A Genetic Clue to T2DM in Bangladesh: The *TCF7L2* rs12255372 (G/T) Variant

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Abstract

Objectives: The transcription factor 7 like 2 (TCF7L2) gene has been emerged as the most promising T2DM causing gene and the intronic variant rs12255372 of the TCF7L2 gene has demonstrated robust association with T2DM across various ethnic groups. The aim of this study was to determine the frequency of rs12255372 polymorphism in Bangladeshi adult population with T2DM and without diabetes.

Methods: This cross-sectional study was conducted in the Endocrinology department, BSMMU. Eighty patients with T2DM, and 80 normoglycemic controls without diabetes were studied. The rs12255372 polymorphism was genotyped using the PCR-RFLP technique.

Results: An allelic odds ratio of 3.29 (95% CI 1.78-6.05, p<0.001) was found for the minor T allele of rs12255372, which significantly increases the risk of T2DM. A significant difference in both TT and GT genotype was noted between participants with T2DM and normoglycemic controls (OR 5.26, 95% CI 1.39-19.9; p=0.008 and OR 3.00, 95% CI 1.33-6.75; p= 0.007 respectively). The dominant model appears to be the most suitable for representing the susceptibility gene effect.

Conclusions: The minor T allele frequency of rs12255372 among T2DM and NGT participants were about one-fourth and one-tenth respectively, indicating the rs12255372 polymorphism of the TCF7L2 gene may be associated with the risk of T2DM in the studied Bangladeshi population.

Keywords: Diabetes; Gene mutation; TCF7L2; rs12255372.

Introduction

Diabetes encompasses a group of heterogenous metabolic diseases characterized by chronic hyperglycemia. This hyperglycemia results from defects in insulin secretion, insulin action, or both. These defects are caused by a complex interplay of genetic and environmental factors which is linked to long-term damage, dysfunction and failure of different organs, particularly the eyes, blood vessels, heart, nerves and kidneys.[1] Majority of the diabetic patients are of type 2 diabetes mellitus (T2DM).[2] Usually, it develops as a result of interactions of genetic and environmental risk factors, which lead to relative deficiency of insulin with coexisting resistance to its activity.[1] Besides that, certain ethnic groups have an increased risk of developing T2DM, which has been declared as global epidemic by International Diabetes Federation. This multifaceted metabolic disorder stands out due to the inherent genetic predisposition within its population, a predisposition further exacerbated by the ongoing process of economic transition and rapid urbanization. Southeast Asia accommodates more than 90 million people with diabetes. Presently, Bangladesh ranks eighth in the list of countries with the highest prevalence of diabetes among adults aged 20 to 79, with 13.1 million documented cases. It is anticipated to ascend to seventh place by the year 2045.[2,3]

The etiology of T2DM is multifactorial, influenced by environmental, pathophysiological, and genetic factors. Genome-wide association studies have pinpointed numerous genes involved in T2DM, with the transcription factor 7-like 2 gene (*TCF7L2*) being one of the most susceptible.[4] TCF7L2 stands out for its strong influence on T2DM risk and has been extensively studied in genetic linkage studies.[5-9] Transcription factors are proteins that regulate gene expression by turning genes on or off. Located on chromosome 10q25.3, TCF7L2 is expressed in various organs, including fat cells, the liver, the gut, and pancreatic cells.[10] TCF7L2 is a key player in the Wnt signaling pathway, which plays a crucial role in cell development and function.[10] While the exact mechanism by which TCF7L2 polymorphisms contribute to T2DM remains under investigation, these variations are located in non-coding regions (introns) of the gene.[11] TCF7L2 and GLP-1 are known to be involved in blood sugar control, and it's hypothesized that TCF7L2 mutations may affect insulin action, increasing T2DM susceptibility.[12-14] Studies also suggest TCF7L2 polymorphisms might influence pancreatic beta cells, leading to problems with glucose production and tolerance.[15] The human *TCF7L2* gene has at least four well-studied polymorphic markers associated with T2DM. Notably, among the well-studied *TCF7L2* polymorphisms linked to T2DM are rs12255372 (G>T) in intron 4 and rs7903146 (C>T) in intron 3.[7]

