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Original Article

Distribution and causal relationship of FTO rs9939609 and leptin rs7799039 nucleotide variants with type 2 diabetes in a subset of the Bangladeshi population

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Abstract

Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disorder in which blood glucose level remains persistently higher than the physiologically normal range. Two important genes, namely fat mass and obesity-related gene (FTO) and Leptin (LEP), are involved with obesity and progression to diabetes. The aim of this study is to identify the underlying genetic make-up that predisposes individuals to T2DM, namely, rs9939609 and rs7799039 variants of FTO and LEP genes, respectively, were analyzed in a subset of the Bangladeshi population. DNA samples were collected from Blood samples, and the genetic polymorphisms were identified using allele-specific polymerase chain reactions (AS-PCR). The data collected by a standard questionnaire were then analyzed by SPSS for Windows. A total of 257 Bangladeshi individuals were included in the study, among whom 158 were T2DM patients, and 99 were non-diabetic healthy controls. Logistic regression analysis showed that the homozygous FTO rs9939609 variant genotype is significantly associated with type 2 diabetes (P=0.006) in the Bangladeshi population. Meanwhile, the Leptin rs7799039 variant is significantly associated with T2DM in males (P=0.006) but not in females. No association was observed for these variants with body mass index (BMI) and hypertension. The inheritance model analyses showed that the FTO co-dominant, dominant and recessive models are associated with T2D. The study revealed that the FTO rs9939609 variants have a significant role, and Leptin rs7799039 variants have a male-specific effect on T2D of Bangladeshi subjects.

Keywords: FTO, rs9939609, leptin, rs7799039, type 2 diabetes, BMI.

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Introduction

Type 2 diabetes mellitus (T2DM) is a widespread metabolic disorder in which patients experience an elevated level of blood glucose due to the impairment of insulin action [1]. Multiple genetic, environmental, and biochemical factors are implicated in the development of T2DM [2]. A lot of studies identified important diabetes susceptibility genes and associated variants linked to disease progression [3–9]. The effect and importance of the risk variants to different ethnic groups may vary as the frequencies of these genetic variations are often highly unlikely. The genetic predisposition makes the South Asian population more vulnerable to T2DM than the Europeans [10]. It has been found in the recent past that the prevalence of prediabetes was 22.4%, and diabetes was 9.7% in Bangladesh [11]. Prevalence is increasing in Bangladesh at such a rate that it will be the top 8th diabetic populous country in the world by 2030 [12]. It is, therefore, important to investigate the frequencies and associations of the susceptibility variants in the South Asian population, including Bangladesh.

The Fat mass and obesity-related gene [Fe (II)- and 2-oxoglutarate-dependent dioxygenases] is a member of the non-heme dioxygenase superfamily. This enzyme consists of two distinct domains: an amino-terminal AlkB-like domain and a carboxy-terminal domain [13]. The catalytic activity of this enzyme is exclusively dependent on the interaction between these two domains [14]. Genome-wide association studies showed that SNPs in intron 1 (including rs9939609, rs3751812, rs17817449, rs9930506, and rs1421085) of FTO are linked to obesity in Caucasians [6, 15, 16]. On the other hand, leptin is a metabolic hormone produced predominantly by the white adipose tissue [17, 18]. The primary functions of this hormone are body mass control, angiogenesis, bone remodeling, reproduction, wound healing, immunity, and cardiac function [17, 19]. Plasma Leptin concentration is strikingly increased in obese subjects that are proportionate to body adiposity [17]. A number of studies have reported that the Leptin gene (LEP) polymorphisms may play a critical role in the pathophysiology of human obesity [20, 21]. The rs7799039 SNP in the Leptin gene has been shown to be associated with perturbations in plasma Leptin concentration and body mass index (BMI) in obese subjects [22–25]. The G-2548A LEP polymorphism has been implicated as being responsible for influencing Leptin expression, probably at the transcriptional level and, hence, also at the secretion level by the adipose tissue [26]. Moreover, this LEP polymorphism is related to the variation of serum leptin levels as well as the obesity level in overweight and obese individuals. However, the existing data are still conflicting [22–24, 26]. A plethora of studies reported a strong correlation of Obesity with T2DM [27, 28]. To the best of our knowledge, there is no study on these polymorphisms in the Bangladeshi population. This study explores the epidemiological aspects of FTO rs9939609 and Leptin rs7799039 variants with T2DM in a subset of the Bangladeshi population.

