68-275)	93 (54-251)	92 (68-194)	0.84
HDL-Cholesterol (mg/dl) Median(range)	35 (17-48)	35 (10-77)	36 (18-42)	0.70
LDL-Cholesterol (mg/dl) Median(range)	80 (29-205)	86.6 (42-193)	90.8 (51-160)	0.41

Data are presented as mean ± SD for parametric tests, median (range) for non-parametric tests, or number and percentage for qualitative data. **BMI** for body mass index, **HDL** for high density lipoprotein, **LDL** for low density lipoprotein,
[#] post hoc for this parameter is non-significant between the three groups
[$] p value for post hoc test TT vs GG groups, [$$] p value for post hoc test TT vs GG groups,

DISCUSSION

Type 2 diabetes mellitus (T2DM) is a complex genetic disorder in terms of interaction between environmental effects, lifestyle factors and genetic variants [9].

The present study tested the impact of TCF7L2 SNP rs7903146 and FTO SNP rs17817449 on the risk of T2DM in a cohort of Egyptians recruited from Upper Egypt. It was found that TCF7L2 SNP rs7903146 is significantly associated with T2DM, (OR=1.89, 95% CI: (1.29-2.76). The effect was observed when testing additive, dominant and recessive modes of inheritance. However, the recessive mode of inheritance comparing TT carrier to both CC and CT carriers conferred more susceptibility to T2DM, (OR=3.18 recessive, 95% CI: (1.35-7.45). An adjusted OR for age, sex and BMI well known diabetic co-founders was carried out and the risk for T2DM attributed to TCF7L2 SNP rs7903146 remained significant.

These results are consistent with those reported by **Grant et al.** [10] in 2006 as well as numerous articles that followed in Europeans, West Africans, Mexican, African Americans, Indians, and Japanese populations. The rs7903146 variant was also reported as risk for T2D T2DM in population from North African population from Tunisia by **Turki et al.** [11], and Algeria by **Ouhaibi et al.** [12]. It has also been reported in other Arab and other Middle Eastern population from; Turkey [13], Sudan, [14] and Emirate [15]. In a recent study that included 180 diabetics and 210 controls from Delta and Cairo, Egypt, the authors noted the same findings in terms of MAF and the effect size as our data. However, **Alsmadi et al.** [16] found no correlation among TCF7L2 variations and type 2 diabetes mellitus in an Arab population of Saudi origin in a study of TCF7L2 variants in those population upon analysis of two SNPs (including rs7903146). This could be explained by the genetic diversities between Caucasians, Asians, and Africans.

Multiple underlying mechanisms implicated TCF7L2 variants in the pathogenesis of T2DM. **Gaulton et al.** [17] reported that, in human islets TCF7L2 locus is more open in chromosomes carrying the rs7903146 T allele suggesting it is transcriptionally more active. It was found that rs7903146 T allele carriers higher levels of TCF7L2 mRNA in human pancreatic islets, and that was related to impaired insulin secretion and incretin effects. In that regard a putative TCF-binding motif within the promoter region of the gene encoding glucagon-like peptide 1 was found suggested that TCF7L2 was capable of regulating the expression of GP-1 from the gut endocrine L cells and is involved in glucose homeostasis. Moreover, T allele carriers had enhanced rates of hepatic gluconeogenesis [18].

T allele of the rs7903146 was not linked with impaired HOMA-IR or fasting insulin secretion in the control group. This contradicts **Damcott et al.** [19] but it agrees with **Saadi et al.** on insulin sensitivity [20].

Furthermore, TCF7L2 risk TT genotypes showed lower age of onset of diabetes although p value was only suggestive. This was in line with **Saxena et al.** [21] but contrary to the study carried out by **Silbernagel et al.** [22] that included 1021 diabetic subjects and reported early age at onset with T allele of rs7903146. Also, the frequency of diabetic complications (diabetic nephropathy, retinopathy, or peripheral vascular disease), and the response to treatment measured by HBA1c were equally distributed between the wild type homozygous CC, heterozygous CT and the minor genotype TT. In contrast to these findings, **Rattanatham et al.** [23] identified T allele as a risk factor for diabetic complications such as diabetic nephropathy and cardiovascular problems. In diabetics with the TT genotype, LDL cholesterol was surprisingly lower and triglycerides were greater. Similarly, rs12255372, an SNP in perfect linkage disequilibrium with rs7903146, was linked to low LDL in an American Mexican population [24] and high TG in a Cameroonian community.

The genotypes and allele frequencies distribution of the FTO rs17817449 T/G in Egyptians were found to confer increased risk of T2DM, an association that was attenuated after adjustment for BMI alone or BMI combined with age and sex, with (G) being the high risk allele and (T) being the low risk allele. This is consistent with the findings of **Hertel et al.** [25] and **Shaikh et al.** [26] in East and South Asian populations that the link of FTO with type 2 diabetes is partly dependent on its effect on BMI. These findings were also consistent with those found in the European population, suggesting that FTO is substantially linked to T2DM, but that this link was lost once BMI was taken into account.

In fact, FTO mRNA levels are linked to the expression of genes involved in gluconeogenesis in the liver. Moreover, adenoviral overexpression of FTO increases basal protein kinase B phosphorylation, enhances lipogenesis and oxidative stress, and reduces mitochondrial oxidative function, a cluster of metabolic defects linked to type 2 diabetes [27].

Carriers of the minor genotype GG of the FTO SNP rs17817449 were less likely than GT or TT genotypes to have a family history of diabetes. These findings contradict with those introduced by **Sabarneh et al.** in Palestinians [28]. In the control group, it was apparent that GG carriers had a higher BMI than the GT and TT genotypes. These findings are consistent with those of **Khella et al.** [29], who studied Egyptian communities, and **Saber-Ayad et al.**, who studied Emirati populations [30]. Furthermore, these findings are consistent with previous research that suggests the FTO gene has a role in body weight management since it is highly expressed in the

hypothalamus, where it is impacted by appetite and hunger and regulated by the hypothalamus[31].

CONCLUSION

In conclusion, this study demonstrated the conferred risk by the genetic variants TCF7L2 (rs7903146) and FTO rs17817449 on the risk of T2DM in Upper Egyptian population. Such an effect was modified by interaction between the genetic risk factors and other biological traits like BMI, age, and sex. The study also demonstrated the effect of these genetic variants on certain metabolic traits like HOM-IR, TG, LDL-C and HDL-C.

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