Several studies investigated the association of genetic variants including *TCF7L2* that could increase the risk of developing T2DM. The three SNPs of *TCF7L2* (rs7903146 - intron 3, rs12255372 and rs11196205 - intron 4), individually and/or as a group, were found to be associated with T2DM in a wide spectrum of populations of different countries: Finnish,[5] U.S., Polish, Scandinavian, Chinese, Brazil, Japanese,[16] Swedish, U.K., Dutch, Palestinian, Iranian,[6] Arab,[17] Tunisian, Pakistan[9] as well as Indian populations.[8,18]

The link between T2DM and the SNP rs12255372, located in intron 4 of the *TCF7L2* gene, was initially identified through a microsatellite study conducted by Grant et al. in 2006. Subsequently, this correlation has been corroborated in numerous studies conducted on diverse populations worldwide, as evidenced by research conducted by Tong et al. (2009) and Peng et al. (2012).[7,19] However, it's worth noting that studies conducted by Alsmadi et al. (2008) and Guo et al. (2007), did not detect any significant association in this particular context.[20,21] Furthermore, a pharmacogenetic study conducted by Pearson et al. (2007) suggested that individuals harboring the rs12255372 variant of *TCF7L2* exhibited a suboptimal response to the widely prescribed oral anti-diabetic medication, Sulfonylurea.[22] These diverse outcomes underscore the imperative for further research into *TCF7L2* variants among individuals from various ethnic backgrounds, including the population of Bangladesh.

To date, published data regarding the role of *TCF7L2* SNPs in diabetes risk for the Bangladeshi population is scanty. Therefore, this study was aimed to determine the prevalence of rs12255372 (G>T) of *TCF7L2* in the adult Bangladeshi population with T2DM along with association of *TCF7L2* rs12255372 (G>T) with T2DM in this population.

Methods

This cross-sectional study included 80 T2DM patients and 80 normoglycemic controls without diabetes. The T2DM patients were recruited from the outpatient facilities of the department of Endocrinology of a medical university. The normoglycemic controls were age and sex-matched with the case population. T2DM-patients were diagnosed based on the standard criteria of American Diabetes Association, 2021. The study protocol was approved by Institutional Review Board of medical university. Written informed consent was obtained from each participant prior to their inclusion in the study. Patients diagnosed with type 1 diabetes and those with diabetes secondary to endocrinopathies, pancreatic diseases, or drug-induced diabetes were excluded from the study.

A comprehensive medical evaluation that included age, gender, blood pressure, and anthropometric measurements (weight, height, and body mass index) was performed on all T2DM patients and normoglycemic control participants. The weight in kilograms divided by the square of the height in meters was used to determine the body mass index or BMI. BMI was categorized as underweight (< 18.5 kg/m^2), normal weight (18.5–22.9 kg/m^2), or overweight (23.0–27.4 kg/m^2) or obese (≥ 27.5 kg/m^2).[23]

Genomic DNA was extracted from peripheral blood leukocytes using Gene JET Whole blood Genomic DNA Purification Mini Kit (Thermo Fisher, USA). Using a Qubit 2.0 fluorometer (Invitrogen, UK) and the Qubit dsDNA BR test kit 10, the amount of DNA was measured. Using Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP), the rs12255372 polymorphism was genotyped. In brief, the targeted region was amplified using the following primers: Forward 5′- CTG GAA ACT AAG GCG TGA GG -3′; Reverse 5′- GGG TCG ATG TTG TTG AGC TT -3′. The PCR cycles comprised of as follows: 5 minutes at 94°C, followed by 35 cycles of 30s at 94°C, 30s at 54°C and 30s at 72°C. The final extension was carried out at a temperature of 72°C for a duration of 10 minutes. Subsequently, 10 μL of the 346 bp amplified product was digested with the Tsp509I restriction enzyme (Thermo Fisher Scientific, Lithuania) for 3 h at 65°C. Following treatment with restriction enzymes, the amplicons underwent 2% agarose gel electrophoresis and were subsequently stained with ethidium bromide. The studied region usually has two restriction sites, the G>T allele change creating a new one. Direct sequencing was used to validate some genotyping results. The expected product sizes following digestion were as follows: for the normal homozygote GG, 143 bp and 104 bp; for the mutant homozygote TT, 126 bp and 104 bp; and for the heterozygote GT, 143 bp, 126 bp, and 104 bp, respectively. Fragments of the digested product that were smaller than 100 bp were not visible. DNA extraction and molecular genotyping was carried out at ‘Genetic Research Laboratory’ in Department of Anatomy, BSMMU, Bangladesh.