Material and methods

Sample collection

The data were collected from 267 subjects. However, data from 13 subjects were missing/incomplete for the FTO genotype, and 10 were missing/incomplete for the leptin genotype (Table S1). The participants who agreed to be involved in this study signed an informed consent form. Among them, 158 (54.6%) of the participants were in the patient group with type 2 diabetes, and 99 (45.4%) were non-diabetic healthy individuals (control). The samples were collected from the Bangladesh University of Health Science (BUHS), Tangail Diabetic Association (TDS), Jessore Diabetic Association, and Students and officers of Mawlana Bhashani Science and Technology University (MBSTU) [29, 30]. The guidelines of this study were approved by the ethical review committee of the Bangladesh Medical Research Council (Approval no: BMRC/NREC/2013-2016/806). Three to 4 milliliters of blood were collected from each individual by an expert phlebotomist. Plasma samples were analyzed to measure the level of random blood glucose (200 mg/dL or above) to confirm their diabetic conditions.

Pregnant women and patients who take insulin regularly, were excluded from this study. We have collected the anthropometric and demographic data such as age, gender, height, weight, family history of diabetes, systolic blood pressure (SBP), diastolic blood pressure (DBP), and socio-economic status. The data were recorded in a standard questionnaire approved by the BMRC.

DNA extraction

Genomic DNA was extracted from the blood cells using Invitrogen™ PureLink™ Genomic DNA Mini Kit (Cat. No. K182002) accompanying the instructions of the manufacturer. The quality of purified DNA and

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Table 1: Primers used in allele specific PCR of FTO rs9939609 and Leptin rs7799039 alleles.

Gene		Primer Sequences	Product size	Annealing temperature used
FTO	FTO_T_Foward	"CAAAATGGCAACACACACTCT	243 bp	65.0
	FTO_T_Reverse	"AGACTATCCAAGTGCATCA CA		
	FTO_A_Foward	CCTTGCGACTGCTGTGAATT TA	206 bp	65.0
	FTO_A_Reverse	CAAGTCACACTCAGCCTCT		
Leptin	LEP_G_Foward	TGTTTTGCGACAGGGTTGCG	262 bp	65.0
	LEP_G_Reverse	TCACAGTGGTCCTGAGGTGACG		
	LEP_A_Foward	TGGTTCAAGGGCTGGGAACT	250 bp	68.5
	LEP_A_Reverse	ACTGAGGCGGGAGGATCAGT		

its quantity were measured using a NanodropTM 2000 spectrophotometer (Thermo Scientific, DE, USA).

Genotyping by allele-specific polymerase chain reaction (PCR)

Allele-specific primers were designed using the Web-based Allele-Specific PCR Primer designing tool (WASP) [31] for rs9939609 and rs7799039 SNPs of FTO and Leptin genes, respectively. Primers were obtained through the commercial services of Integrated DNA Technologies Inc. (IDT, USA). Primer sequences, along with other relevant information, are given in Table 1. PCR was performed in a total volume of 25 µL with an initial denaturing step at 95˚C for 5 minutes, followed by 35 cycles- each for 30 s at 95˚C, 30 s at the annealing temperature (shown in Table 1), 40 s at 72˚C, and a final extension at 72˚C for 5 minutes. The PCR products were separated in 2% agarose gel and visualized upon incubation with Ethidium Bromide in a gel documentation system.

DNA sequencing

A different set of primers were designed using the Primer-BLAST tool to sequence the regions of FTO and Leptin genes harboring the rs9939609 and rs7799039 SNPs. DNA from five randomly selected samples was amplified using these primers shown in Figures 1 and 2. Amplified products were purified and sequenced

