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RESEARCH

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Association of *SLC30A8* rs13266634 gene polymorphism with type 2 diabetes mellitus (T2DM) in a population of Noakhali, Bangladesh: a case–control study

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Abstract

Background Type 2 diabetes mellitus (T2DM) is considered to be a polygenic disorder that emerges as a result of complicated gene-environment interactions. Several investigations revealed that *SLC30A8* rs13266634 polymorphism elevates T2DM risk. T2DM and hypertension (HTN) are often found to be coexist. Compared to normotensive non-diabetic controls, T2DM patients with HTN have a fourfold increased risk of cardiovascular disease (CVD). The average age of T2DM diagnosis is decreasing, and 'early onset of T2DM' in adolescents and young adults is an emerging worldwide health concern. The objective of this study was to examine the potential correlations of *SLC30A8* rs13266634 polymorphism with T2DM and T2DM-related CVD and HTN as well as 'early onset of T2DM' in the Noakhali region.

Methods This case–control study involved 163 T2DM patients and 75 healthy controls for analysis of *SLC30A8*-rs13266634 polymorphism. Genotyping of this polymorphism was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) method. MedCalc and Gene Calc programs were used for statistical analysis.

Results A statistically significant association of *SLC30A8* rs13266634 ($P < 0.05$) with T2DM was found in dominant, over dominant and allele models. But this study found no evidence of a connection between *SLC30A8*-rs13266634 with CVD, HTN, or 'early onset of T2DM' in any models. Furthermore, T2DM patients had higher total cholesterol (TC) and triglyceride (TG) levels than non-diabetics individuals.

Conclusions This study revealed a substantial association between the variation in *SLC30A8*-rs13266634 and the increased risk of developing T2DM within a sample of the Noakhali population in Bangladesh. However, no significant associations were observed between *SLC30A8*-rs13266634 and T2DM-related cardiovascular disease (CVD), hypertension (HTN), or the early onset of T2DM within this specific population.

Keywords *SLC30A8*, rs13266634, Polymorphism, Type 2 diabetes mellitus, PCR–RFLP

Background

Type 2 diabetes mellitus (T2DM) is a lifelong and chronic health concern that is greatly exacerbated by hereditary factors, peripheral insulin resistance, insufficient or inefficient pancreatic insulin synthesis and secretion, and environmental variables [1–4]. According to the

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International Diabetes Federation (IDF), about 463 million people, or 9.3% population of the world, had diagnosed with diabetes as of 2019, and incidence of diabetes is predicted to rise to 700 million by 2045 [5, 6]. Diabetes has recently become an alarming epidemic within the region of South East Asia [1, 7–10]. In Bangladesh, 8.4 million individuals have been diagnosed with diabetes in 2019; by 2045, around 15.0 million persons are anticipated to have this disease [5, 11]. T2DM was previously known as disease of older age, however, the current scenario of T2DM diagnosis is different. Now-a-days children and teenagers are also being affected by T2DM [12–17]. The estimated number of prediabetics in Bangladesh in 2019 was 3.8 million [5, 11]. Moreover, the co-occurrence of diabetes mellitus (DM) and hypertension (HTN) is also alarmingly increasing around the globe [5, 18–20]. HTN is seen in more than 50% of DM patients [18, 20, 21]. DM patients with HTN pose fourfold higher risk of cardiovascular disease (CVD) than normotensive non-diabetic controls [19, 20, 22].

T2DM is thought to be a polygenic disorder that arises from a complex interplay between a number of genes and environmental variables [23–26]. It is more likely that this genetic component is caused by single-nucleotide polymorphisms (SNPs) in a number of functionally important genes [23]. The zinc transporter solute carrier family 30 member 8 gene (*SLC30A8*) (Accession No: NM_001172811), has received significant interest as a putative candidate gene for T2DM risk due to its high expression in the pancreatic islets and β cells [1, 27, 28]. Under normal physiological conditions, the ZNT8 protein, encoded by the *SLC30A8* gene, assumes a pivotal role in preserving zinc homeostasis within pancreatic β cells. ZNT8 plays a crucial role in transporting zinc ions into insulin-containing vesicles, thereby participating in the formation and stability of insulin crystals [29]. These complexes of zinc and insulin are indispensable for the accurate storage of insulin and its controlled release in response to glucose levels. The ZNT8-mediated control of zinc within β cells constitutes an integral aspect of the physiological process of insulin secretion, ensuring the steadfastness and functionality of insulin before its eventual release into the bloodstream [30].

In the context of T2DM, the ZNT8 protein's significance lies in its association with genetic variations in the *SLC30A8* gene. Changes in ZNT8 function may contribute to the pathogenesis of T2DM by affecting zinc homeostasis within pancreatic β cells [31]. Altered ZNT8 activity could lead to disruptions in insulin crystallization, storage, and secretion, contributing to β cell dysfunction. The genetic link between *SLC30A8* and T2DM underscores the importance of ZNT8 in the intricate molecular mechanisms underlying the disease, providing insights into potential targets for therapeutic interventions aimed at preserving β cell function and glycemic control [1, 31].

