Heliyon 7 (2021) e08376

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Risk of type 2 diabetes mellitus and cardiovascular complications in *KCNJ11*, *HHEX* and *SLC30A8* genetic polymorphisms carriers: A case-control study

Tutun Das Aka [a,1], Urmi Saha [a,1], Sayara Akter Shati [a,1], Md. Abdul Aziz [a,b], Mobashera Begum [a], Md. Saddam Hussain [a], Md. Shalahuddin Millat [a,b], Mohammad Sarowar Uddin [a,b], Mohammad Safiqul Islam [a,b,*]

[a] Department of Pharmacy, Faculty of Science, Noakhali Science and Technology University, Sonapur, 3814, Noakhali, Bangladesh
[b] Laboratory of Pharmacogenomics and Molecular Biology, Department of Pharmacy, Noakhali Science and Technology University, Sonapur, 3814, Noakhali, Bangladesh

ARTICLE INFO

Keywords:
Type 2 diabetes mellitus
Cardiovascular disease
KCNJ11
HHEX
SLC30A8
Polymorphism

ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) are two deadly diseases caused by the complex interaction of multiple genetic loci, lifestyle and environmental factors. Genome-wide association studies described hundreds of susceptibility loci for T2DM and T2DM-related CVD, but it remains uncertain due to geographic and ethnic variations. The objective of this study was to evaluate the associations of *KCNJ11* rs5219, *SLC30A8* rs13266634 and *HHEX* rs1111875 polymorphisms with T2DM and related CVD.

Methods: Genotyping of all three polymorphisms was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method on 250 T2DM cases and 246 healthy controls. Both descriptive and inferential statistical methods were applied using MedCalc and IBM SPSS software programs for statistical analyses.

Results: A significantly increased association of KCNJ11 rs5219 ($p<0.05$) with T2DM was found in dominant, recessive, heterozygote, homozygote, and allele model (aOR = 2.23, 2.03, 1.90, 3.09, and 1.80, respectively). For *SLC30A8* rs13266634, only dominant, heterozygote, and allele model (aOR = 3.37, 3.59, and 1.79, respectively) showed significantly increased association with T2DM. SNP rs1111875 (*HHEX*) also revealed 2.08, 4.18, 5.93, and 2.08-times significant association in dominant, recessive, homozygote, and allele models. Besides, a significantly reduced correlation of *KCNJ11* rs5219 was found with T2DM-related CVD in the recessive and allele model (aOR = 0.40 and 0.65, respectively). Again, a significant difference was observed between T2DM-related CVD and non-CVD patients in terms of gender distribution, fasting blood glucose (FBG), systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol (TC), and triglycerides (TG).

Conclusions: Our investigation indicates that *KCNJ11* rs5219, *SLC30A8* rs13266634 and *HHEX* rs1111875 polymorphisms are associated with T2DM. Moreover, *KCNJ11* rs5219 polymorphism is correlated with the risk of T2DM-related CVD.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a global chronic and lifelong health problem mainly caused by genetic factors, deficiency or ineffectiveness of pancreatic insulin production and secretion, peripheral insulin resistance, and environmental factors [1, 2, 3]. The prevalence of T2DM patients has been drastically increasing around the world, more specifically, in low to middle economic countries. In 2017, more than 69 million patients were Bangladeshis out of 425 million diagnosed patients with diabetes worldwide, with a prevalence of 6.9%, and surprisingly 90% of them had T2DM [4, 5]. It has recently turned into a shocking epidemic in South East Asia, where the Indian subcontinent, including Bangladesh and China, has the highest incidence rate and the number is rapidly rising [6, 7, 8].

* Corresponding author.
E-mail address: research_safiq@yahoo.com (M.S. Islam).
[1] These authors contributed equally.

https://doi.org/10.1016/j.heliyon.2021.e08376
Received 12 July 2021; Received in revised form 15 October 2021; Accepted 10 November 2021

Different environmental and genetic factors as well as their complex interactions are involved in T2DM development [9, 10, 11]. T2DM creates a significant burden on public health care by producing a variety of complications that can damage the heart, kidney, eyes, and others. Genome-wide association studies (GWAS) have recently identified more than 100 loci for susceptibility to T2DM and identified different genetic markers that are significantly associated with obesity, diabetes, and cardiovascular disease (CVD) [12, 13, 14, 15, 16, 17]. Moreover, many other studies have shown that different genes are involved in the metabolism of glucose, beta-cell function, and insulin secretion pathways in a single and combined way, which ultimately results in T2DM [18, 19].

The electrical activity of insulin secretion can be limited by different ion channels of plasma membrane-like ATP-sensitive potassium (KATP) channel [20]. Potassium inwardly-rectifying channel (*KCNJ11*)-11th member of J subfamily gene belongs to the potassium ion channel. *KCNJ11* makes up the compartment of the KATP channel, up-regulating insulin secretion in their inhibition condition [21, 22]. After point mutation in the rs5219 locus of *KCNJ11*, thymine (T) takes the place of cytosine (C) by substitution at the NH_2- terminal of Kir6.2 and 15^{th} intron, respectively that attenuate the sensitivity of the channel to ATP [3, 22]. Previous studies have evaluated the role *KJNJ11* polymorphisms and the risk of T2DM and associated diseases [3, 8, 15].