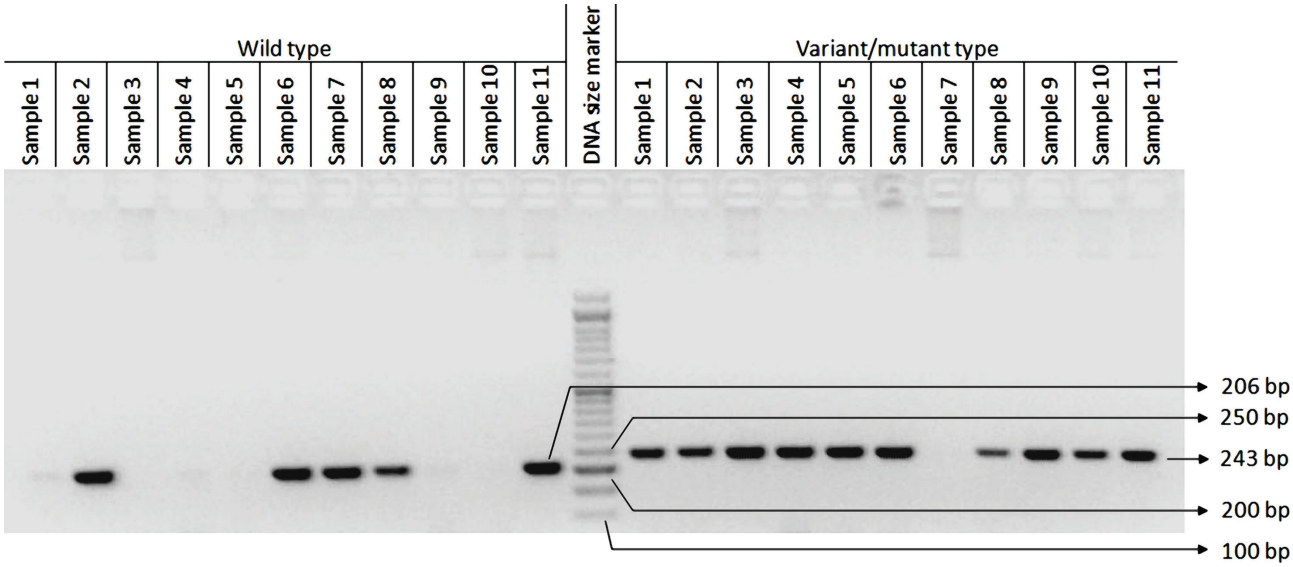

Figure 1: AS-PCR with FTO rs9939609 specific primers. Wild-type and variant-specific amplicon sizes are 206 and 243 base pairs, respectively. 11 samples (1-11) were amplified by wild-type and variant allele-specific primer pairs. In this representative figure, sample 7 has homozygous wild-type (A/A), samples 3, 5 and 10 have homozygous variant (T/T), and samples 1, 2, 4, 6, 8, 9, and 11 have heterozygous (A/T) genotype at the rs9939609 locus. DNA size markers are used at the center.

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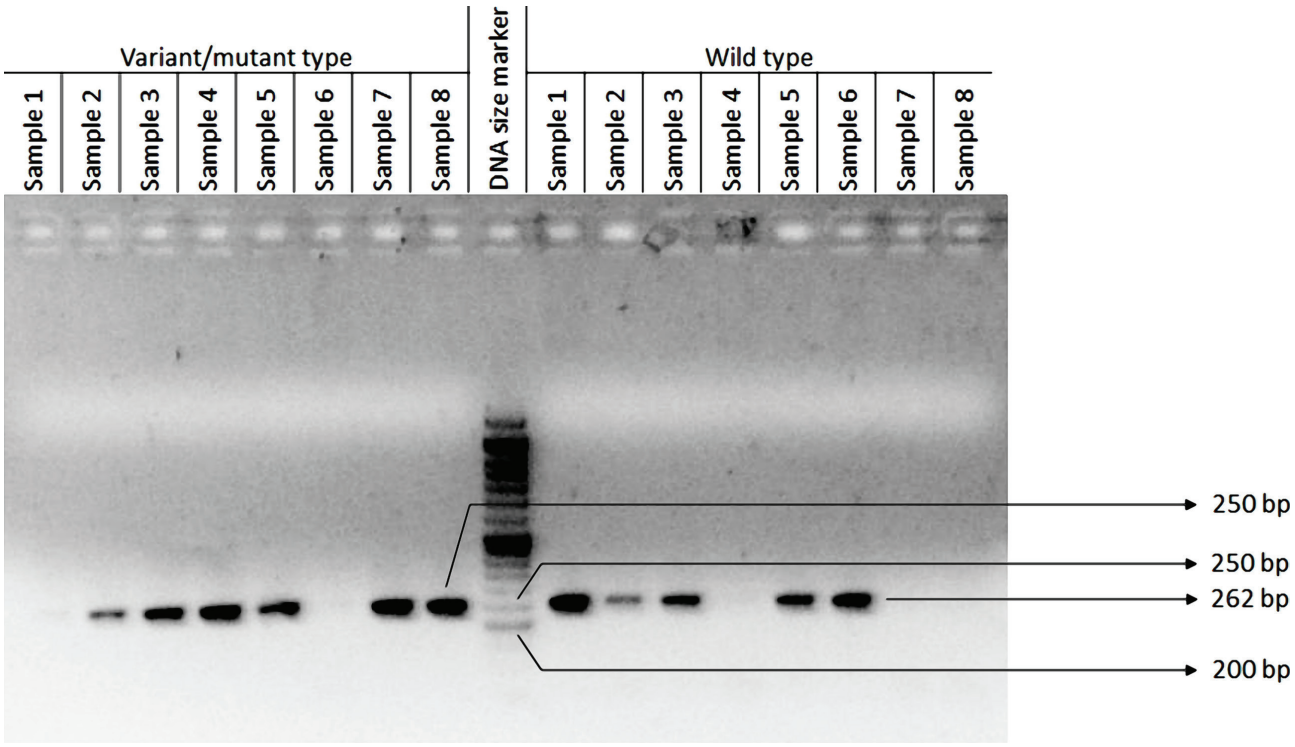