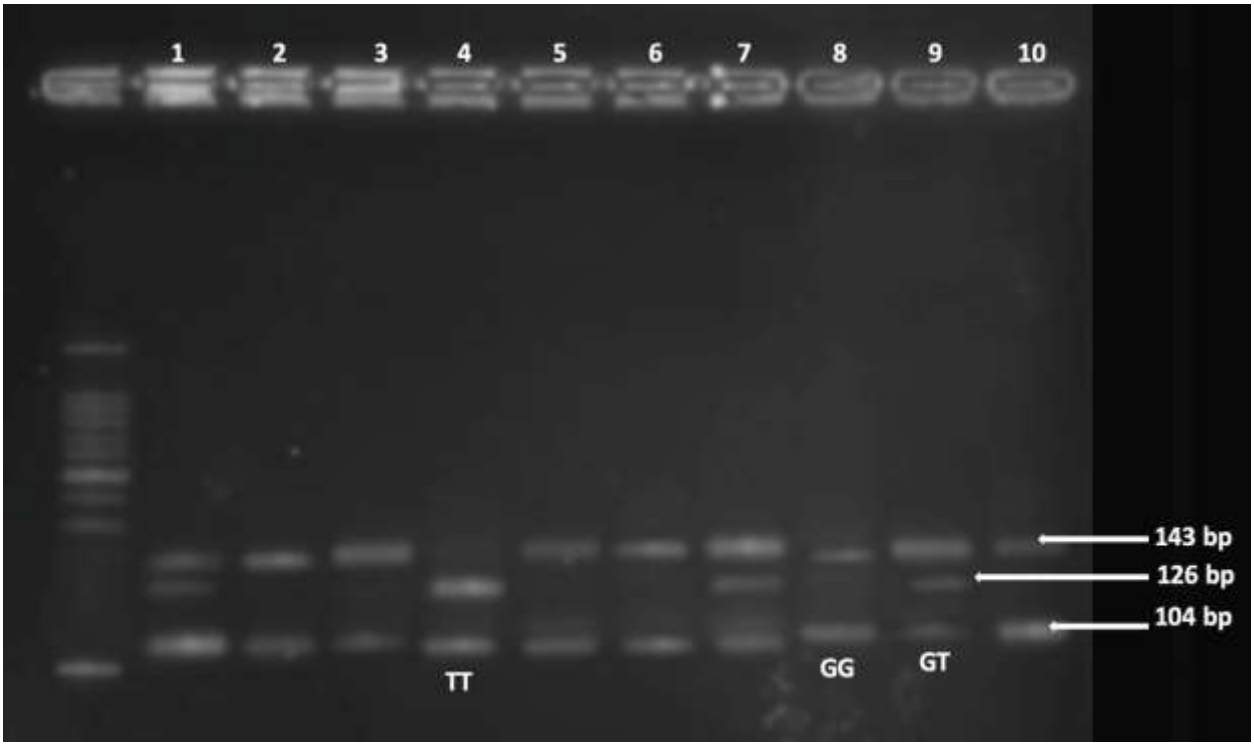

Figure 1: The PCR-RFLP analysis of *TCF7L2* rs12255372 (G>T) on 2% agarose gel using Tsp509I restriction enzyme in T2DM group. In the gel electrophoresis results, the presence of bands at 143 bp and 104 bp in lane GG signifies the wild genotype. Lane GT, with bands at 143 bp, 126 bp, and 104 bp, represents the heterozygous genotype. Lane TT, showing bands at 126 bp and 104 bp, corresponds to the homozygous (mutant) genotype.