In human chromosome 10, many polymorphisms of the 350 kb linkage disequilibrium (LD) block are inextricably associated with insulin secretion, T2DM development, and increased glucagon secretion. Haematopoietically expressed homeobox or *HHEX* is one of the three genes of LD block, and it contains an insulin-degrading enzyme [23, 24, 25]. The rs1111875 is a single nucleotide polymorphism (SNP) in the 3′ flanking regions of the *HHEX* gene and after polymorphism, it causes T2DM [26]. The eighth member of solute carrier family 30 (*SLC30A8*) or Zinc transporter 8 (*ZNT8*), mainly expressed in beta cells of pancreatic islets, a novel *ZNT* family member which responsibly transports zinc into insulin-containing granules from the cytoplasm and subsequently forms insulin crystal for secretion, maturation and storage [27]. At 325 position, rs13266634 of *SLC30A8* gene forms tryptophan (W) in the place of arginine (R) (Arg325Trp) after polymorphism that may be correlated with impaired glucose regulation [28]. Investigations have provided evidence of the association between *SLC30A8* gene variants and the risk of T2DM and cardiovascular complications [28].

Unfortunately, there is a scarcity in a genetic association of T2DM and related cardiovascular complications related research in Bangladesh. To date, there is no single study that evaluated the impact of genetic variants on the risk of T2DM and T2DM-related CVD. Therefore, we have identified three single nucleotide polymorphisms (SNPs) in three candidate genes, namely *KCNJ11* rs5219, *HHEX* rs1111875, and *SLC30A8* rs13266634 for the present study, which were previously known to be involved with T2DM and T2DM-related CVD among different ethnic groups. Our present study aimed to provide evidence of the possible link of these SNPs with T2DM and T2DM-related CVD in the Bangladeshi population.

2. Methods

2.1. Statistical analysis

Different demographic data of the T2DM patients including, age, BMI, FBG level, 2-hour postprandial blood glucose level, blood pressure, total cholesterol, triglycerides, and serum creatinine and related data of healthy controls (age, BMI and FBG level), and T2DM patients with T2DM-related CVD and without CVD were presented as average ± standard deviations and compared with a one way ANOVA test.

Hardy-Weinberg equilibrium (HWE) values were found from the Hardy-Weinberg equilibrium test. To find the genotypic associations of all SNPs with type 2 diabetes, we have tested different genetic models such as heterozygote model (Aa vs. AA), homozygote model (aa vs. AA), dominant model (Aa + aa vs. AA), recessive model (AA + Aa vs. aa), and allele model (a vs. A). Association of risk of T2DM and T2DM-related CVD was presented as adjusted odds ratio (aOR) that were adjusted based on the age, gender and BMI, and 95% confidence intervals (95% CI) based on χ^2-test, which were evaluated by MedCalc software. All other calculations including HWE were performed by SPSS (Version 25, IBM). $p < 0.05$ was considered statistically significant for all of the analyses in this study. Again, statistical power was estimated for three SNPs using OSSE online tool (http://osse.bii.a-star.edu.sg/).

2.2. Ethical statement

The study protocol and questionnaire were approved by the ethical committee of the Noakhali Science and Technology University (ID# 03/2018). Furthermore, written informed consent was obtained prior to the investigation from all the cases and healthy controls.

2.2.1. Clinical parameters

T2DM-related CVD has been diagnosed in 250 patients with T2DM according to clinical features. Among them, the presence of at least one of the following pathological conditions has been found: diabetic cardiomyopathy, heart failure and coronary heart disease. T2DM-related CVD was found in a total of 116 patients (46.40%). Based on the presence or absence of hypertension, 250 patients with T2DM were divided into two groups (116 with T2DM-related CVD and 134 without CVD). Hypertension was defined based on the presence of ≥140/90 mmHg blood pressure or the use of antihypertensive drugs.

All clinical features were documented in a questionnaire form, and our current analysis has been conducted in accordance with the Helsinki Declaration and its subsequent amendments [30]. The full genetic analysis was conducted in the Laboratory of Pharmacogenomics and Molecular Biology, Department of Pharmacy, Noakhali Science and Technology University, Bangladesh.

2.3. Study design and subject recruitment

This case-control study consisted of 250 patients with T2DM and 246 healthy volunteers matching age and sex with the patients. T2DM patients were recruited from Al-Haj Sirajul Islam Diabetic and General Hospital, Maijdee, Noakhali, Bangladesh. According to the WHO criteria (fasting blood glucose or FBG level >7.0 mmol/l or random plasma glucose level >11.1 mmol/l), these patients were diagnosed. In the presence of expert physicians, the detailed physical (sex, age, body mass index or BMI) and clinical (fasting blood glucose level, 2-hour postprandial blood glucose level, blood pressure, total cholesterol, triglycerides and serum creatinine) history of all cases were taken by a trained nurse from the personal interview and medical records between the period of August 2018 to April 2019 [29]. By matching sex, age, and BMI, healthy non-diabetic controls with a <6.2 mmol/L of FBG level were selected from different parts of the Noakhali region, and the FBG levels of controls were detected with a portable Quick Check Glucometer. Normal glucose tolerance, no family history of severe diseases like kidney disease, ocular disease, cancer, and heart disease were present in their body. Subjects with other chronic illnesses were excluded during recruitment.

