Diabetes Ther (2020) 11:2145–2157
https://doi.org/10.1007/s13300-020-00896-w

ORIGINAL RESEARCH

Role of FTO and MC4R Polymorphisms in Escalating Obesity and Their Indirect Association With Risk of T2D in Indian Population

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Received: June 3, 2020 / Published online: August 1, 2020

ABSTRACT

Introduction: Obesity plays a pivotal role in the development of metabolic syndrome—excessive body fat, spikes in blood glucose levels and hypertension—and ultimately leads to cardiovascular diseases and type 2 diabetes (T2D), if left unattended. The present study aimed to investigate the associated risk of T2D with obesity risk alleles of fat mass and obesity-associated (FTO) and melanocortin 4 receptor (MC4R) genes.

Methods: The study includes 400 subjects (300 T2D diabetic cases and 100 healthy controls). Genetic analysis was done by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods.

Results: The findings of the study show no significant increase in odds of diabetes associated with the prevalence of FTO and MC4R minor alleles. Rare allele frequencies for "A" of FTO rs9939609 were 0.34 and 0.30 in cases and controls, respectively. Rare allele frequencies for A of MC4R rs12970134 were found to be more common in controls (0.45) than cases (0.41), but the difference was insignificant (p 0.246); however, an increase in body weight with the presence of allele "A" of the FTO gene (p value < 0.001) was found, indicating indirect involvement in the development of T2D. In addition, these were also correlated with the demographic/lifestyle and clinico-pathological parameters between T2D cases and controls. We found that T2D patients with a history of smoking and high consumption of alcohol, fast foods and sweetened beverages are at high risk of T2D compared to healthy controls ($p < 0.01$*).

Conclusion: The present study concludes that there is no direct association of rs9939609 of the FTO gene with the occurrence of diabetes in the Indian population, but its role in T2D development cannot be overlooked altogether.

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Digital Features To view digital features for this article go to https://doi.org/10.6084/m9.figshare.12706535.

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Furthermore, we conclude that the rs9939609 of FTO carries a potential risk of obesity and because of this FTO rs9939609 T > A is widely considered an obesity-associated allele/single-nucleotide polymorphism (SNP).

Keywords: FTO; Obesity; MC4R; RFLP; SNP; Type 2 diabetes

Key Summary Points
Genotype, environmental factors and ethnicities cause phenotypic alterations.
Genetic alterations in the fat mass and obesity-associated FTO are associated with increased risk of obesity.
Single-nucleotide polymorphism (SNP) analysis showed no direct involvement of FTO and melanocortin 4 receptor (MC4R) polymorphism with T2D in an Indian population.

INTRODUCTION

Obesity, a global phenomenon, is also known as corpulence or fatness. This leads to excessive accumulation of body fat, usually due to higher intake of calories than utilisation by the body. However, many other risk factors are involved in the development of T2D, including a sedentary lifestyle [1], lack of exercise, dysregulation of the appetite hormone (leptin) and glucose metabolism hormone (insulin) [2], genetic factors [3], environmental factors, smoking [4] and excessive alcohol consumption. Excess glucose/calories are stored as fat or adipose tissue in the belly contributing to obesity. Sedentary lifestyles and advanced food production technologies add to the problem, as people prefer off-the-shelf processed food. Thus, in the past 2 decades, the pandemic of obesity in the world has been an invited guest [5].

Obesity plays a pivotal role in the development of metabolic syndrome—excessive body fat, spikes in blood glucose levels and hypertension—ultimately leading to cardiovascular diseases and type 2 diabetes (T2D) as people get older, if left unattended [6, 7]. T2D alone accounts for about 90% of the cases worldwide [8–11]. In the last decade, advances in SNP genotyping technologies have facilitated genome-wide association studies (GWAS) to determine various risk loci/SNPs associated with increased risk of obesity and T2D [12]. The FTO and MC4R genes are known to play a pivotal central role in energy regulation and appetite, leading to better management of obesity [13, 14]. The FTO and MC4R genes are located on chromosomes 16q12.2 and 18q21.3, respectively, which uphold cell physiology by maintaining different biochemical features in the body. The important obesity-associated risk alleles (ORAs) FTO rs9939609A and MC4R rs12970134A are often studied in different ethnic groups and populations across the globe. These are linked to a greater risk of obesity, higher body mass index (BMI) and T2D in the UK [15–18], Asia [16, 19, 20], Palestine [21], Iran [22], Italy [23], Japan [24] and Arabic countries [25]. While the developing countries account for about three-fourths of the total T2D population, India and China alone have one-third of the world's diabetic population [26]. Therefore, considering these facts, the current case-control study aimed to evaluate the independent and combined association of the FTO rs9939609A and MC4R rs12970134A ORAs with obesity and onset of T2D in an Indian population. Furthermore, we correlated various clinico-pathological parameters and demographic characteristics to understand the implications for T2D onset. The findings of our study may help to understand the underlying genetic variations in these genes for better management of obesity and reduced risk of T2D.