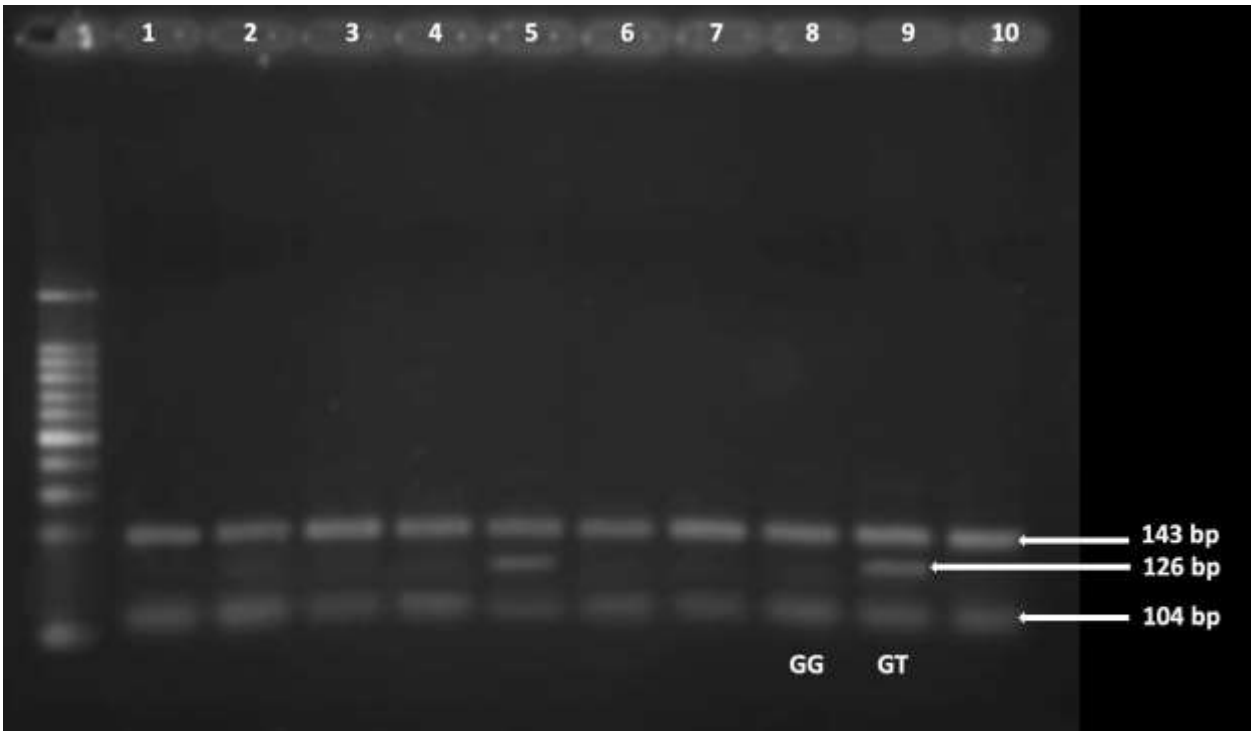

Figure 2: The PCR-RFLP analysis of *TCF7L2* rs12255372 in control group.

RFLP results were analyzed by gel electrophoresis to get the frequency of rs12255372 polymorphism. Qualitative values were expressed as frequencies and proportions. Quantitative values with a normal distribution were presented as mean (±SD). Quantitative variables with skewed distribution were described using median (IQR) values. The characteristics of cases and controls were compared using the Chi-square ($\chi 2$) test of independence, independent sample Student's t-test, and Mann–Whitney U test, as appropriate. The independent segregation of alleles was tested for Hardy-Weinberg equilibrium using the Chi-square ($\chi 2$) test of goodness of fit. The Hardy-Weinberg principle assumes that a population is found to be in equilibrium when the population is large, homogenous and is not experiencing notable mutation, migration, natural selection, or sexual selection and genetic variations will remain constant across generations.[24] The frequency of genotype between the T2DM and NGT groups were compared using the Chi-square ($\chi 2$) test of independence within different genetic models. Statistical significance was determined by *P* values that were less than 0.05, and the analysis of the data was conducted using SPSS Statistical software (version 26, SPSS).

Results

This cross-sectional study encompassed 80 T2DM patients (cases) and age and sex matched 80 participants with NGT (controls) to see the frequencies of SNP rs12255372 (G>T) genotype in them and to compare between the two groups including observing the allele frequency and standing with Hardy Weinberg equilibrium.

The median age of the studied participants was 44 (37.5- 50) years, and 50.6% of them were female. The age and gender of participants didn't differ between T2DM patients and NGT control group.