2.4. Genotyping of single nucleotide polymorphisms (SNPs) of candidate genes

From all the cases and healthy controls, 3 ml venous blood samples were collected in ethylenediaminetetraacetic acid (EDTA)-Na_2 containing sterile tubes, and until DNA extraction, it was stored at -80 °C. After extraction of all genomic DNA following the previously established method [31], DNA amplification was done using the primers as described earlier [32, 33]. To genotype *KCNJ11* rs5219, *SLC30A8* rs13266634 and *HHEX* rs1111875 polymorphisms, the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method

was used. By running on 1% agarose gel, PCR products (210 bp, 256 bp and 161 bp for rs5219, rs13266634 and rs1111875 polymorphisms, respectively) were confirmed. Then with the restriction endonucleases (*BanII*, *MspI* and *XbaI*, respectively) successively for rs5219 (Figure 1), rs13266634 (Figure 2) and rs1111875 (Figure 3) SNPs, five microliters of confirmed PCR products were digested at proper conditions, and after ethidium bromide staining, fragments were visualized on 2% agarose gel. We have reanalyzed all mutant homozygotes and 20% of heterozygotes twice that confirmed our findings. PCR conditions, number of cycles and the number of fragments for wild-type homozygote (AA), heterozygote (Aa) and mutant homozygote (aa) of all SNPs are described in Table 1.

3. Results

3.1. Characteristic features of cases and healthy controls

In this case-control study, a total of 250 T2DM patients and 246 healthy controls were recruited for the analysis. Among them, 124 (49.60%) were males, and 126 (50.40%) were females in the patient group, whereas 140 (56.91%) were males and 106 (43.09%) were females in the control group. All clinical data and demographic characteristics of study populations are summarized in Table 2. Again, significant differences were observed between T2DM with CVD patients and T2DM without CVD in terms of gender distribution, fasting blood glucose (FBG), systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol (TC), and triglycerides (TG) were found (p-value: <0.001, 0.020, 0.011, 0.034, 0.009, 0.039, respectively) that are presented briefly in Table 3.

3.2. Influences of the variants in T2DM

For all SNPs, we tested the Hardy-Weinberg equilibrium (HWE) in cases and healthy controls. We evaluated the association between risk of T2DM and all selected SNPs (Table 4). Genotype distributions for *KCNJ11* rs5219, *SLC30A8* rs13266634 and *HHEX* rs1111875 for T2DM group and healthy control group are presented in Supplementary Table 1. Among the three variants from three different loci, rs5219 of *KCNJ11* showed significant association in heterozygote model, homozygote model, allele model, dominant model, and recessive model ($p = 0.004$, <0.0001, <0.0001, <0.0001, and 0.001; aOR = 1.90, 3.09, 1.80, 2.23, and 2.03, respectively). On the other hand, *SLC30A8* rs13266634 demonstrated a significant relationship with T2DM in heterozygote, allele, and dominant models ($p < 0.0001$ for all models; aOR = 3.59, 1.79, and 3.37, respectively). Another variant, rs1111875 of *HHEX*, showed significant correlation in allele model, homozygote model, dominant, and recessive model ($p < 0.0001$, <0.0001, 0.001, and <0.0001; aOR = 2.08, 5.93, 2.08, and 4.18, respectively).

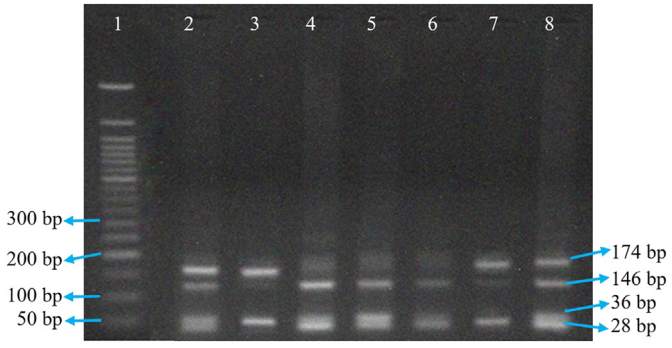

Figure 1. Restriction endonuclease (BanII) digestion fragments of *KCNJ11* rs5219 polymorphism (2% agarose gel). Lane-1: 50 bp molecular marker; Lanes 2 and 8: heterozygous (CT) form (28, 36, 146 and 174 bp); Lanes 3 and 7: mutant (TT) form (36 and 174 bp) and lanes 4, 5, and 6: wild-type (CC) form (28, 36 and 146 bp).

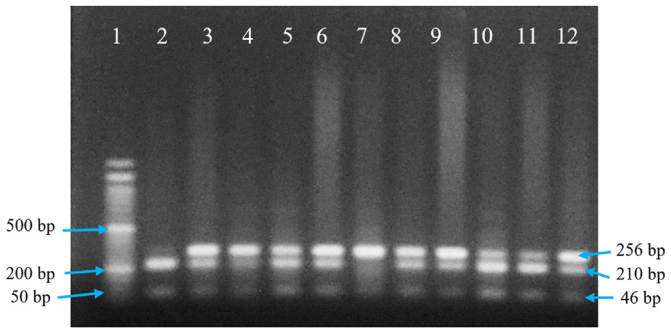

Figure 2. Restriction Endonuclease (MspI) digestion fragments of rs13266634 allele of *SLC30A8* gene (1% agarose gel). Lane-1: 50 bp molecular ruler; Lanes-3-6 and 8-12: heterozygous (CT) form (46, 210 and 256 bp); Lane- 7: mutant (TT) form (256 bp); lane- 2: wild-type (CC) form (46 and 210 bp).