METHODS

Study Design and Sample Collection

The present case-control study was conducted at the Medical Biotechnology Laboratory, Department of Biotechnology, Jamia Millia

Islamia, New Delhi, India. In this study, 400 subjects (300 newly diagnosed diabetic and 100 non-diabetic) were included after due ethical clearance from the institute's ethics committee. The cases were selected according to the inclusion and exclusion criteria; samples were collected after obtaining consent from cases as well as controls and relevant case history information was collected through a standardised pretexted questionnaire, which was maintained in the database throughout the course of the study. Blood samples (3 ml) were taken, of which 1 ml was collected in ethylenediaminetetraacetic acid (EDTA) vials for deoxyribonucleic acid (DNA) isolation and 2 ml in plain vials for biochemical parameter analyses: fasting plasma glucose (FPG), postprandial glucose (PPG), cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), haemoglobin *A1c* test (HbA1c), fasting plasma insulin (FPI), blood pressure (BP) and triglycerides. Demographic information, including physical activity, smoking and consumption of alcohol, fast foods, sweetened beverages, non-vegetarian food and caloric intake, was gathered using standardised questionnaires by trained interviewers. Weight (in kg) was measured with the subjects in light clothes and barefoot and height was measured on a Frankfurt plane. BMI was calculated using the formula: weight (kg)/height $(m)^2$. Institutional Ethics Committee of Jamia Millia Islamia (J.M.I.), New Delhi, India (proposal no. 17/9/13/J.M.I./I.E.C./2015 dated 14/01/2016) approved this study. Written informed consent was obtained before inclusion in the study. This study was conducted in accordance with the Helsinki Declaration.

DNA Extraction and Genotyping

Genomic DNA was isolated from samples by using the phenol–chloroform method. Genomic DNA was analysed on 1% agarose gel electrophoresis and absorbance was observed at 260/280 nm to estimate the purity of the isolated DNA. The polymerase chain reaction-based restriction fragment length polymorphism (PCR–RFLP) analysis was done for genotyping. FTO rs9939699 T > A SNP was detected by PCR amplification using specific primers (forward: 5′-GGCTCTTGAATGAAATAGGA-3′ and reverse: 5′-AGAGACTATCCAAGTGCAG TAC-3′) followed by restriction digestion using the ScaI restriction enzyme. Touchdown PCR was used for FTO SNP genotyping and the PCR reaction included initial denaturation at 94 °C for 10 min, followed by 20 cycles of denaturation at 94 °C for 30 s, annealing at 61 °C (− 0.5 c/cycle) for 30 s and elongation at 72 °C for 30 s. Then, we followed up with 15-cycle denaturation at 94 °C for 30 s, annealing at 51 °C for 30 s and elongation at 72 °C for 30 s culminating in the final elongation at 72 °C for 10 min. The PCR product was found to be 170 bp long. It was digested overnight at 37 °C with one unit of ScaI restriction enzyme (New England Biolabs, Beverly, MA, USA), which produced two fragments of A-allele (150 bp and 20 bp) and an undigested 70-bp-long fragment of T-allele. These were analysed using 2.5% agarose gel electrophoresis.