In the studied population, it was observed that the minor T allele of the *TCF7L2* rs12255372 variant significantly raised the risk of T2DM, with an allelic odds ratio (OR) of 3.29 (95% CI 1.78-6.05, p <0.001). T allele frequency was 28% in T2DM patients and 11% in normoglycemic controls.

Table 1: Genotype and allele frequency distribution of rs12255372 *TCF7L2* (G>T) in T2DM cases and normoglycemic controls.

Variables	**T2DM**	**NGT**	**OR (95% CI)**	**$\chi 2$**	***p*-value**
GG (%)	46 (57%)	66 (82%)	Ref.		
GT (%)	23 (29%)	11 (14%)	3.00† (1.33-6.75)	7.39	0.007*
TT (%)	11 (14%)	03 (04%)	5.26† (1.39-19.9)	7.06	0.008*
Major allele frequency (G)	115 (72%)	143 (89%)	Ref.		
Minor allele frequency (T)	45 (28%)	17 (11%)	3.29† (1.78-6.05)	15.68	<0.001*

(Within parenthesis are percentages over column total)
* P- *values were calculated using Chi-square test*
† OR (Odds ratio) was calculated using GG genotype as reference
TCF7L2*: Tranascription factor 7 like 2*
T2DM: Type 2 Diabetes mellitus NGT: normal glucose tolerance
G: Guanine T: Thymine

Comparison of genotype frequencies in patients with T2DM compared to normoglycemic controls revealed significant differences in the GT and TT genotypes, with odds ratios (OR) of 3.00 (95% CI 1.33-6.75; p = 0.007) and 5.26 (95% CI 1.39-19.9; p = 0.008) respectively. (Table 1).

To investigate which model would fit the effect of *TCF7L2* rs12255372, comparison of genotype frequencies between T2DM and NGT groups in different genetic models were considered (Table 2). Significant differences were observed between T2DM and NGT across all four genetic models used. Based on Akaike Information Criterion (AIC), the dominant genetic model was the best fit here, as AIC value was lowest in this model. According to the dominant genetic model, variant homozygotes + heterozygote (TT+GT) frequencies were 42.5% in T2DM and 17.5% in NGT group. Thus, the highest risk was observed under the dominant model with an odds ratio (OR) 3.48 (95% CI 1.68-7.21; p <0.001).

Table 2: Comparison of rs12255372 (G>T) genotype frequencies between T2DM and NGT (N=160).

Genetic Model	Total (N=160) n (%)	T2DM (n=80) n (%)	NGT (n=80) n (%)	OR (95% CI)	*p*-value	AIC
Co-dominant						
G/G	112 (70)	46 (57)	66 (82)	1.00	**0.001**	215
G/T	34 (21)	23 (29)	11 (14)	3.00 (1.33-6.75)		
T/T	14 (9)	11 (14)	03 (4)	5.26 (1.39-19.9)		
Over-dominant						
G/G + T/T	126 (78.7)	57 (71.2)	69 (86.2)	1.00	**0.019**	220.3
G/T	34 (21.3)	23 (28.8)	11 (13.8)	2.53 (1.13-5.63)		
Dominant						
G/G	112 (70)	46 (57.5)	66 (82.5)	1.00	**<0.001**	**213.6**
G/T + T/T	48 (30)	34 (42.5)	14 (17.5)	**3.48 (1.68-7.21)**		
Recessive						
G/G + G/T	146 (91)	69 (86.2)	77 (96.2)	1.00	**0.036**	220.5
T/T	14 (9)	11 (13.8)	03 (3.8)	4.09 (1.09-15.27)		

Genotype frequency expressed in 'n' & (%)
Comparison of genotype frequency between diabetes mellitus and control is done by Chi-square ($\chi 2$) test of independence.
AIC: Akaike Information Criterion

Genotype frequencies violated the Hardy-Weinberg equilibrium in T2DM ($\chi 2 = 6.67$, df = 1, $p = 0.01$) and control subjects ($\chi 2 = 6.09$, df = 1, $p = 0.01$).