Several studies reported that variations in the *SLC30A8* gene have been linked to an increased risk of T2DM and cardiovascular complications [27, 28, 31]. The *SLC30A8* gene's 13th exon carries a missense SNP termed rs13266634 that alters the nucleotides C to T, converting the amino acids arginine (R) to tryptophan (W) at position 325 (R325W) [1, 30–33]. Extensive studies revealed that there is a strong association between *SLC30A8*-rs13266634 (C > T) and T2DM in different population [27, 34–39]. However, there is a significant lack of knowledge on the genetic relatedness between T2DM and the related cardiovascular problems in Bangladeshi population. The prevalence of diabetic patients is continuously rising in Noakhali region of Bangladesh. In addition, young individuals in this region are now more likely to have T2DM than they were in the prior. To the best of our knowledge, no research has been conducted to determine whether *SLC30A8*-rs13266634 polymorphism is associated with 'early onset of T2DM' or HTN in the population of Noakhali region. As *SLC30A8* rs13266634 has been indicated as a risk factor for T2DM and CVD in a variety of ethnic groups, hence, this study aims to investigate the correlation of *SLC30A8* rs13266634 polymorphism with T2DM, T2DM-related CVD, HTN, and 'early-onset T2DM' in the population of Noakhali region of Bangladesh.

Material and methods

Study design and subject recruitment

This case–control study included 75 healthy controls and a total of 163 T2DM patients. The recruitment of T2DM patients was conducted at the Al-Haj Sirajul Islam Diabetic Hospital in Maijdee, Noakhali, Bangladesh. The case group comprised 163 patients diagnosed with T2DM, selected based on the standard criteria of the World Health Organization (WHO), which includes fasting blood glucose (FBG) levels greater than 7.0 mmol/l or random blood glucose (RBG) levels exceeding 11.1 mmol/l. From February 2022 to September 2022, in the presence of qualified physicians, a trained nurse conducted in-person interviews with each patient, obtaining detailed clinical information such as FBG, 2-h postprandial blood glucose (2 h PBG), RBG, and blood pressure (BP), along with physical details including sex, age, and BMI. Additionally, medical records were thoroughly reviewed during this period.

Patients with type I diabetes, liver failure, cancer, or other medical conditions predisposing them to hyperglycemia, such as thyroid disease, infections, glucose-altering medications, surgeries, women with gestational

diabetes, and patients in critical condition precluding their involvement in interviews, were exempted from this study. A control group consisting of 75 healthy non-diabetic individuals (median age, 39.74 ± 7.56) was carefully chosen from diverse locations within the Noakhali region. These individuals exhibited fasting blood glucose levels below 6.2 mmol/l and had no reported history of kidney or heart disease, as well as any other chronic conditions.

The study's purposes, its secrecy, the participants' ability to revoke their participation, and their responsibilities were well explained to the participants of this study. Prior to the assessment, each patient and healthy control submitted written informed consent. The Ethical Clearance Committee of the Science Faculty of Noakhali Science and Technology University approved the protocol of the study. The complete genetic analysis was carried out in the Laboratory of Molecular Biology, Department of Biotechnology and Genetic Engineering, Noakhali Science and Technology University, Bangladesh.

Clinical parameters

Patients with T2DM-related CVD who had at least one of the following pathological conditions -diabetic cardiomyopathy, coronary heart disease, or heart failure—were recruited. Out of 163 T2DM patients, T2DM-related CVD was identified in 66 patients (40.49%). If a patient had a blood pressure reading of 140/90 mmHg or above or was taking any antihypertensive medication, they were assumed to have hypertension. T2DM-related HTN was present in 86 patients (52.76%). 'Early onset of T2DM' had diagnosed in patients with diagnostic age < 40 years. We identified a total number of 45 patients (27.61%) with 'early onset of T2DM'. To document each clinical aspect, a questionnaire form was used.

Sampling and biochemical assays

Three ml of venous blood were drawn from each patient and healthy control by a skilled nurse and placed in sterile tubes containing ethylenediaminetetraacetic acid (EDTA)-Na_2. In the departmental laboratory, the samples were then separated into two distinct microcentrifuge tubes. One tube of whole blood samples was stored at -20 °C until DNA was extracted for SNP genotyping. The blood samples of the second tube were used for isolation of blood serum by centrifuging at 12,000 rpm for 10 min. The separated serum samples were then kept in a labeled microcentrifuge tube and used for the analysis of total cholesterol (TC) and triglyceride (TG). The TC and TG levels were assessed using the spectrophotometric approach with commercially available kits (Human-GmbH, Germany). The assays were performed in triplicate.

Molecular genotyping

The molecular genotyping of the *SLC30A8* rs13266634 polymorphism was carried out in both T2DM patients and the control sample using the Restriction Fragment Length Polymorphism (RFLP) method. Genomic DNA necessary for this analysis was extracted from whole blood samples of both patients and the control group, employing the FavorPrep Blood Genomic DNA Extraction Mini Kit from FAVORGEN BIOTECH CORP, Taiwan. Extracted genomic DNA was employed for the amplification of a specific region of *SLC30A8* (256 bp) by polymerase chain reaction (PCR). PCR was carried out using PCR Master Mix (2X) (GoTaq Green Master Mix-Promega Corporation) and the following primers:

Forward Primer: 5`- GAAGTTGGAGTCAGAGCA GTC-3'.