The detection of MC4R rs12970134 G > A was performed by PCR using specific primers (forward primer: GACTCTTACCAAACAAAGC CTG and reverse primer: TGCTAGGTTGGT CCTGGTTG). The reaction included denaturation at 94 °C for 10 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 45 s, elongation at 72 °C for 45 s and a final elongation step at 72 °C for 5 min. The amplified PCR product of 124 bp length was digested at 37 °C using one unit of DdeI (NEB, Beverly, MA, USA) restriction enzyme. This enzyme recognises the CTG sequence, originating in two fragments of 104 bp and 20 bp length. The wild-type allele (GG) produced one band (124 bp) while the wild type/variant allele (GA) produced three bands of 124 bp, 104 bp and 20 bp. The variant allele (AA) produced two bands of 104 bp and 20 bp length when run on 2.5% agarose gel electrophoresis.

Statistical Analysis

Genotype frequencies between the cases and controls were evaluated using the chi-square test. Allele frequency was calculated by the Hardy-Weinberg equilibrium (HWE) equation.

Means ± standard deviation (SD) and percentage were compared using analysis of variance (ANOVA). The associations between genotypes and risk of T2D were calculated by computing the odds ratios (ORs) with 95% confidence intervals (CIs). $p < 0.05$ was considered statistically significant, while p between 0.05 and 0.1 was regarded as a trend towards association.

RESULTS

Genotype, Allele Frequency Distribution and T2D Risk

The allelic and genotypic distributions of FTO T > A and MC4R G > A SNP among cases and controls were not found to be statistically significant (p 0.117 and 0.246, respectively) in the studied population (Table 1). Furthermore, different genetic models (recessive, dominant and co-dominant) were applied to evaluate the risk associated with the FTO and MC4R polymorphisms. We found no real increase in odds of diabetes associated with the prevalence of FTO and MC4R minor allele (Table 2). Rare allele frequencies were found to be 0.34 and 0.30 for the "A" allele of rs9939609 FTO in cases and controls, respectively. However, the rare allele frequencies for the "A" allele of MC4R rs12970134 was found to be more common in controls (0.45) than cases (0.41) although the difference was not significant (p 0.246).

Correlation with Clinico-Pathological Parameters

The clinico-pathological parameters between T2D cases and controls were studied and we found that most of the clinico-pathological parameters including age, BMI, FPG, PPG, HbA1c, cholesterol), LDL, triglycerides, and diastolic and systolic blood pressure were significantly (p 0.001*) associated with the development of T2D (Table 3). The association of rs9939609 and MC4R rs12970134 FTO genotypes with the investigated clinical parameters is shown in Table 4. In the present study, a higher BMI was significantly associated with the presence of FTO risk allele "A" compared to the wild "TT" genotype (p 0.001*) in T2D patients. A total of 300 diabetic cases were analysed, in which 180 (60%) were men and 120 (40%) women. The mean age of the group was 39.46 ± 9.99 years and 38.63 ± 8.74 years, respectively (Table 5). The women showed higher BMIs (29.64 ± 5.53) compared to men (27.99 ± 5.25) and the difference was statistically significant (p 0.001*).

Table 1 Genotype distribution and allele frequencies of FTO (rs9939609) and MC4R (rs12970134) gene polymorphism between T2D cases and controls

FTO/variables	TT, *n* (%)	AA + TA, *n* (%)	*p* value	Allele frequency	
				T allele	A allele
Patients (n = 300)	129 (43%)	171 (57%)	0.117	0.66	0.34
Controls (n = 100)	52 (52%)	48 (48%)		0.70	0.30
MC4R/variables	**GG, *n* (%)**	**AA + GA, *n* (%)**	***p* value**	**Allele frequency**	
				G allele	**A allele**
Patients (n = 300)	106 (35.33%)	194 (64.67%)	0.246	0.59	0.41
Controls (n = 100)	29 (29%)	71 (71%)		0.55	0.45

Significant at $p < 0.05$

Table 2 Association of FTO rs9939609 T > A and MC4R rs12970134 G > A variants under different genetic models