Figure 2: AS-PCR with Leptin rs7799039 specific primers. Wild-type and variant amplicon sizes are 262 and 250 base pairs, respectively. In this figure, 8 samples (1-8) were amplified by variant and wild-type primer pairs. Individuals 1 and 6 are homozygous wild type (G/G), individuals 4, 7, and 8 are homozygous mutant (A/A), and individuals 2, 3, and 5 are heterozygous (G/A) at the rs7799039 locus. DNA size markers are used at the center.

through the commercial service of DNA solutions Ltd. (Panthapath, Dhaka-1205, Bangladesh).

Statistical analyses

All statistical analyses were carried out using the software SPSS for Windows (version 17.0 SPSS Inc, Chicago, IL, USA). A Chi-square test was performed to assess the association between disease status and polymorphisms. Unconditional logistic regression analyses were performed to determine the odds ratios (ORs) and 95% confidence intervals (95% CI). The odds ratio (OR) was adjusted for two confounding factors: age and gender. BMI, systolic, and diastolic blood pressure were compared between the two groups using analyses of variance (ANOVA), and the values are shown as Mean±SD.

Table 2: Prevalence of FTO rs9939609 alleles in Bangladeshi population.

Variables	FTO genotype			Chi square	P-value
	Homozygous A N (%)	Heterozygous N (%)	Homozygous T N (%)		
Age					
11–20	2 (66.67)	0 (0.00)	1 (33.33)		
21–30	19 (32.76)	33 (56.90)	6 (10.34)		
31–40	15 (20.27)	23 (31.08)	36 (48.65)	36.59	<0.05
41–50	13 (20.63)	34 (53.97)	16 (25.40)		
>50	10 (17.86)	17 (30.36)	29 (51.79)		
Gender					
Male	30 (20.69)	66 (45.52)	49 (33.79)	1.93	0.381
Female	29 (26.61)	41 (37.61)	39 (35.78)		

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Table 2: Continued.

Variables	FTO genotype			Chi square	P-value
	Homozygous A N (%)	Heterozygous N (%)	Homozygous T N (%)		
Occupation type					
On desk	17 (20.48)	36 (43.37)	30 (36.14)	0.53	0.769
Non desk	42 (24.56)	71 (41.52)	58 (33.92)		
Disease type					
Normal subjects	30 (31.25)	41 (42.71)	25 (26.04)	7.59	0.023
Type 2 diabetes patients	29 (18.35)	66 (41.77)	63 (39.87)		
Obesity type					
Normal	36 (24.00)	63 (42.00)	51 (34.00)	3.62	0.728
Over weight	18 (22.50)	33 (41.25)	29 (36.25)		
Obese	3 (15.00)	9 (45.00)	8 (40.00)		
Under weight	2 (50.00)	2 (50.00)	0 (0.00)		
Total	59 (23.22)	107 (42.13)	88 (34.65)		

All analyses were performed at a 95% confidence interval (95% CI), and all tests were two-tailed.

Results

Frequency of FTO rs9939609 and Leptin rs7799039 alleles in the Bangladeshi population

The data were collected from 267 subjects. However, data from 13 subjects were missing/incomplete for the FTO genotype, and 10 were missing/incomplete for the Leptin genotype. The distributions of FTO rs9939609 and Leptin rs7799039 alleles are shown in Tables 2 and 3, respectively. The frequency of FTO homozygous A genotype, heterozygous AT genotype, and homozygous T genotype were 23.22% (59/254), 42.13% (107/254), and 34.65% (88/254), respectively. We observed a significant association of FTO rs9939609 with T2D (P-value <0.05). 18.35% (29/158) of the T2D patients have a homozygous A genotype in comparison to 39.87% (63/158) patients who have a homozygous T

Table 3: Prevalence of Leptin rs7799039 alleles in Bangladeshi population.