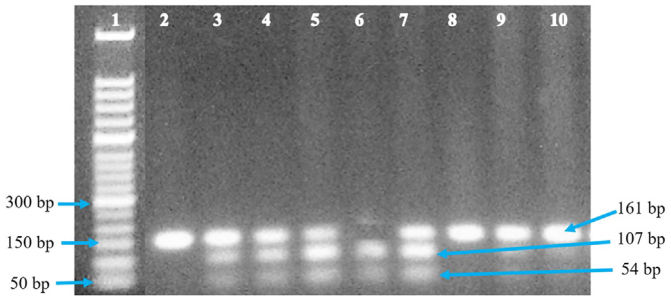

Figure 3. Restriction endonuclease (XbaI) digestion fragments of *HHEX* rs1111875 polymorphism (2% agarose gel). Lane-1: 50 bp molecular marker; Lanes- 3, 4, 5 and 7: heterozygous (CT) form (54, 107 and 161 bp), Lane- 6: homozygous (TT) form (54, 107 bp), and Lanes- 2, 8, 9, and 10: wild-type (CC) form (161 bp).

3.3. Association with CVD in T2DM patients

We also examined the link of T2DM-related CVD in *KCNJ11* rs5219 and *SLC30A8* rs13266634 SNPs in T2DM patients (Table 5). Genotype distributions for *KCNJ11* rs5219, and *SLC30A8* rs13266634 for T2DM-without-CVD group and T2DM-with-CVD group in Supplementary Table 2. Between them, only rs5219 of *KCNJ11* showed significant correlation in recessive and allele model ($p = 0.002$ and 0.017; aOR = 0.40 and 0.65, respectively). *SLC30A8* rs13266634 did not show any significant association to T2DM-related CVD risk in any evaluated genetic association models.

3.4. Statistical power analysis

After completing the laboratory-based genetic analysis using the online sample size estimator (OSSE), the statistical power was estimated, setting a 5% significance level. The outcome of the analysis revealed that *KCNJ11* rs5219 had 88.10%, *SLC30A8* rs13266634 had 87.30%, and *HHEX* rs1111875 had 98.00% statistical power.

4. Discussion

T2DM is a multifactorial disease along with various genetic and environmental factors. Previous studies have shown that 30–70% of T2DM patients are genetically at higher to moderate risk, the reason for which are different genes and their numerous combinations leading to the development of T2DM [11, 12]. For the development of T2DM, South Asian populations, i.e., especially people of Bangladesh, India, Sri Lanka, Bhutan, seem to be at high risk. In South Asia, almost 120.9 million people will be diabetic patients by 2030. Among them, T2DM patients will be 90–95%, which will be more than 50% of all affected European or

Table 1. Primers, conditions of PCR, restriction enzymes, digestion condition and estimated DNA fragments on digestion to the genotype of selected SNPs.

SNPs	Primers (5'-3')	PCR condition	No. of cycles	SAF (bp)	RE	Digestion condition	Fragments of DNA
KCNJ11 rs5219	**F:** ACGTTGCAG TTGCCTTTCTT **R:** GACTCTGCA GTGAGGCCCTA	95 °C 30 s 62 °C 30 s 72 °C 30 s	35	210	BanII (Takara, Japan)	37 °C (incubated 4 h)	AA: 28, 36, 146 Aa: 28, 36, 146, 174 aa: 36, 174
SLC30A8 rs13266634	**F:** GAAGTTGGAG TCAGAGCAGTC **R:** TGGCCTGTCA AATTTGGGAA	94 °C 30 s 59 °C 27 s 72 °C 40 s	35	256	MspI (NEB, England)	37 °C (incubated overnight)	AA: 46, 210 Aa: 46, 210, 256 aa: 256
HHEX rs1111875	**F:** GCTGCTTATGG AAACTGCATTACT **R:** CATCATAACTT CTCACTCCCTTCC	94 °C 1 min 61 °C 1 min 72 °C 1 min	35	161	XbaI (Takara, Japan)	37 °C (incubated overnight)	AA: 161 Aa: 54, 107, 161 aa: 54, 107

SAF: size of amplification fragment; ***RE:*** restriction endonuclease; ***AA:*** wild-type homozygote; ***Aa:*** heterozygote; ***aa:*** mutant homozygote; ***F:*** forward; ***R:*** reverse.

Table 2. Sociodemographic and clinical characteristics of T2DM patients and healthy controls.

Variables	T2DM Patients (n = 250)	Healthy Controls (n = 246)	Normal Range
Age (years) (±SD)	53.83 ± 12.03	52.30 ± 10.84	NA
Age range (years)	25–80	25–77	NA
Sex (male/female)	124/126	140/106	NA
BMI (kg/m^2) (±SD)	25.33 ± 3.90	23.54 ± 2.64	18.5–24.9
FBG (mmol/l) (±SD)	10.06 ± 1.95	5.76 ± 0.56	3.9–5.6
2 h PBG (mmol/l) (±SD)	14.90 ± 3.89	NA	<7.8
SBP (mmHg) (±SD)	124.52 ± 12.71	NA	120–129
DBP (mmHg) (±SD)	81.25 ± 10.04	NA	80–84
TC (mg/dl) (±SD)	200.96 ± 38.34	NA	<200
TG (mg/dl) (±SD)	192.46 ± 31.30	NA	<150
SC (mg/dl) (±SD)	1.03 ± 0.19	NA	0.7–1.2

Data are expressed as mean ± SD, median (interquartile range), or percentage values (%); ***T2DM:*** type 2 diabetes mellitus; ***BMI:*** body mass index; ***FBG:*** fasting blood glucose; ***2 h PBG:*** 2 h postprandial blood glucose; ***SBP:*** systolic blood pressure; ***DBP:*** diastolic blood pressure; ***TC:*** total cholesterol; ***TG:*** triglycerides; ***SC:*** serum creatinine; ***NA:*** not available.