Gene	Model	Genotype	Number of Cases	Number of controls (n = 100)	Odds ratio (95% CI)	p value
FTO (T > A)	Recessive	AA	34	11	1.0342 (0.5029–2.1268)	0.9272
		TA + TT	266	89		
	Dominant	TA + AA	171	48	1.4360 (0.9120–2.2613)	0.118
		TT	129	52		
	Co-dominant	TA	137	37	1.4311 (0.8987–2.2788)	0.1310
		TT + AA	163	63		
MC4R (G > A)	Recessive	AA	54	18	1.0000 (0.5548–1.8023)	1.0000
		GA + GG	246	82		
	Dominant	GA + AA	194	71	0.7475 (0.4568–1.2234)	0.2469
		GG	106	29		
	Co-dominant	GA	140	53	0.7759 (0.4930–1.2212)	0.2729
		GG + AA	160	47		

OR odds ratio, *CI* confidence interval, *n* number of samples
*Significant at $p < 0.05$

Correlation with Demographic/Lifestyle Parameters

The demographic characteristics of the study group (T2D cases and controls) were correlated during the study period (Table 6). We found that lifestyle-related and dietary habits were closely associated and statistically significant with the prevalence of diabetes compared to healthy controls. Habits such as smoking and consumption of alcohol, fast food and sweetened beverages were found to be higher in T2D patients than in healthy controls (p value < 0.01*), and these parameters were found to be associated with the development of diabetes. In addition, healthy controls (52%) were more involved in routine exercise compared to T2D patients (42%), indicating a decreased risk of T2D.

Correlation of Genotypes with Obesity

To further validate the association of genotype variation with the obesity, chi-square analysis was carried out (Table 7). Our study found a clear increase in body weight with the presence of rare allele "A" of the FTO gene (p 0.001), while for the MC4R gene we did not find any significant association (p 0.263) indicating indirect involvement of FTO (rs9939609 T > A) in the development of disease as obesity is one of the major risk factors for T2D.

DISCUSSION

The various genome-wide association studies (GWAS) in the past decades have identified several genetic variants related to obesity and their roles in obesity-related vascular diseases [27]. Frayling et al. [13] were the first to observe the role of the FTO SNP rs9939609 on the upsurge in BMI and the result was successfully replicated in many populations [28–31]. In the present study we also did not find significant obesity-associated risk for allele "A" of FTO. MC4R rs12970134 SNP did not show any significant association, but minor allele "A" of

Table 3 Comparative analyses of the investigated clinical parameters between T2D cases and controls, $n = 400$

	T2D	Control	*t* test for equality of means	
			t	Sig. (2-tailed)
N	300	100		*p* values
Age (years)	39.13 ± 9.51	38.11 ± 8.44	4.55	< **0.0001**
BMI (kg/m^2)	28.5 ± 5.2	24.8 ± 2.3	6.94	< **0.0001**
FPG (mg/dl)	135.1 ± 26.0	90.2 ± 7.1	17.09	< **0.0001**
PPG (mg/dl)	204.2 ± 35.9	136.2 ± 4.5	18.89	< **0.0001**
HbA1c	7.1 ± 1.1	5.7 ± 0.5	12.40	< **0.0001**
FPI	9.6 ± 1.4	8.7 ± 0.7	6.23	< **0.0001**
Systolic BP (mmHg)	145.1 ± 17.3	106.1 ± 10.4	21.57	< **0.0001**
Diastolic BP (mmHg)	102.4 ± 15.8	75.9 ± 10.9	15.84	< **0.0001**
T-Cholesterol (mg/dl)	245.5 ± 15.2	152.6 ± 18.8	51.99	< **0.0001**
HDL (mg/dl)	46.8 ± 11.1	46.2 ± 8.7	0.50	0.615
LDL (mg/dl)	192.3 ± 29.1	106.4 ± 19.9	27.92	< **0.0001**
Triglycerides (mg/dl)	357.9 ± 99.1	141.0 ± 5.5	21.86	< **0.0001**

Data presented as mean ± SD for biochemical parameters and p values calculated by Student's t test. Significant at $p < 0.05$

BMI body mass index, *FPG* fasting plasma glucose, *PPG* postprandial plasma glucose, *HbA1c* haemoglobin *A1c* test, FPI fasting plasma insulin, *BP* blood pressure, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein

MC4R showed a trend towards association with FPI, diastolic BP and triglyceride levels ($p < 0.1$). The diabetic women accumulated more visceral adipose tissue and had significantly higher BMIs (29.64 ± 5.53) compared to males (27.99 ± 5.25). Our findings agreed with the previous studies as the two genders had differences in free fatty acid metabolism, contributing to the accumulation of visceral adipose tissue [32]. A study conducted by Aline et al. [32] reported that T2D was associated with female carriers of the risk allele for the MC4R gene. Some similar studies showed an association of the FTO and MC4R risk alleles with type 2 diabetes [13, 17, 20]. The findings of the few other studies reporting the contribution of these studied polymorphisms in FTO and MC4R genes to type 2 diabetes mellitus were controversial, probably because of the ethnic and lifestyle differences among populations [33]. Furthermore, one of the studies based on the ethnic Chinese Han population with a sample size of 2351 did not show any association of FTO rs9939609 and MC4R rs17782313 SNPs with the development of T2D. However, the risk alleles were reported to be associated with an increase in obesity [34]. Nonetheless, the present study did not show any significant association of T2D with the risk allele of the FTO gene, but it showed a strong association with obesity. These findings perhaps suggest that although these alleles may be associated with increased body weight, they may only be indirectly involved in the development of diabetes, and lifestyle factors may be more indicative of an increased risk of T2D. The FTO gene is highly expressed in the hypothalamus region of the brain, which is involved in the regulation of food intake and energy expenditure [35]. The association studies of FTO variants with obesity are additionally supported by subsequent animal studies. A few of the animal studies on

Table 4 Correlation of clinico-pathological parameters with polymorphisms (FTO and MC4R) using analysis of variance (ANOVA)-one-way ANOVA from summary data

Parameter	FTO (n = 300)			MC4R (n = 300)		
	Genotype	Mean + SD	p value	Genotype	Mean + SD	p value
Age (years)	TT	38.10 ± 8.68	0.108	GG	39.18 ± 9.02	0.945
	TA/AA	39.90 ± 10.04		GA/AA	39.10 ± 9.78	
BMI (kg/m^2)	TT	27.42 ± 5.51	**0.001***	GG	28.53 ± 5.37	0.784
	TA/AA	29.57 ± 5.17		GA/AA	28.71 ± 5.45	
FPI	TT	9.26 ± 1.17	0.505	GG	9.50 ± 1.42	0.068
	TA/AA	9.36 ± 1.34		GA/AA	9.22 ± 1.17	
FPG (mg/dl)	TT	137.02 ± 31.18	0.963	GG	132.92 ± 23.43	0.140
	TA/AA	137.22 ± 40.20		GA/AA	139.44 ± 41.89	
PPG (mg/dl)	TT	201.25 ± 41.38	0.569	GG	198.44 ± 38.02	0.223
	TA/AA	204.56 ± 54.54		GA/AA	205.70 ± 54.36	
HbA1c	TT	6.79 ± 0.70	1.00	GG	6.71 ± 0.67	0.159
	TA/AA	6.79 ± 0.71		GA/AA	6.83 ± 0.72	
Systolic BP (mmHg)	TT	140.92 ± 19.86	0.074	GG	142.71 ± 19.34	0.735
	TA/AA	144.94 ± 18.25		GA/AA	143.49 ± 18.91	
Diastolic BP (mmHg)	TT	101.42 ± 14.78	0.627	GG	103.97 ± 16.90	0.095
	TA/AA	102.32 ± 16.31		GA/AA	100.81 ± 14.85	
Cholesterol (mg/dl)	TT	247.68 ± 15.87	0.762	GG	246.65 ± 15.23	0.248
	TA/AA	248.21 ± 13.90		GA/AA	248.71 ± 14.48	
HDL (mg/dl)	TT	44.98 ± 10.70	0.731	GG	46.02 ± 12.11	0.377
	TA/AA	45.44 ± 11.79		GA/AA	44.81 ± 10.87	
LDL (mg/dl)	TT	202.63 ± 17.46	0.383	GG	202.08 ± 21.43	0.257
	TA/AA	204.54 ± 19.32		GA/AA	204.62 ± 16.74	
Triglycerides (mg/dl)	TT	390.59 ± 81.47	0.333	GG	397.65 ± 88.64	0.062
	TA/AA	380.56 ± 92.16		GA/AA	377.89 ± 86.65	