Variables	Leptin genotype			Chi square	P-value
	Homozygous G N (%)	Heterozygous N (%)	Homozygous A N (%)		
Age					
11–20	2 (66.67)	0 (0.00)	1 (33.33)	9.88	0.273
21–30	18 (30.51)	25 (42.37)	16 (27.12)		
31–40	20 (26.32)	32 (42.11)	24 (31.58)		
41–50	14 (21.88)	21 (32.81)	29 (45.31)		
>50	10 (18.18)	24 (43.64)	21 (38.18)		
Gender					
Male	37 (25.17)	53 (36.05)	57 (38.78)	2.25	0.324
Female	27 (24.55)	49 (44.55)	34 (30.91)		

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Table 3: Continued.

Variables	Leptin genotype			Chi square	P-value
	Homozygous G N (%)	Heterozygous N (%)	Homozygous A N (%)		
Occupation type					
On desk	24 (28.24)	31 (36.47)	30 (35.29)	0.89	0.638
Non desk	40 (23.26)	71 (41.28)	61 (35.47)		
Disease type					
Normal subjects	31 (31.31)	37 (37.37)	31 (31.31)	3.64	0.162
Type 2 diabetes patients	33 (20.89)	65 (41.14)	60 (37.97)		
Obesity type					
Normal	38 (24.68)	69 (44.81)	47 (30.52)	6.11	0.411
Over weight	20 (25.32)	25 (31.65)	34 (43.04)		
Obese	5 (26.32)	7 (36.84)	7 (36.84)		
Under weight	1 (20.00)	1 (20.00)	3 (60.00)		
Total	64 (24.90)	102 (39.69)	91 (35.41)		

genotype. The FTO variant is also diversely distributed in different age groups (P-value <0.05); however, it is not associated with gender, occupation type, or obesity (Table 2).

The frequency of LEP homozygous G genotype, heterozygous GA genotype, and homozygous A genotype were 24.90% (64/257), 39.69% (102/257), and 35.41% (91/257), respectively. No statistically significant association was observed between Leptin rs7799039 alleles and T2DM in the Bangladeshi population. 20.89% (33/158) of the T2D patients have a homozygous G genotype. In comparison, 37.97% (60/158) of patients have a homozygous – a genotype.

Association with T2DM

Type 2 diabetes was significantly associated with age, gender, and FTO rs9939609 genotype (P-value <0.05) but not associated with Leptin rs7799039 genotype (Table S1). As age and gender are significantly associated with type 2 diabetes, we calculated the odds ratio by adjusting the confounding factors: age and gender. FTO homozygous A genotype had a protective role in the case of type 2 diabetes (OR, 0.38; 95% CI, 0.19–0.76, P-value 0.006). Though it was found that the Leptin A/A genotype moderately increases the risk of developing type 2 diabetes (OR, 1.82; 95% CI, 0.95–3.49, P-value 0.073), after adjusting with the confounding factors, it was found not to be significant (OR, 1.45; 95% CI, 0.46–4.61, P-value 0.527) (Table 4).

We have also analyzed the gender-specific effect of these polymorphisms. It was found that both polymorphisms are significantly associated with T2DM in Bangladeshi males (P-value <0.05). FTO rs9939609 was moderately associated [P-value 0.037 (OR, 4.57; 95% CI, 1.09–19.14)], but Leptin rs7799039 was not associated [P-value 0.327 (OR, 0.48; 95% CI, 0.11–2.08)] with T2DM in the females (Table S2).

Relation of different variables with BMI, systolic and diastolic blood pressure

The status of BMI, systolic and diastolic blood pressures in different age groups, gender, occupation type, FTO genotype, and Leptin genotype is shown in Table S3. BMI, systolic and diastolic blood pressures were significantly associated with age, and all of these were significantly higher in older people (P-value <0.05). In the study subjects, the mean systolic blood pressure was 123.12±17.44, the mean diastolic blood pressure was 81.89±7.61, and the mean BMI was 24.55±3.88. Gender was significantly associated with BMI (P-value 0.014) but not associated with systolic blood pressure (P-value 0.942) and diastolic blood pressure (P-value 0.608). The females had a higher BMI than the males. The mean BMI of the female subjects was

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Table 4: The regression analyses to elucidate the relationship of type 2 diabetes with FTO rs9939609 and Leptin rs7799039 polymorphisms.