Reverse Primer: 5`-TGGCCTGTCAAATTTGGG AA-3'.

The following conditions were applied to conduct the PCR: Initial denaturation at 95 °C for 5 min, followed by 35 cycles of (a) 95 °C for 1 min (denaturation), (b) 53 °C for 30 s (annealing), (c) 72 °C for 1 min 30 s (elongation) and final elongation at 72 °C for 5 min.

Following DNA amplification, the resultant DNA fragments underwent restriction digestion utilizing the *Msp*I enzyme (NEB, England) at 37 °C overnight. The reaction mixture (20.2 µl), comprising 15 µl of PCR product, 2 µl of 10X Tango buffer, 0.2 µl of MspI enzyme, and 3 µl of nuclease-free water, facilitated the targeted cleavage of DNA based on the presence or absence of the polymorphism. Subsequently, the digested PCR products were separated on a 2.5% agarose gel containing ethidium bromide via electrophoresis. The resulting band patterns were visualized under a UV transilluminator, enabling the discrimination of various genotypes.

Statistical analysis

The Gene Calc and Medcalc Odd Ratio Calculator were applied for the statistical analysis. Using Gene Calc software's chi-square test (https://gene-calc.pl/), the deviation of variable allele frequencies in the control group from the patient group was evaluated in accordance with Hardy–Weinberg equilibrium (HWE). The genotype and allelic frequencies were presented as a percentage. The odds ratio (OR) and its 95% confidence intervals (CI) were also calculated using the Medcalc Odd Ratio Calculator (https://www.medcalc.org/calc/

odds_ratio.php). $P < 0.05$ was applied to assess the statistically significant value.

Results

Characteristic features of cases and healthy controls

A total of 163 T2DM patients and 75 healthy controls were enrolled in this case–control study. Among them, 61 (37.42%) were males, and 102 (62.58%) were females in the patient group, whereas 45 (60%) were males and 30 (40%) were females in the control group. Table 1 presents a summary of the clinical information and demographic details of the participants.

In terms of SBP, DBP, TC, and TG levels, we found substantial differences between T2DM patients and healthy controls. The control group displayed a mean SBP of 115 mmHg and a mean DBP of 72 mmHg. In contrast, T2DM patients exhibited values of 136.14 ± 17.12 for SBP and 82.71 ± 10.23 for DBP (Table 1). The observed variations in blood pressure could be linked to factors such as the age discrepancy between the control and T2DM patient groups. Notably, the control group, characterized by a mean age of 39.74 ± 7.56, is considerably younger than the T2DM patient group, which has a mean age of 55.28 ± 10.73. This age difference may play a contributory role in the deviations observed in both SBP and DBP values [40]. As it is well-established that blood pressure tends to rise with age, influenced by factors such as changes in blood vessel elasticity and overall cardiovascular health, the relatively younger age of the control group, as compared to the T2DM patient group, may exert an influence on the mean SBP and DBP values in our study [41].

Age-related alterations in lipid and lipoprotein levels are extensively documented and hold considerable importance in cardiovascular health. As individuals age, shifts in lipid metabolism lead to changes in the composition and concentration of diverse lipids and lipoproteins in the bloodstream. Recognizing these changes is crucial, particularly in individuals without pre-existing cardiac disorders [42, 43]. TC levels typically rise with age, partly due to shifts in hormonal profiles, including a decline in estrogen among women and alterations in androgen levels in men. This age-related increase in TC is a concern, as elevated levels are linked to a higher risk of atherosclerosis and cardiovascular diseases. Concurrently, TG levels also tend to rise with age, influenced by factors like changes in dietary habits, physical activity, and hormonal fluctuations. Elevated TG is associated with insulin resistance and metabolic syndrome, both contributing to cardiovascular risk [43, 44]. Hence, aging significantly influences lipid metabolism, contributing to commonly observed changes in lipid profiles, such as increased levels of TC and TG [42]. The T2DM patients group, with a mean age of 55.28 ± 10.73, is expected to exhibit age-related changes in lipid levels. The control group, with a mean age of 39.74 ± 7.56, represents a younger population, and age-related changes in lipid levels may be less pronounced compared to the older T2DM without cardiac disorders group.

Impacts of the SLC30A8-rs13266634 in T2DM

Successful amplification of the targeted *SLC30A8* fragment containing the rs13266634 polymorphism (256 bp) was achieved via PCR for both patient and control samples (Fig. 1). Subsequent application of the *Msp*I enzyme to the PCR product revealed distinct patterns of DNA bands, as detailed in Table 2 and visually represented in Fig. 2.