The clinical and biochemical features of T2DM patients and normoglycemic individuals were categorized based on rs12255372 (G>T) genotypes (Table 3). Those having at least one variant type allele were found to have statistically significantly higher BMI, waist circumference (WC) and waist-hip ratio (WHR) compared to those having pair of wild type allele. (BMI: Wild vs Mutant: 23.5 (21.6, 25.7) vs 26.3 (24.4, 30.2); $p<0.001$, WC: Wild vs Mutant: 85.34 ± 9.00 vs 96.52 ± 10.81; $p<0.001$, WHR: Wild vs mutant: 0.92 ± 0.05 vs 0.97 ± 0.06; $p<0.001$).

Table 3. Association of rs12255372 variants with clinical parameters in study participants.

Variables	Wild type G/G (n=112)	Heterozygous + Variant G/T + T/T (n=48)	*p*-value
BMI (Kg/m^2) †	23.5 (21.6, 25.7)	26.3 (24.4, 30.2)	**<0.001**
WC (cm) #	85.34 ± 9.00	96.52 ± 10.81	**<0.001**
WHR #	0.92 ± 0.05	0.97 ± 0.06	**<0.001**
SBP (mmHg) †	120 (110, 125)	120 (120, 125)	0.98
DBP (mmHg) †	80 (70, 80)	80 (75, 85)	0.054
MBP (mmHg) †	90 (86.7, 95)	93.3 (90, 96.7)	**0.035**

 # Expressed in mean ± SD and comparison is done by independent sample student's t-test
† Data were expressed as median followed by interquartile range in parentheses and P *values were obtained by Mann-Whitney U test*
WC: waist circumference
WHR: Waist hip ratio

Discussion

T2DM and its associated compilations are regarded as significant health challenges of the 21st century.[25] Evidence from numerous studies strongly supports the notion that susceptibility to T2DM is heavily influenced by genetic factors.[26] Though there is high prevalence of T2DM in Bangladesh, limited epidemiological data on genetic predisposing factors of the disease is available.[27] Of all the identified variants, *TCF7L2* genetic variants have shown the most significant impact on the risk of T2DM, as reported in numerous studies.[28-30] The present study explored the relationship between the rs12255372 variant in the *TCF7L2* gene and T2DM within the Bangladeshi population.

In our study, among T2DM participants the observed genotype frequencies of rs12255372 wild type (GG), heterozygous (GT) and homozygous variant (TT) were 57%, 29% and 14% respectively. Among NGT participants, the observed genotype frequencies of rs12255372 wild type (GG), heterozygous (GT) and homozygous variant (TT) were 82%, 14% and 4% respectively. In this polymorphism, G is the major allele and T is the minor allele. The results of our study successfully replicate previous findings regarding the association between rs12255372 and T2DM.[6,25,27] Our study revealed the mutant T allele of rs12255372 had a frequency of 28%, which was comparable to the previous two studies on the Bangladeshi population done by Barman et al. and Salauddin et al., where the frequency was found 29% and 19% respectively[25,27] as well as populations of other countries including South East Asia like Pakistani population 29%,[31] population of Hyderabad, India 24%,[8] Nigerian population 26%,[32] the Iranian Kurdish ethnic group 18%[33] and the natives of India 26%.[34]

The aforementioned T allele was shown to be substantially linked with the incidence of T2DM, with an OR (odd ratio) of 3.29 (95% CI 1.78-6.05; P <0.001). Additionally, our study highlighted that homozygotes carry a significantly higher risk compared to heterozygote carriers. Therefore, our study demonstrates agreement with previous studies regarding the connection between risk alleles. Moreover, it conforms to the multiplicative model of inheritance, suggesting that the risk for homozygotes is higher compared to heterozygotes across all SNPs. When the genotypes were compared, the wild type homozygous GG genotype was predominant in NGT group, the heterozygous GT genotype and mutant TT homozygous genotype were more frequent in T2DM patients and the difference was significant (P <0.05).