Variables	P-value	Odds ratio (95% CI)	P-value*	Odds ratio* (95% CI)
Age				
11–20		1		
21–30	0.309	0.27 (0.02-3.37)		
31–40	0.445	2.59 (0.23-29.75)		
41–50	0.020	20.67 (1.63-262.71)		
>50	0.036	14.86 (1.19-185.92)		
Gender				
Male		1		
Female	<.001	5.55 (3.12-9.90)		
Occupation type				
On desk		1		1
Non desk	<.001	8.60 (4.80-15.41)	<.001	6.28 (2.73-14.45)
FTO polymorphism				
Homozygous A		1		1
Heterozygous	0.120	1.67 (0.88-3.17)	0.041	2.91 (1.05-8.11)
Homozygous T	0.006	2.61 (1.31-5.19)	0.048	2.78 (1.00-7.69)
Leptin polymorphism				
Homozygous G		1		1
Heterozygous	0.122	1.65 (0.86-3.11)	0.229	1.79 (0.69-4.65)
Homozygous A	0.073	1.82 (0.95-3.49)	0.328	1.45 (0.62-4.19)

Note: * – The Odds ratios were adjusted for the confounding factors age and gender.

Table 5: The inheritance model to analyse the association of FTO rs9939609 and Leptin rs7799039 polymorphisms with Type 2 Diabetes.

Model	Genotype	Control (%)	Type 2 Diabetes (%)	Odds ratio (95% confidence interval)	P-value
FTO rs9939609					
Co-dominant	T/T	25 (28.41)	63 (71.59)	1	0.022
	T/A	41 (38.31)	66 (61.68)	0.64 (0.35–1.17)	
	A/A	30 (50.85)	29 (49.15)	0.38 (0.19–0.76)	
Dominant	T/T	25 (28.41)	63 (71.59)	1	0.024
	T/A-A/A	71 (42.77)	95 (57.23)	0.53 (0.30–0.93)	
Recessive	T/T-T/A	66 (33.85)	129 (66.15)	1	0.018
	A/A	30 (50.85)	29 (49.15)	0.49 (0.27–0.89)	
Overdominant	T/T-A/A	55 (37.41)	92 (62.59)	1	0.883
	T/A	41 (38.32)	66 (61.68)	0.96 (0.58–1.61)	

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Table 5: Continued.

Model	Genotype	Control (%)	Type 2 Diabetes (%)	Odds ratio (95% confidence interval)	P-value
Leptin rs7799039					
Co-dominant	G/G	31 (48.44)	33 (51.56)	1	0.162
	G/A	37 (36.27)	65 (63.73)	1.65 (0.87–3.11)	
	A/A	31 (34.07)	60 (65.93)	1.82 (0.94–3.50)	
Dominant	G/G	31 (48.44)	33 (51.56)	1	0.059
	G/A-A/A	68 (35.23)	125 (64.77)	1.73 (0.97–3.06)	
Recessive	G/G-G/A	68 (40.96)	98 (58.04)	1	0.163
	A/A	31 (32.29)	65 (67.71)	1.45 (0.86–2.46)	
Overdominant	G/G-A/A	62 (40.00)	93 (60.00)	1	0.548
	G/A	37 (36.27)	65 (63.73)	1.17 (0.69–1.96)	

25.22±4.45, whereas the mean BMI of the male subjects was 24.04±3.29. Occupation type was not significantly related to BMI, systolic, and diastolic blood pressure. However, systolic blood pressure (P-value <0.001), diastolic blood pressure (P-value <0.05), and BMI (P-value <0.05) were significantly associated with type 2 diabetes. The mean systolic blood pressure of type 2 diabetes patients was 126.89±14.35 in comparison to healthy control subjects with 120.33±10.88. Likewise, the mean diastolic blood pressure of type 2 diabetes patients was 82.76±7.29 in comparison to healthy control subjects with 80.69±7.93. Moreover, the mean BMI of type 2 diabetes patients was 25.12±4.03 in comparison to healthy control subjects with 23.61±3.43. On the other hand, neither FTO rs9939609 nor Leptin rs7799039 was significantly associated with BMI, systolic, and diastolic blood pressures.