For both the case and healthy control groups, we conducted an examination of the Hardy–Weinberg

Table 1 Clinical and socio-demographic variables of T2DM patients and healthy controls

Variables	T2DM patients (n = 163)	Healthy controls (n = 75)	Normal range
Age (years) (± SD)	55.28 ± 10.73	39.74 ± 7.56	NA
Sex (male/female)	61/102	45/30	NA
BMI (kg/m^2) (± SD)	25.32 ± 6.62	22.43 ± 3.19	18.5–24.9
Age of Onset of T2DM (years) (± SD)	44.84 ± 12.18	NA	≥ 40
FBG (mmol/l) (± SD)	10.0 ± 3.82	NA	3.9–5.6
2 h PBG (mmol/l) (± SD)	14.54 ± 4.83	NA	< 7.8
RBG (mmol/l) (± SD)	12.69 ± 4.59	NA	< 11.1
SBP (mmHg) (± SD)	136.14 ± 17.12	115 ± 7.07	120–129
DBP (mmHg) (± SD)	82.71 ± 10.23	72 ± 7.07	80–84
TC (mg/dl) (± SD)	202.62 ± 74.47	156.51 ± 57.24	< 200
TG (mg/dl) (± SD)	192.35 ± 105.94	148.53 ± 114.46	< 150

BMI body mass index, *FBG* fasting blood glucose, *2 h PBG* 2 h postprandial blood glucose, *RBG* random blood glucose, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *TC* total cholesterol, *TG* triglycerides, *NA* not available

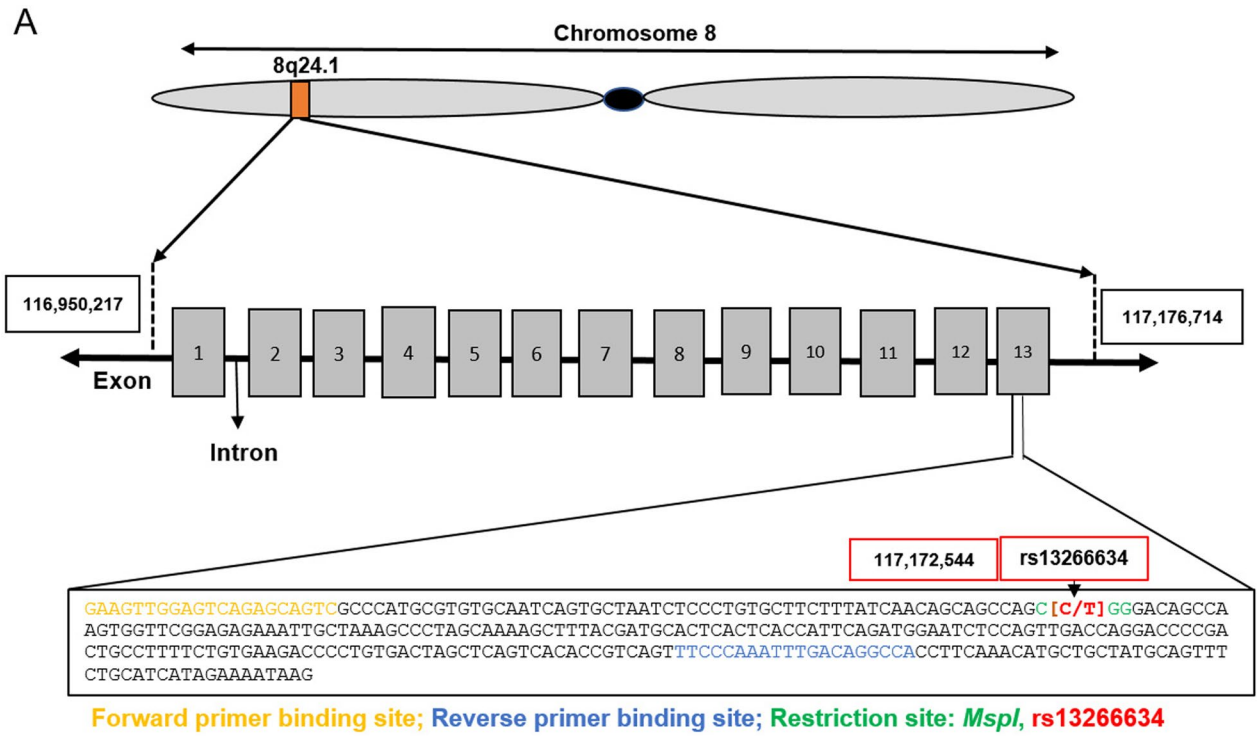

Fig. 1 **A** Physical map of *SLC30A8*-rs13266634 with *MspI* restriction site. The *SLC30A8* gene is located on the long arm of human chromosome 8, at position 8q24.11 (occupied on 116,950,217 – 117,176,714 location). *SLC30A8* gene has 13—exon (dark boxes). The last exon carries SNP rs13266634 (location; 117,172,544). Introns are indicated in linear line. Nucleotides marked in orange color indicates the binding site of forward primer, Nucleotides in blue color denotes the binding site of reverse primer, green color nucleotides indicates restriction site of *MspI* (#CCGG) and red color nucleotide represents rs13266634 of *SLC30A8* gene. **B** Amplified PCR product of rs13266634 covering *SLC30A8* gene fragment (256 bp). Samples were electrophoresed on 2.5% agarose gel. Lane M: 100 bp Ladder, Lane (1–19): PCR products (256 bp)

equilibrium (HWE), and our findings confirm that the genotype frequencies of all participants were in accordance with Hardy–Weinberg equilibrium (Table 3).