Significant at $p < 0.05$

mouse models also reported that FTO is an important candidate gene for obesity [36]. Loss of function or expression of FTO is more relevant for a lean phenotype, whereas, overexpression results in obesity [36]. However, the MC4R gene showed high expression in the mesentery of obese and diabetic rats compared to lean rats [18]. In addition, some studies also supported that the association of FTO rs9939609 T > A and the MC4R rs12970134 G > A polymorphism with type 2 diabetes and obesity could be modified by lifestyle and diet [23]. In the present study, high consumption of sugar-sweetened beverages, not eating vegetables and eating fast food were observed among T2D cases compared to controls. The T2D

Table 5 Comparative analysis of characteristics between male and female patients in the study groups

Variables	Male T2D patients $N = 180$ (100%)	Female T2D patients $N = 120$ (100%)	p value
Age	39.46 ± 9.99	38.63 ± 8.74	0.46
BMI (kg/m^2)	27.99 ± 5.25	29.64 ± 5.53	0.01*
FPI	9.27 ± 1.24	9.39 ± 1.30	0.421
FPG (mg/dl)	138.08 ± 35.30	135.73 ± 38.43	0.586
PPG (mg/dl)	206.66 ± 44.60	197.85 ± 55.31	0.129
HbA1c	6.77 ± 0.70	6.81 ± 0.71	0.63
Systolic BP (mmHg)	143.36 ± 18.74	142.99 ± 19.55	0.869
Diastolic BP (mmHg)	102.48 ± 16.33	101.10 ± 14.60	0.455
Cholesterol (mg/dl)	248.47 ± 13.51	247.26 ± 16.48	0.487
HDL (mg/dl)	44.52 ± 9.66	46.33 ± 13.40	0.175
LDL (mg/dl)	204.28 ± 15.71	202.88 ± 22.16	0.532
Triglycerides (mg/dl)	390.38 ± 85.02	376.61 ± 91.37	0.183
Non-vegetable consumption			
Yes	132 (73.33%)	92 (76.67%)	
No	48 (26.67%)	28 (23.33%)	0.515
High fast food consumption			
Yes	61 (33.89%)	41 (34.17%)	
No	119 (66.11%)	79 (65.83%)	0.96
Smoking status			
Yes	113 (62.78%)	25 (20.83%)	0.001*
No	67 (37.22%)	95 (79.17%)	
Alcoholism			
Yes	120 (66.67%)	19 (15.83%)	0.001*
No	60 (33.33%)	101 (84.17%)	
High sugar-sweetened beverage consumption			
Yes	33 (18.33%)	29 (24.17%)	0.221
No	147 (81.67%)	91 (75.83%)	
High-calorie intake			
Yes	49 (27.22%)	33 (27.5%)	0.957
No	131 (72.78%)	87 (72.5%)	
Daily exercise			
Yes	75 (41.67%)	51 (42.5%)	0.886
No	105 (58.33%)	69 (57.5%)	

Data presented as mean ± SD for biochemical parameters and p values calculated by Student's t test. p values of demographic characteristics are calculated by chi-square test. Significant at $p < 0.05$

Table 6 Comparative analyses of the demographic characteristics/lifestyles between T2D cases and controls

Variables	T2D patients *n* (%)	Healthy controls *n* (%)	*p* value
Total number	300 (100%)	100 (100%)	
Gender			
Males	180 (60%)	68 (68%)	0.238
Females	120 (40%)	32 (32%)	
Non-vegetable consumption			
Yes	224 (74.67%)	38 (38%)	0.001*
No	76 (25.33%)	62 (62%)	
High fast food consumption			
Yes	102 (34%)	5 (5%)	0.001*
No	198 (66%)	95 (95%)	
Obesity			
Yes	133 (44.33%)	4 (4%)	0.001*
No	167 (55.67%)	96 (96%)	
Smoking status			
Yes	138 (46%)	15 (15%)	0.001*
No	162 (54%)	85 (85%)	
Alcoholism			
Yes	139 (46.33%)	10 (10%)	0.001*
No	161 (53.67%)	90 (90%)	
High sugar sweetened beverage consumption			
Yes	62 (20.67%)	5 (5%)	0.001*
No	238 (79.33%)	95 (95%)	
High calorie intake			
Yes	82 (27.33%)	14 (14%)	0.006*
No	218 (72.67%)	86 (86%)	
Daily exercise			
Yes	126 (42%)	52 (52%)	0.08
No	174 (58%)	48 (48%)	
Family history			
Yes	125 (41.67%)	39 (39%)	0.124
No	175 (58.33%)	61 (61%)	

p values of demographic characteristics are calculated by chi-square test. Significant at $p < 0.05$