The dominant, recessive and codominant models were used to assess the associated risk between rs12255372 (G>T) genotypes of *TCF7L2* gene and T2DM. In all three models, the TT and GT genotypes were found to confer a significant risk of susceptibility to T2DM. However, the dominant model showed the greatest risk of T2DM with an OR of 3.48 (95% CI: 1.68-7.21, P < 0.001). Overall, this study showed strong association between the rs12255372 (G>T) polymorphism of *TCF7L2* gene and T2DM and the finding is consistent with previous reports in Bangladesh and South-Asia region as well as other different ethnic and geographical populations across the world. However, a modest association was noted in West-Africa[35] and among Afro-Americans[36] with odds ratios (OR) below 2, whereas no association was detected in studies conducted on the Egyptian population,[37] Venezuelan population,[38] Arabic populations, South-African Zulu population,[39] Tunisian population[40] and in Brazilian population.[41] The observed regional variation may be attributed to a combination of genetic and environmental factors. Different populations may have distinct genetic backgrounds that influence the impact of this genetic variant. Additionally, lifestyle factors, dietary habits, rapid urbanizations and socioeconomic conditions can interact with genetic predisposition, contributing to varying T2DM susceptibility across regions.

The observed genotype frequencies were compared with expected counts for determining Hardy Weinberg Equilibrium of *TCF7L2* rs12255372 polymorphisms. Chi-square ($\chi 2$) test for Hardy Weinberg equilibrium for *TCF7L2* rs12255372 revealed a statistically significant P value. So, the genotype distribution of this polymorphism deviated from Hardy Weinberg Equilibrium. As interpreting RFLP-PCR data can be challenging at times, we opted to exclude all doubtful genotypes from our analysis. This was also reported in some of the previous studies.[42] This observation may be due to the limited sample size in our study or ongoing evolutionary processes.[24] A study by Dalhat et al. conducted on 567 Pakistani population revealed genotype frequencies of rs12255372 that was not in Hardy Weinberg equilibrium (HWE). Deviation from HWE in that study was confirmed by replicated genotyping using fresh reagents which yielded the same outcome.[31] Another study by Nanfa et al. on the Cameroonian population was also not in harmony with HWE.[42] After validating their genotype analysis by sequencing, authors concluded that the deviation from HWE could be due to small sample size or this polymorphism might have accumulated over generations which led to deviation from HWE. Therefore, it is imperative to replicate the findings of our study using

a larger sample size and more sensitive genotyping techniques, such as direct sequencing. This is particularly important given the occasional challenges in interpreting RFLP-PCR data. Careful selection of controls is necessary to prevent population stratification from creating confounding. Nevertheless, the high odds ratio and statistically significant findings in our study strongly suggest an association between rs12255372 *TCF7L2* and T2DM within our population.

To determine whether there is any interaction between rs12255372 (G>T) genotypes and clinical characteristics, the association between genotypes and covariates in study participants was studied. Our study found that participants carrying the GT + TT genotypes of the rs12255372 variation had a significantly higher BMI and waist-hip ratio (WHR) as compared with those with the GG genotype. This result is in accordance with the previously reported findings in an Iranian Kurdish ethnic group.[33] However, several other studies didn't demonstrate any association between *TCF7L2* rs12255372 polymorphism and obesity in the European and US populations.[43,44] A possible explanation for this incongruity could be difference in ethnic background or the effects of environmental factors. South Asian populations have experienced a shift towards Westernized diets, high in processed foods and low in nutrient-rich foods. Additionally, sedentary lifestyles, urbanization, and socioeconomic disparities have contributed to the rise in these health issues.

As a preliminary study, one of the limitations was the small sample size. This limitation might have restricted the distribution of genotypes in the population. Another limitation was the design of our study was cross-sectional study. To see association, case-control study would be better. However, all our participants were sourced from a public hospital offering services to individuals from diverse socioeconomic backgrounds. Hence, our subjects had a strong potential to be representative of the general population of Bangladesh.

Conclusion

Since there has been very scarce prior genetic exploration of the population in this region, especially regarding complex genetic disorders like T2DM, it becomes imperative to investigate the involvement of various candidate genes in the development of T2DM. We found the minor T allele frequency of rs12255372 (G>T) among T2DM and NGT participants were about one-fourth and one-tenth respectively, indicating the rs12255372 (G>T) polymorphism of the *TCF7L2* gene may be associated with the risk of T2DM in the studied Bangladeshi population. Further explorations should be aimed to uncover the diversity within the genetic predisposition of the population in this region, which is characterized by significant variations in geography, ethnicity, culture, and genetics.

Disclosure

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