The frequency of (CC–CT–TT) genotypes for the patients' group was (68.09%–27.61%–4.29%), and for the control group was (34.67%–58.67%–6.67%), and the difference of CT genotype between the two groups was statistically significant (p value < 0.0001).

To evaluate the effect of *SLC30A8*- rs13266634 polymorphism on T2DM, dominant, recessive, over dominant and allele models were tested.

Table 2 Restriction digestion of PCR products of *SLC30A8* gene with *MspI* enzyme

Fragment Name	Fragment size (bp)
CC	46 bp, 210 bp
CT	46 bp, 210 bp, 256 bp
TT	256 bp

SLC30A8 rs13266634 demonstrated a significant relationship with T2DM in dominant, over dominant and allele models (p value < 0.0001). The odds ratio (OR) and p value were calculated for each model (Table 4). The frequency of (C–T) alleles was 81.90–18.09% in the patients' group, and 64 – 36% in the control group, respectively. The difference was shown to be statistically significant (p value < 0.0001) (Table 4). This suggests that the C allele is more prevalent among individuals with T2DM. Upon conducting additional analysis, it was found that the odds ratio (OR) associated with the CC genotype is greater than 1, signifying a statistically significant association with an increased predisposition to T2DM.

Genotypic association of CVD in T2DM patients

We investigated the association between T2DM-related CVD and the *SLC30A8* rs13266634 polymorphism in T2DM patients. In both the T2DM with CVD and T2DM without CVD groups, we assessed the Hardy–Weinberg equilibrium (HWE), and we observed that all participant genotype frequencies were in alignment with HWE (Additional File 1: Table S1).

To evaluate the impact of the *SLC30A8*-rs13266634 polymorphism on T2DM-related CVD, various genetic models including dominant, recessive, over-dominant, and allele models were examined. Despite analyzing each model, neither the odds ratio nor the p-value indicated

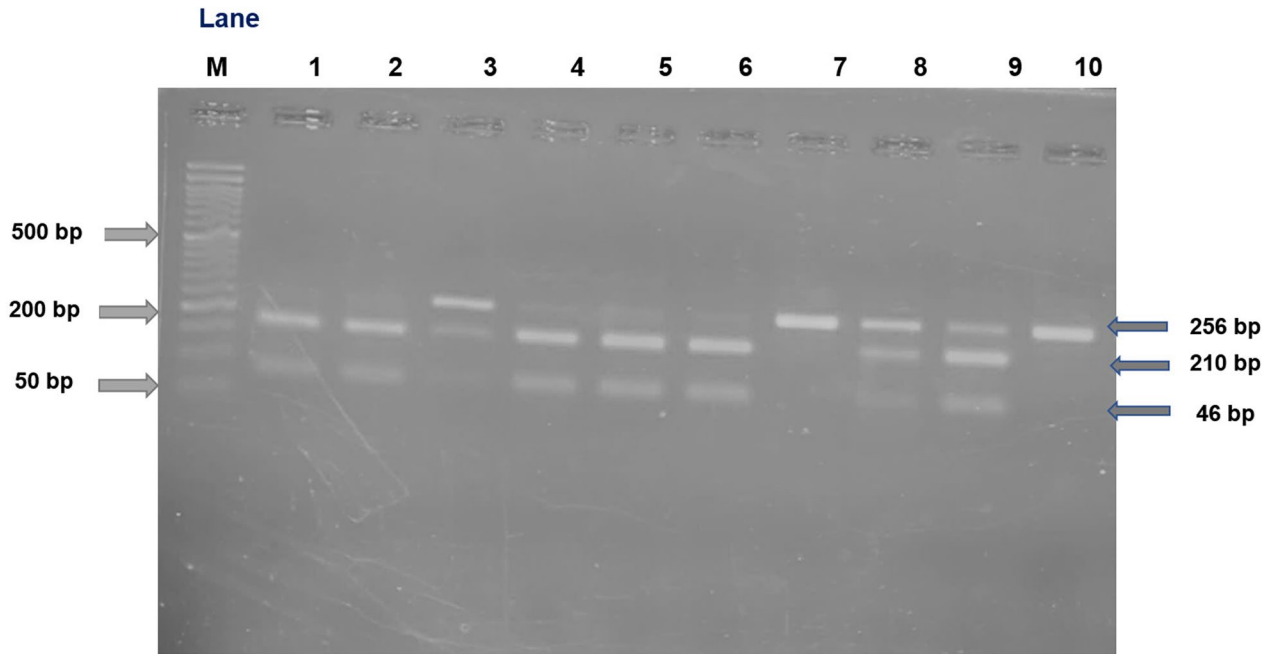

Fig. 2 Restriction digestion products of *SLC30A8*-rs13266634 PCR fragment by *MspI* restriction enzyme. Samples were electrophoresed on 2.5% agarose gel, Lane M: 50 bp ladder, Lane (1, 2, 4, 5, 6): CC form (46 and 210 bp), Lane (3, 8, 9): CT form (46, 210 and 256 bp), and Lane 7, 10: TT form (256 bp)