Inheritance model to analyze the SNP with T2D and overweight

We have analyzed the inheritance model to assess the association between the FTO and leptin polymorphism with Type 2 Diabetes. The model shows that both the dominant and recessive model in rs9939609 is significantly related to Type 2 Diabetes. The odds ratio dictates the protective role of the A-genotype in disease morbidity. While the rs7799039 has no significant relationship with Type 2 Diabetes, only moderate significance (P-value 0.059) has been found in the case of a dominant model (Table 5). We have also analyzed the inheritance model to assess the association between the FTO and Leptin polymorphism in overweight. We excluded the underweight samples and compared the overweight samples (BMI>25) with control samples (BMI<25). It has been found that both the FTO rs9939609 and Leptin rs7799039 have not significantly associated with being overweight. In the case of the Leptin rs7799039 recessive model, the A-genotype is moderately associated with the overweight phenotype (P-value 0.066) (Table 6).

Discussion

Type 2 diabetes mellitus (T2DM) is known to occur by impaired insulin action and/or abnormal pancreatic beta-cell function [32]. A number of genome-wide association studies (GWAS) have identified 94 independent T2D genetic variants and 47 diabetes-related traits [33]. In this study, we investigated the distribution and association of two such predisposing single nucleotide polymorphisms (SNPs) in FTO and Leptin genes with T2DM in a subset of the Bangladeshi population. FTO was one of the first genes described to be associated with obesity, having an extensive influence on increased BMI to date [6, 15], and leptin has been reported to be potentially involved in the progression of obesity with the potential to be engaged in the elicitation of type 2 diabetes [34].

In this study, the frequency of FTO rs9939609 polymorphism has been found to be significantly diverse in different age groups but not in gender groups and occupation types (Table 2). We have found that FTO

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Table 6: The inheritance model to analyse the association of FTO rs9939609 and Leptin rs7799039 polymorphisms with obesity/overweight.

Model	Genotype	Control (%)	Overweight (%) (BMI>25)	Odds ratio (95% confidence interval)	P-value
FTO rs9939609					
Co-dominant	T/T	51 (57.95)	37 (42.05)	1	0.823
	T/A	63 (60.00)	42 (40.00)	0.92 (0.52–1.63)	
	A/A	36 (63.16)	21 (36.84)	0.80 (0.41–1.59)	
Dominant	T/T	51 (57.95)	37 (42.05)	1	0.626
	T/A-A/A	99 (61.11)	63 (38.89)	0.88 (0.52–1.49)	
Recessive	T/T-T/A	114 (59.07)	79 (40.93)	1	0.579
	A/A	36 (63.16)	21 (36.84)	0.84 (0.46–1.55)	
Overdominant	T/T-A/A	87 (60.00)	58 (40.00)	1	1
	T/A	63 (60.00)	42 (40.00)	1 (0.60–1.67)	
Leptin rs7799039					
Co-dominant	G/G	38 (60.32)	25 (39.68)	1	0.109
	G/A	69 (68.32)	32 (31.68)	0.71 (0.37–1.36)	
	A/A	47 (53.41)	41 (46.59)	1.33 (0.69–2.55)	
Dominant	G/G	38 (60.31)	25 (39.68)	1	0.881
	G/A-A/A	116 (61.38)	73 (38.62)	0.96 (0.53–1.71)	
Recessive	G/G-G/A	107 (65.24)	57 (34.76)	1	0.066
	A/A	47 (53.41)	41 (46.59)	1.64 (0.96–2.78)	
Overdominant	G/G-A/A	85 (56.29)	66 (43.71)	1	0.054
	G/A	69 (68.32)	32 (31.68)	0.59 (0.35–1.01)	

polymorphism is significantly associated with type 2 diabetes (P-value <0.05) (Table 2) but not associated with obesity and hypertension (Table 2 and Table S3). The AA genotype at the rs9939609 locus of FTO was found to be protective for T2DM. This result is consistent in the case of the inheritance model (Table 5). The result is consistent with the North-East Indian population [35]. A similar association has been found between the Chinese Han population and the Japanese [36, 37]. This, however, affects T2DM in the Scandinavian and Vietnamese populations independent of the effect on BMI [38, 39]. The result is also very different from the Caucasian populations, in which this is a strong marker for obesity [15] as well as diabetes [6]. A study on Pakistani females showed that rs9939609 was associated with higher fasting blood glucose (FBG), plasma insulin, and Leptin levels and predisposed them to obesity and type 2 diabetes [40]. The A/A genotype increased the risk of obesity in adult females [40]. Some studies in China found no association of this polymorphism with diabetes [41, 42]. However, studies on European children revealed that the A/A genotype decreased the BMI at early ages [43]. In the population of Iraq, the FTO rs9939609 was shown to develop insulin resistance and the occurrence of T2DM in obese patients. The homozygous T/T genotype significantly increased the risk of T2DM concerning those of the A/A genotype after adjustment for age, gender, and BMI [44, 45]. We have adjusted the odds ratios for the FTO genotype with age and gender in a binary logistic regression model and found that both heterozygous (A/T) and homozygous (T/T) alleles increased the risk of T2DM (Table 4). There are several conflicting reports in the South Asian population on FTO rs rs9939609 polymorphism in metabolic disorders. This variant is associated with the obesity phenotype but not related to T2D in the north Indian population [46]. It has been shown that the FTO A-genotype was involved in the body weight increase, though not