Table 3. Selected characteristics of T2DM patients with T2DM-related CVD and without CVD.

Variables	T2DM with CVD (n = 116) (%)	T2DM without CVD (n = 134) (%)	*p*-value
Gender			
Male	68 (58.62)	56 (41.79)	0.059
Female	48 (41.38)	78 (58.21)	**<0.001**
Age (years)			
Mean age, n (±SD)	53.74 ± 11.42	53.92 ± 12.59	0.284
Range	28–80	25–80	
BMI (kg/m^2) (±SD)	26.04 ± 3.66	24.72 ± 4.01	0.872
FBG (mmol/l) (±SD)	10.73 ± 1.76	9.48 ± 1.92	**0.020**
2 h PBG (mmol/l) (±SD)	15.48 ± 4.17	14.41 ± 3.58	0.069
SBP (mmHg) (±SD)	132.01 ± 11.65	118.10 ± 9.75	**0.011**
DBP (mmHg) (±SD)	86.25 ± 8.46	76.96 ± 9.31	**0.034**
TC (mg/dl) (±SD)	223.38 ± 37.16	181.75 ± 27.48	**0.009**
TG (mg/dl) (±SD)	210.29 ± 24.37	177.18 ± 28.45	**0.039**
SC (mg/dl) (±SD)	1.11 ± 0.20	0.95 ± 0.14	0.146

Data are expressed as mean ± SD, median (interquartile range), or percentage values (%); ***T2DM:*** type 2 diabetes mellitus; ***BMI:*** body mass index; ***FBG:*** fasting blood glucose; ***2 h PBG:*** 2 h postprandial blood glucose; ***SBP:*** systolic blood pressure; ***DBP:*** diastolic blood pressure; ***TC:*** total cholesterol; ***TG:*** triglycerides; ***SC:*** serum creatinine. *p*-value <0.05 was considered significant (Bold).

North American [34]. However, in 2011, the prevalence of T2DM in the Bangladeshi population was higher (9.6%) than in other countries of South Asia. Previous GWAS in the South Asian population (except Bangladesh) found 20 independent SNPs in type 2 diabetes patients, and so we took this initiative to find out the susceptible SNPs for developing T2DM in Bangladeshi populations [34].

To take the candidate SNPs, we selected those genes which are- 1) found from the previous GWAS due to the relation with insulin resistance or T2DM development and 2) related to the pathway of insulin secretion or other different diabetic-related complications like hypertension and/or CVD. Under different genetic models like recessive, dominant, homozygote, and heterozygote, our present study was performed as suggested by Salanti et al. [35] to eliminate strong biases in searching and reporting the level of association. For the very first time in the Bangladeshi population, a significant association was identified between T2DM and the studied SNPs, including *KCNJ11* rs5219, *SLC30A8* rs13266634 and *HHEX* rs1111875.

Table 4. Association of candidate polymorphisms with T2DM cases and healthy controls.

SNP		rs5219	rs13266634	rs1111875
Chromosome		11p15.1	8q24.11	10q23.33
Position		17388025	117172544	92703125
Gene		*KCNJ11*	*SLC30A8*	*HHEX*
N (Patient/Control)		250/246		
Major/Minor allele		C/**T**	C/**T**	C/**T**
HWE *p*-value	Patient	0.010	0.000	0.218
	Control	0.005	0.456	0.009
aOR (95% CI)	Heterozygote model	1.90 (1.23–2.95)	3.59 (2.39–5.37)	1.38 (0.88–2.17)
	Homozygote model	3.09 (1.86–5.13)	2.40 (1.12–5.11)	5.93 (3.25–10.79)
	Allele model	1.80 (1.40–2.32)	1.79 (1.37–2.34)	2.08 (1.62–2.68)
	Dominant model	2.23 (1.50–3.31)	3.37 (2.28–4.98)	2.08 (1.36–3.19)
	Recessive model	2.03 (1.33–3.10)	1.12 (0.56–2.22)	4.18 (2.60–6.73)
p-value	Heterozygote model	**0.004**	**<0.0001**	0.157
	Homozygote model	**<0.0001**	0.024	**<0.0001**
	Allele model	**<0.0001**	**<0.0001**	**<0.0001**
	Dominant model	**<0.0001**	**<0.0001**	**0.001**
	Recessive model	**0.001**	0.747	**<0.0001**

p-value <0.05 was considered significant (Bold); aOR = adjusted odds ratio.

Table 5. Association of alleles or genotypes with risk of T2DM-related CVD in Bangladeshi T2DM patients.