Table 3 Genotype distribution of *SLC30A8*-rs13266634 among T2DM patients and controls

SLC30A8 rs13266634	Patients (n = 163)	χ2	*p* value	Controls (n = 75)	χ2	*p* value
CC	111 (68.09%)	0.7704	0.68031	26 (34.67%)	5.59574	0.06094
CT	45 (27.61%)			44 (58.67%)		
TT	7 (4.29%)			5 (6.67%)		

If $p > 0.05$, it is consistent with Hardy Weinberg Equilibrium

Table 4 *SLC30A8*-rs13266634 genotypes characteristics among T2DM patients and controls

***SLC30A8* rs13266634**	**Patients (n = 163)**	**Controls (n = 75)**	**OR (95% CI)**	***p* value**
CC	111(68.09%)	26 (34.67%)	Reference	
CT	45 (27.61%)	44 (58.67%)	0.2396 (0.1320–0.4347)	$p < 0.0001$
TT	7 (4.29%)	5 (6.67%)	0.3279 (0.0964–1.1158)	$p = 0.0743$
Dominant model (CT + TT vs. CC)				
CC	111 (68.09%)	26 (34.67%)	Reference	
CT + TT	52 (31.90%)	49 (65.33%)	0.2486 (0.1394 -0.4433)	$p < 0.0001$
Recessive model (TT vs. CC + CT)				
CC + CT	156 (95.71%)	70 (93.33%)	Reference	
TT	7 (4.29%)	5 (6.67%)	0.6282 (0.1927–2.0481)	$p = 0.4407$
Over dominant model (CC + TT vs. CT)				
CC + TT	118 (72.38%)	31 (41.34%)	Reference	
CT	45 (27.61%)	44 (58.67%)	0.2687 (0.1514–0.4769)	*p < 0.0001*
Allele model				
C	267 (81.90%)	96 (64%)	Reference	
T	59 (18.09%)	54 (36%)	0.3928 (0.2539–0.6079)	$p < 0.0001$

Here, if $p < 0.05$, it is considered as statistically significant; OR: Odds Ratio; 95% CI: 95% Confidence Intervals

Table 5 Association of *SLC30A8*-rs13266634 alleles or genotype with risk of T2DM-related CVD in T2DM patients of Noakhali

***SLC30A8* rs13266634**	**T2DM with CVD (n = 66)**	**T2DM without CVD (n = 97)**	**OR (95% CI)**	***p*-value**
CC	43 (65.15%)	68 (70.10%)	Reference	
CT	20 (30.30%)	25 (25.77%)	1.2651 (0.6275–2.5505)	0.5109
TT	3 (4.55%)	4 (4.12%)	1.1860 (0.2530–5.5597)	0.8286
Dominant model (CT + TT vs. CC)				
CC	43 (65.15%)	68 (70.10%)	Reference	
CT + TT	23 (34.85%)	29 (29.89%)	1.2542 (0.6435–2.4445)	0.5059
Recessive model (TT vs. CC + CT)				
CC + CT	63 (95.45%)	93 (95.88%)	Reference	
TT	3 (4.55%)	4 (4.12%)	1.1071 (0.2396–5.1169)	0.8963
Over dominant model (CC + TT vs. CT)				
CC + TT	46 (69.70%)	72 (74.22%)	Reference	
CT	20 (30.30%)	25 (25.77%)	1.2522 (0.6251–2.5082)	0.5258
Allele model				
C	106 (80.30%)	161 (82.98%)	Reference	
T	26 (19.69%)	33 (17.01%)	1.1967 (0.6771–2.1149)	0.5366

Here, if $p < 0.05$, it is considered as statistically significant

OR Odds Ratio, *95% CI* 95% Confidence Intervals

a significant association between *SLC30A8*-rs13266634 and the risk of T2DM-related CVD (Table 5).

Genotypic association of HTN in T2DM patients

The correlation between T2DM-related HTN and the *SLC30A8*-rs13266634 polymorphism in T2DM patients was also investigated. We assessed the HWE in both the T2DM with HTN and T2DM without HTN groups, and we found that all participant genotype frequencies were in HWE (Additional File 1: Table S2).

The *SLC30A8*-rs13266634 polymorphism's effects on T2DM-related HTN were investigated employing dominant, recessive, over dominant and allele models. For each model, the odd ratio and *p* value were calculated. *SLC30A8* rs13266634 did not demonstrate a significant

Table 6 Association of *SLC30A8*-rs13266634 alleles or genotype with risk of T2DM-related HTN in T2DM patients of Noakhali