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directly related to T2D [47]. In the Indian Bengali population, this polymorphism has no association with either T2D or obesity [48]. However, FTO A-genotype was associated with T2D in the western Indian population of Pune, the south Indian population of Hyderabad, as well as the Tamil population of Srilanka [49–51].

Previous studies reported that the LEP G-2548A polymorphism may affect Leptin gene expression and thereby stimulate the secretion of leptin by adipocytes [26]. So, this variant may influence the elevated BMI in obese/overweight subjects [23]. However, the present findings indicate that the LEP G-2548A polymorphism is not an appropriate obesity marker for the Bangladeshi population. We have not found any statistically significant difference in LEP gene polymorphism between the diabetic and control groups (P>0.05) (Tables 3 and 4). However, the inheritance model showed that a moderate relationship existed in the case of a recessive model with a BMI (P-value 0.066). That means the A-genotype might be responsible for the overweight phenotype (Table 6). Increasing the sample size might give a clear picture of the association between this phenotype with BMI. This result is consistent with previous studies on obesity in Turkish and Malaysian populations [52, 53] and metabolic syndromes in the Thai population [54]. On the other hand, this finding is not supported by the study carried out on the Mongolians, as an association of G-2548A of LEP polymorphism was observed with elevated body mass index (BMI) and FBG, as well as an independent risk factor for Metabolic syndrome [55]. An association of this polymorphism with increased BMI was also reported in overweight Europeans [56] as well as in Taiwanese and Pakistani individuals with obesity [57, 58]. Studies in the Indian population reported that the risk allele of Leptin rs7799039 was associated with T2D and BMI [59, 60]. These inconsistencies among the results might arise due to the different genetic backgrounds and/or non-genetic factors and sample size of the study population. Analyses of variance showed that both the FTO A/T polymorphism (rs9939609) and Leptin G-2548A polymorphism (rs7799039) were not significantly associated with BMI, systolic, and diastolic blood pressure.

We have analyzed the gender-specific effect of these polymorphisms. It was found that Leptin rs7799039 variants are significantly associated with T2DM in Bangladeshi males (P-value <0.05). This type of gender (male) specific effect of leptin G-2548A polymorphism (rs7799039) was reported earlier in several studies. Leptin G-2548A was shown to be associated with human longevity phenotype in Jordanian males [61]. Multiple linear regression analyses showed that the A/A genotype at Leptin rs7799039 significantly affected systolic and diastolic blood pressures among the Tunisian obese males [62]. Our study showed that the subjects with the G/A genotype at this locus have 2.58 times higher risk, and the A/A genotype imparts even more (4.28 times) for type 2 diabetes in Bangladeshi males (Table S2).

Conclusion

In conclusion, these results from a subset of the Bangladeshi population support the involvement of the FTO gene in the susceptibility of T2DM but not in obesity and hypertension. The Leptin rs7799039 genotype was only associated with T2DM in Bangladeshi males. However, our study is limited to a small number of the patient as well as control subjects. The second limitation was the samples are not evenly from different regions of Bangladesh. This study demands a further study in a larger cohort to additional validation of the current results.

These data may have potentially important scientific, clinical, and public health implications by informing clinicians for the better management of susceptible patients as well as healthy controls. Further genetic and functional studies are required in large populations on the other FTO and Leptin variants, as well as other susceptible genes of obesity. It will help to clarify the physiological mechanisms of the pathogenesis of T2DM and obesity in the Bangladeshi population.

Conflict of interest

The authors declare no conflict of interest.

Ethics approval

The guidelines of this study were approved by the ethical review committee of the Bangladesh Medical Research Council (Approval no: BMRC/NREC/2013-2016/806). All human procedures followed were in accordance with the guidelines of the Helsinki Declaration of 1975.

Consent to participate

Written informed consent was obtained from all the participants.

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