SNPs	Genes		Association models				
			Heterozygote model	Homozygote model	Dominant model	Recessive model	Allele model
rs5219	*KCNJ11*	*p*-value	0.240	0.066	0.799	**0.002**	**0.017**
		aOR (95% CI)	1.48 (0.77–2.87)	0.51 (0.25–1.05)	0.93 (0.51–1.68)	0.40 (0.22–0.71)	0.65 (0.45–0.93)
rs13266634	*SLC30A8*	*p*-value	0.509	0.708	0.617	0.467	0.981
		aOR (95% CI)	1.22 (0.68–2.20)	0.80 (0.24–2.63)	1.16 (0.65–2.08)	0.68 (0.24–1.94)	1.00 (0.70–1.44)

Bold values indicate statistically significant ($p < 0.05$); aOR = adjusted odds ratio.

Polymorphism in the rs5219 of *KCNJ11* gene causes unregulated insulin secretion as well as congenital hyperinsulinism. It may also be associated with autosomal dominant T2DM through the polymorphism of rs5219 (E23K), where the T allele or lysine (K) instead of C allele or glutamate (E) is suppressed insulin secretion through decreasing the ATP sensitivity of the KATP channel [36]. As stated earlier in this study, we found a strong association of *KCNJ11* ($p < 0.0001$, <0.0001, 0.001, 0.004, and <0.0001; aOR = 1.80, 2.23, 2.03, 1.90, and 3.09; by turn, in allele, dominant, recessive, heterozygote, and homozygote model) with T2DM patients of Bangladesh as like Chinese ($p < 0.05$, OR = 1.72. 95% CI = 1.12–2.63 in the dominant model) and Iranian ($p = 0.048$, OR = 2.50. 95% CI = 1.01–6.14 in allele model) populations, though no such association was found in the case of the South Indian population [21, 32, 37].

On the other hand, through the activation of hepatocyte nuclear factor 1a, *the* HHEX gene regulates pancreatic beta-cell development and function. It is reliably assumed that the risk allele reduces cell mass and decreases the secretory capacity of beta-cell to raise T2DM [38, 39]. Our study showed a strong association of *HHEX* rs1111875 polymorphism with T2DM ($p < 0.0001$ for all models; aOR = 1.79, 3.37, and 3.59, in allele, dominant, and heterozygote model, respectively) like the population of the Dutch Breda cohort (mutant homozygosity is 39.6% and 37% for cases and healthy controls, respectively) [25]. However, our results are not consistent with some other studies; for example, in the Indian and European populations, no association of *HHEX* rs1111875 polymorphism was found with T2DM development [40, 41].

Moreover, zinc is essential for the process of insulin maturation to secretion and *SLC30A8* gene- located in secretory granules of insulin, plays an important role in zinc transportation to the container vesicles of insulin. After non-synonymous polymorphism in *SLC30A8* rs13266634, a newly formed amino acid (arginine) affects its regular function and puts down the formation and secretion of insulin, which lowers the amount of insulin in the body as a whole [27, 28]. From our study, we observed a strong association of rs13266634 in our population to develop T2DM ($p < 0.0001$, 0.001, <0.0001, and <0.0001; aOR = 2.08, 2.08, 4.18, and 5.97, respectively in allele, dominant, recessive, and homozygote model) and a similar association was reported in several meta-analyses for Asian and European population [42, 43]. Moreover, most of the *SLC30A8* carriers in our studied population are taking either combined medicine or insulin due to the extreme reduction of insulin in the body.

Hypertension, high cholesterol, and triglyceride level in T2DM patients highly elevate the risk of cardiovascular complications. Again, a diabetic patient with T2DM-related CVD tends to lead to chronic kidney disease [44]. Therefore, here we studied *KCNJ11* rs5219 and *SLC30A8* rs13266634 SNPs for the risk of cardiovascular complications in our T2DM patient. Between them, rs5219 of *KCNJ11* showed significant association in recessive and allele model ($p = 0.002$ and 0.017; aOR = 0.40 and 0.65, respectively). However, we did not observe any significant link of *SLC30A8* with cardiovascular complications in our population, which is further needed to be evaluated. Furthermore, we have observed significant differences between T2DM with CVD patients and T2DM without CVD in terms of gender distribution, FBG, SBP, DBP, TC, and TG status (*p*-value: <0.001, 0.020, 0.011, 0.034, 0.009, 0.039, respectively).

It is worth mentioning that we carried out this study on a comparatively small scale population from the Noakhali district of Bangladesh, which might affect the significance level to some extent. Besides, the inclusion of more clinicopathological information of patients may provide a more practical outcome. For a better conclusion and concrete outcome, a large prospective study is required from the different regions of Bangladesh along with the gene-gene and gene-environment interactions in the future.

5. Conclusion

In conclusion, our investigation indicates that *KCNJ11* rs5219, *SLC30A8* rs13266634 and *HHEX* rs1111875 polymorphisms are associated with T2DM. Moreover, *KCNJ11* rs5219 polymorphism is correlated with the risk of T2DM-related CVD. Large-scale investigations are warranted to validate the results of our study.

Declarations

Author contribution statement

Tutun Das Aka: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Urmi Saha, Sayara Akter Shati: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Md. Abdul Aziz, Mobashera Begum: Performed the experiments; Wrote the paper.

Md. Saddam Hussain, Md. Shalahuddin Millat, Mohammad Sarowar Uddin: Analyzed and interpreted the data; Wrote the paper.

Mohammad Safiqul Islam: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This work was supported by the Research Cell of Noakhali Science and Technology University.

Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Appendix

Supplementary Table 1. Genotype distributions for *KCNJ11* rs5219, *SLC30A8* rs13266634 and *HHEX* rs1111875 for T2DM group and healthy control group

Genotypes	Cases (n = 250) (%)	HWE χ^2	HWE p-value	Healthy Controls (n = 246) (%)	HWE χ^2	HWE p-value
***KCNJ11* rs5219**						
CC	64 (13.64)			102 (16.67)		
CT	104 (44.54)	6.70	0.010	96 (41.67)	7.97	0.005
TT	82 (41.82)			48 (41.67)		
C	232 (46.40)			300 (60.98)		
T	268 (53.60)			192 (39.02)		
***SLC30A8* rs13266634**						
CC	70 (10.91)			134 (13.89)		
CT	162 (82.73)	31.44	0.00	92 (77.78)	0.55	0.456
TT	18 (6.36)			20 (8.33)		
C	302 (60.40)			360 (73.17)		
T	198 (39.60)			132 (26.83)		
***HHEX* rs1111875**						
CC	48 (10.91)			78 (13.89)		
CT	112 (82.73)	1.52	0.218	138 (77.78)	6.81	0.009
TT	90 (6.36)			30 (8.33)		
C	208 (41.60)			294 (59.76)		
T	292 (58.40)			198 (40.24)		

Supplementary Table 2. Genotype distributions for *KCNJ11* rs5219, and *SLC30A8* rs13266634 for T2DM-without-CVD group and T2DM-with-CVD group

Genotypes	T2DM with CVD (n = 116) (%)	HWE χ^2	HWE p-value	T2DM without CVD (n = 134) (%)	HWE χ^2	HWE p-value
***KCNJ11* rs5219**						
CC	30 (10.91)			34 (13.89)		
CT	60 (82.73)	0.148	0.70	42 (77.78)	16.64	0.00
TT	26 (6.36)			58 (8.33)		
C	120 (51.72)			110 (41.04)		
T	112 (48.28)			158 (58.96)		
***SLC30A8* rs13266634**						
CC	30 (10.91)			40 (13.89)		
CT	80 (82.73)	22.56	0.00	82 (77.78)	10.49	0.001
TT	6 (6.36)			12 (8.33)		
C	140 (60.34)			162 (60.45)		
T	92 (39.66)			106 (39.55)		

Acknowledgements

We would like to thanks the authority of Al-Haj Sirajul Islam Diabetic and General Hospital for their support in patient recruitment. Furthermore, we would like to express our gratitude to the authority of the Laboratory of Pharmacogenomics and Molecular Biology of the Department of Pharmacy, Noakhali Science and Technology University, for giving permission to conduct this study.