SLC30A8 rs13266634	T2DM with HTN (n = 86)	T2DM without HTN (n = 77)	OR (95% CI)	*p*-value
CC	63 (73.26%)	48 (62.34%)	Reference	
CT	18 ((20.93%)	27 (35.06%)	0.5079 (0.2510–1.0278)	0.0596
TT	5 (5.81%)	2 (2.59%)	1.9048 (0.3542–10.2436)	0.4528
Dominant model (CT + TT vs. CC)				
CC	63 (73.26%)	48 (62.34%)	Reference	
CT + TT	23 (26.74%)	29 (37.66%)	0.6043 (0.3112 -1.1735)	0.1369
Recessive model (TT vs. CC + CT)				
CC + CT	81 (94.19%)	75 (97.40%)	Reference	
TT	5 (5.81%)	2 (2.59%)	2.3148 (0.4359–12.2925)	0.3245
Over dominant model (CC + TT vs. CT)				
CC + TT	68 (79.07%)	50 (64.93%)	Reference	
CT	18 (20.93%)	27 (35.06%)	0.4902 (0.2436–0.9864)	0.0457
Allele model				
C	144 (83.72%)	123 (79.87%)	Reference	
T	28 (16.28%)	31 (20.13%)	0.7715 (0.4386–1.3572)	0.3680

Here, if $p < 0.05$, it is considered as statistically significant

OR Odds Ratio, *95% CI* 95% Confidence Intervals

correlation to T2DM-related HTN risk in any examined genetic association models, (Table 6).

Genotypic association of 'early onset of T2DM' in T2DM patients

We explored the association between the 'early onset of T2DM' and the *SLC30A8*-rs13266634 polymorphism in T2DM patients. In both the 'T2DM with early onset' and 'T2DM without early onset' groups, we assessed HWE, and our analysis indicated that the genotype frequencies of all participants in both groups were consistent with HWE (Additional File 1: Table S3).

To investigate the effect of *SLC30A8*-rs13266634 polymorphism on 'early onset of T2DM,' dominant, recessive, over dominant and allele models were examined. For each model, we calculated the odds ratio (OR) and *p*-value. However, none of the analyzed genetic association models revealed a significant relationship between *SLC30A8*-rs13266634 and the risk of 'early onset of T2DM' (Table 7).

Discussion

T2DM is a significant medical issue and one of the most prominent non-infectious illnesses in the world. It is thought that T2DM is caused by a variety of intricate genetic variables [23, 45, 46]. Thus, it is crucial to look into these genetic variants that are linked to the risk of developing T2DM. According to earlier GWAS studies, *SLC30A8* has been connected to the emergence of T2DM, insulin resistance, as well as other diabetic-related disorders like CVD and the insulin secretion pathway [1, 27]. Moreover, GWAS have demonstrated a correlation between T2DM and specific gene loci, such as rs13266634 in the *SLC30A8* gene [1, 27, 28, 47]. The amino acid shift from arginine (R) to tryptophan (W) at position 325 (Arg325Trp) caused by the non-synonymous SNP rs13266634 of *SLC30A8* may contribute to impaired glucose regulation. Hence, a sample of the Noakhali population was included in this study to examine the association between the *SLC30A8*-rs13266634 polymorphism and T2DM since there hasn't been much genetic research on this disease on the population of this area in Bangladesh.

This study found that the frequency of CT genotype between T2DM patients and controls groups were 27.61% and 58.67%, respectively. It was higher in controls group than in the patients' group and the difference was statistically significant ($P < 0.0001$) (Table 4). Unlike our findings, among the Chinese and Indian populations, it was discovered that the frequency of the CT genotype was higher in the patient group than in the control group [28, 35]. However, our findings are comparable with the result of western Asian populations, such as Palestinian and Jordanian populations where controls' CT genotype was found greater than the patients' group [33, 48]. In our study population, C allele frequency was found higher in the patient group (81.90%) compared to the control group (64%). In contrast, T allele frequency was found higher in the control group (36%) than in the patient group (18.09%) (Table 4).

Table 7 Association of alleles or genotype with risk of 'T2DM-related early onset' in T2DM patients of Noakhali

SLC30A8 rs13266634	T2DM with 'early onset of T2DM' (n = 45)	T2DM without 'early onset of T2DM' (n = 118)	OR (95% CI)	*p*-value
CC	32 (71.11%)	79 (66.95%)	Reference	
CT	12 (26.67%)	33 (27.97%)	0.8977 (0.4124–1.9544)	0.7858
TT	1 (2.22%)	6 (5.08%)	0.4115 (0.0476–3.5554)	0.4196
Dominant model (CT + TT vs. CC)				
CC	32 (71.11%)	79 (66.95%)	Reference	
CT + TT	13 (28.89%)	39 (33.05%)	0.8229 (0.3887–1.7423)	0.6106
Recessive model (TT vs. CC + CT)				
CC + CT	44 (97.78%)	112 (94.91%)	Reference	
TT	1 (2.22%)	6 (5.08%)	0.4242 (0.0496–3.6260)	0.4335
Over dominant model (CC + TT vs. CT)				
CC + TT	33 (73.33%)	85 (73.03%)	Reference	
CT	12 (26.67%)	33 (27.97%)	0.9366 (0.4322–2.0298)	0.8682
Allele model				
C	76 (84.44%)	191 (80.93%)	Reference	
T	14 (15.56%)	45 (19.07%)	0.7819 (0.4057–1.5068)	0.4623

Here, if $p < 0.05$, it is considered as statistically significant

OR Odds Ratio, *95% CI* 95% Confidence Intervals

The dominant, over-dominant, and allele models ($p < 0.0001$) were identified as statistically significant when various models were employed to assess the association between the *SLC30A8* rs13266634 polymorphism and the risk of T2DM. In dominant model CT + TT genotype showed association with the T2DM risk compared with CC genotype with an OR ratio of 0.2486 (95% CI 0.1394 -0.4433), and the result was statistically significant ($p < 0.0001$). In over dominant model CT genotype showed association with the T2DM risk compared with CC + TT genotype with an OR ratio of 0.2687 (95% CI 0.1514–0.4769) and the result was statistically significant *($p < 0.0001$).* In allele model T allele showed association with T2DM risk compared with C allele with an OR ratio of 0.3928 (95% CI 0.2539–0.6079), and result was statistically significant ($p < 0.0001$) (Table 4).