Table 7 Correlation of body weight distribution of patients with FTO (rs9939609 T > A) and MC4R (rs1297034 G > A) genotypes in the study groups

FTO				
Body weight/genotype	**TT**	**TA**	**AA**	***p* value**
Normal (82)	46	31	5	0.001*
Overweight (85)	45	34	6	
Obese (133)	38	72	23	
MC4R				
Body weight/genotype	**GG**	**GA**	**AA**	***p* value**
Normal (82)	30	32	20	0.263
Overweight (85)	33	41	11	
Obese (133)	43	67	23	

p values are calculated by Chi-square test
*Significant at $p < 0.05$

patients also had a history of higher caloric intake and were less (42%) physically active compared to controls (52%) (Table 4). The present study included 300 T2D patients, 60% males and 40% females, and found that most of the lifestyle and dietary-related parameters did not vary greatly between genders, while smoking and consumption of alcohol were found to be important factors among males. However, the social taboo probably weighed in favour of females in the Indian population.

There may be some limitations to the present study. First, the sample size was small although India is a geographically vast country with diverse ethnic populations having different cultures, lifestyles and eating habits. However, a study including most regions with a larger, more diverse sample size might provide greater insight into the association of the genetic variability of these genes with obesity and T2D. Second, an intervention-based follow-up study on diabetic subjects could help better understand the role of these SNPs in obesity and T2D.

In conclusion, the present study did not find a direct association of rs9939609 in FTO and rs12970134456 in MC4R genes with the occurrence of diabetes, but their role in T2D development cannot be overlooked altogether. This study further concludes that although the rs12970134 MC4R polymorphism did not show an association with obesity, the rs9939609 of FTO carries a potential risk of obesity because this FTO rs9939609 T > A is widely considered an obesity-associated allele/SNP. These findings further suggest that these alleles may be indirectly involved in the development of diabetes, and lifestyle factors may be more indicative of an increased risk of T2D. However, a detailed understanding of the genetic variants in metabolism regulation and their functional consequences can provide a better management strategy for developing a solution to curb obesity. In turn, this can help to reduce the risk of type 2 diabetes and cardiovascular diseases.

ACKNOWLEDGEMENTS

We acknowledge all the study participants who were included in this study.

Funding. There was no funding for this study. The Rapid Service Fee was funded by the authors.

Authorship. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Authorship Contributions. Conceptualization, KD, AKV and SK; Methodology, SK, AKV and VK; Software, SK, AKV; Validation, KD; Formal analysis, SK, VK, DB and AKV; Data curation, SK, AKV and VK; Writing-original draft preparation, SK, AKV; Writing-review and editing, SR, DB and AKV; Visualization, KD; Supervision, KD.

Disclosures. Shahbaz Khan, Amit Kumar Verma, Vasiuddin Khan, Deepti Bhatt, Sahar Rafat, Mohammed A. Alsahli, Arshad Husain Rahmani, Babita Meena, Irshad Husain Naqvi and Kapil Dev have nothing to disclose.

Compliance with Ethics Guidelines. Institutional Ethics Committee of Jamia Millia Islamia (J.M.I.), New Delhi, India (proposal no. 17/9/13/J.M.I./I.E.C./2015 dated 14/01/2016) approved this study. Written informed consent was obtained before inclusion in the study. This study was conducted in accordance with the Helsinki Declaration.

Data Availability. We confirm that the data used during the research will not be shared with anyone/broadcast in any public domain, since it is impermissible as per the policy instructions of J.M.I. New Delhi, India. The metadata supporting the study outcomes can be obtained from J.M.I. New Delhi, India, with proper consent; however, privileged data with restricted open accessibility require institutional authorization and discretion. Other related information and data can also be retrieved from the authors with permission from J.M.I., New Delhi, India.

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