Coexistence of T2DM with HTN is frequently observed [5, 19, 20, 49]. Diabetes patients often have high BP since the prevalence of it relies on the kind and length of the diabetes, as well as on gender, age, BMI, and the presence of renal disease [50]. Having diabetes and HTN together elevates the likelihood of mortality by 7.2 times, with a larger mortality risk in developing nations [18]. As a result, we investigated the association of *SLC30A8* rs13266634 polymorphism for the risk of HTN in our T2DM patient. However, we found no significant evidence of a substantial association between *SLC30A8*-rs13266634 and HTN in our population.

The risk of cardiovascular complications is greatly increased in T2DM patients due to HTN, high TC, and TG levels [1, 51]. According to research on DM patients, having HTN raises the risk of death and cardiovascular events by 44% and 41%, respectively, as compared to 7% and 9% for those who have only diabetes [18]. Nearly 32.2% of patients with T2DM worldwide are affected by CVD which is the most common cause of death for T2DM patients [22, 52]. Due to this reason, we were interested to investigate the association of *SLC30A8*-rs13266634 polymorphism for the risk of cardiovascular complications in our T2DM patients. However, we found no conclusive evidence that *SLC30A8*-rs13266634 was associated with the cardiovascular problems in this population.

T2DM was often considered an illness only affecting the elderly, but as it becomes more prevalent, adolescents and teenagers are increasingly impacted [12, 14]. Due to the lack of routine checkups, the age of onset of T2DM in the Noakhali population is not clearly anticipated. T2DM patient's sometimes goes untreated for three to ten years before suddenly becoming chronic diabetic patient with elevated glucose levels [53, 54]. As a result, we examined *SLC30A8* rs13266634 polymorphism for the risk of 'early onset of T2DM' in our T2DM patients. But in our population, we found no statistically significant evidence of a correlation between *SLC30A8*-rs13266634 and 'early onset of T2DM'.

It is well understood that T2DM is a complex disorder affected by a number of genetic and environmental factors, and that individual variances in environmental factors cause different people to experience the same

genetic factor's effects on the development of T2DM differently [23]. Given that 60.93% of the participants in this study hail from rural areas, it is noteworthy that they generally exhibit suboptimal nutritional habits and possess limited awareness of health issues— with 12% being illiterate and 24% enrolled in primary school. The pronounced impact of these environmental factors could potentially amplify the influence of genetic factors among carriers of risk alleles. It is important to note that our study was conducted on a relatively small population from the Noakhali district of Bangladesh, which could have an impact on the level of significance. Large prospective studies from the different regions of Bangladesh, as well as future research on gene–gene and gene-environment interactions, are needed for a more in-depth conclusion and practical result.

Conclusion

This study demonstrated that *SLC30A8*-rs13266634 is a significant risk factors for T2DM in a sample of the Noakhali population. However, *SLC30A8*-rs13266634 was not identified as a risk factor for T2DM-related CVD, HTN, or 'early onset of T2DM' in this sample. In conclusion, it is important to acknowledge the significant limitation of this study was small sample size. Despite this constraint, our study provides valuable preliminary insights into the potential association of rs13266634 with cardiovascular changes and early-onset T2DM.

Abbreviations

BMI	Body mass index
BP	Blood pressure
CVD	Cardio vascular disease
DM	Diabetes mellitus
DBP	Diastolic blood pressure
FBG	Fasting blood glucose
HTN	Hypertension
RBG	Random blood glucose
RFLP	Restriction fragment length polymorphism
SBP	Systolic blood pressure
SNP	Single nucleotide polymorphism
T2DM	Type 2 diabetes mellitus
TC	Total cholesterol
TG	Tri-glyceride
2 h PBG	2 H postprandial blood glucose

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43042-024-00484-8.

Additional file 1. Supplementary Tables 1–3.

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Author contributions

Conceptualization: FSM & SDG. Data curation: FSM & SDG. Formal analysis: FSM. Methodology: FSM. Writing – original draft: FSM. Writing – review & editing: SDG, MMH, MMI, SCD, DNB.

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Availability of data and materials

All data analyzed during this study are included in this article.

Declarations

Ethics approval and consent to participate

The study protocol and questionnaire were approved by the Ethical Clearance Committee, Faculty of Science of the Noakhali Science and Technology University. Furthermore, both the cases and controls gave their signed consent to take part in this study after a briefing session.

Consent for publication

Not applicable.

Competing interests

No potential conflict of interest relevant to this article was reported.

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