References

[1] J. Tremblay, M. Haloui, R. Attaoua, et al., Polygenic risk scores predict diabetes complications and their response to intensive blood pressure and glucose control, Diabetologia (2021).
[2] K. Blaslov, F.S. Naranđa, I. Kruljac, I.P. Renar, Treatment approach to type 2 diabetes: past, present and future, World J. Diabetes 9 (12) (2018) 209–219.
[3] P. Haghvirdizadeh, Z. Mohamed, N.A. Abdullah, P. Haghvirdizadeh, M.S. Haerian, B.S. Haerian, KCNJ11: genetic polymorphisms and risk of diabetes mellitus, J Diabetes Res 2015 (2015) 908152.
[4] T. Biswas, A. Islam, L.B. Rawal, S.M. Islam, Increasing prevalence of diabetes in Bangladesh: a scoping review, Publ. Health 138 (2016) 4–11.
[5] P. Saeedi, I. Petersohn, P. Salpea, et al., Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: results from the International Diabetes Federation Diabetes Atlas, Diabetes Res. Clin. Pract. 157 (2019) 107843, 9th edition.
[6] A. Afroz, K. Alam, L. Ali, et al., Type 2 diabetes mellitus in Bangladesh: a prevalence based cost-of-illness study, BMC Health Serv. Res. 19 (2019) 601.
[7] Y. Zheng, S.H. Ley, F.B. Hu, Global aetiology and epidemiology of type 2 diabetes mellitus and its complications, Nat. Rev. Endocrinol. 14 (2) (2018) 88–98.
[8] L. Yang, X. Zhou, Y. Luo, et al., Association between KCNJ11 gene polymorphisms and risk of type 2 diabetes mellitus in East Asian populations: a meta-analysis in 42,573 individuals, Mol. Biol. Rep. 39 (1) (2012) 645–659.
[9] Z. Li, Cy Ye, T.Y. Zhao, et al., Model of genetic and environmental factors associated with type 2 diabetes mellitus in a Chinese Han population, BMC Publ. Health 20 (2020) 1024.
[10] F.B. Hu, Globalization of diabetes: the role of diet, lifestyle, and genes, Diabetes Care 34 (6) (2011) 1249–1257.
[11] J. Gulcher, K. Stefansson, Clinical risk factors, DNA variants, and the development of type 2 diabetes, N. Engl. J. Med. 359 (2009) 2220–2232.
[12] C. Hu, W. Jia, Diabetes in China: epidemiology and genetic risk factors and their clinical utility in personalized medication, Diabetes 67 (1) (2018) 3–11.
[13] K. Läll, R. Mägi, A. Morris, A. Metspalu, K. Fischer, Personalized risk prediction for type 2 diabetes: the potential of genetic risk scores, Genet. Med. 19 (3) (2017) 322–329.
[14] X. Lei, S. Huang, Enrichment of minor allele of SNPs and genetic prediction of type 2 diabetes risk in British population, PLoS One 12 (11) (2017), e0187644.
[15] M.H. Wang, J. Li, V.S. Yeung, et al., Four pairs of gene-gene interactions associated with increased risk for type 2 diabetes (CDKN2BAS-KCNJ11), obesity (SLC2A9-IGF2BP2, FTO-APOA5), and hypertension (MC4R-IGF2BP2) in Chinese women, Meta Gene 2 (2014) 384–391.
[16] G. Abraham, M. Inouye, Genomic risk prediction of complex human disease and its clinical application, Curr. Opin. Genet. Dev. 33 (2015) 10–16.
[17] The Emerging Risk Factors Collaboration, Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies, Lancet 375 (9733) (2010) 2215–2222.
[18] S.S. Rich, J.M. Norris, J.I. Rotter, Genes associated with risk of type 2 diabetes identified by a candidate-wide association scan: as a trickle becomes a flood, Diabetes 57 (11) (2008) 2915–2917.
[19] R.W. Schwenk, H. Vogel, A. Schürmann, Genetic and epigenetic control of metabolic health, Mol. Metab. 2 (4) (2013) 337–347.
[20] A.L. Gloyn, E.R. Pearson, J.F. Antcliff, et al., Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir 6.2 and permanent neonatal diabetes, N. Engl. J. Med. 350 (18) (2004) 1838–1849.
[21] L. Zhuang, Y. Zhao, W. Zhao, et al., The E23K and A190A variations of the KCNJ11 gene are associated with early-onset type 2 diabetes and blood pressure in the Chinese population, Mol. Cell. Biochem. 404 (1-2) (2015) 133–141.
[22] M. Harakalova, J.J. van Harssel, P.A. Terhal, et al., Dominant missense mutations in ABCC9 cause Cantú syndrome, Nat. Genet. 44 (7) (2012) 793–796.
[23] J. Zhang, L.B. McKenna, C.W. Bogue, K.H. Kaestner, The diabetes gene Hhex maintains δ-cell differentiation and islet function, Genes Dev. 28 (8) (2014) 829–834.
[24] Y. Cai, J. Yi, Y. Ma, D. Fu, Meta-analysis of the effect of HHEX gene polymorphism on the risk of type 2 diabetes, Mutagenesis 26 (2) (2011) 309–314.
[25] J.V. van Vliet-Ostaptchouk, N.C. Onland-Moret, T.W. van Haeften, et al., HHEX gene polymorphisms are associated with type 2 diabetes in the Dutch Breda cohort, Eur. J. Hum. Genet. 16 (5) (2008) 652–656.
[26] C.C. Lu, Y.T. Chen, S.Y. Chen, et al., Hematopoietically expressed homeobox gene is associated with type 2 diabetes in KK Cg-A^y/J mice and a Taiwanese Han Chinese population, Exp. Ther. Med. 16 (1) (2018) 185–191.
[27] F. Hosseini-Esfahani, P. Mirmiran, G. Koochakpoor, M.S. Daneshpour, K. Guity, F. Azizi, Some dietary factors can modulate the effect of the zinc transporters 8 polymorphism on the risk of metabolic syndrome, Sci. Rep. 7 (1) (2017) 1649.
[28] F. Dong, B.H. Zhang, S.L. Zheng, et al., Association between SLC30A8 rs13266634 polymorphism and risk of T2DM and IGR in Chinese population: a systematic review and meta-analysis, Front. Endocrinol. 9 (2018) 564.
[29] A.J. Monsuur, P.I. de Bakker, B.Z. Alizadeh, et al., Myosin IXB variant increases the risk of celiac disease and points toward a primary intestinal barrier defect, Nat. Genet. 37 (12) (2005) 1341–1344.
[30] World Medical Association declaration of Helsinki: ethical principles for medical research involving human subjects, J. Am. Med. Assoc. 310 (2013) 2191–2194.
[31] M.S. Islam, M.U. Ahmed, M.S. Bin Sayeed, et al., Lung cancer risk in relation to nicotinic acetylcholine receptor, CYP2A6 and CYP1A1 genotypes in the Bangladeshi population, Clin. Chim. Acta 416 (2013) 11–19.

[32] A. Rastegari, M. Rabbani, H.M. Sadeghi, E.F. Imani, A. Hasanzadeh, F. Moazen, Association of KCNJ11 (E23K) gene polymorphism with susceptibility to type 2 diabetes in Iranian patients, Adv. Biomed. Res. 4 (2015) 1.
[33] F.A. Sharif, M.E. Shubair, M.M. Zaharna, et al., Genetic polymorphism and risk of having type 2 diabetes in a Palestinian population: a study of 16 gene polymorphisms, Adv. Diabetes Endocrinol. 3 (1) (2018) 1–6.
[34] U.P. Gujral, R. Pradeepa, M.B. Weber, K.M. Narayan, V. Mohan, Type 2 diabetes in South Asians: similarities and differences with white Caucasian and other populations, Ann. N. Y. Acad. Sci. 1281 (1) (2013) 51–63.
[35] G. Salanti, L. Southam, D. Altshuler, et al., Underlying genetic models of inheritance in established type 2 diabetes associations, Am. J. Epidemiol. 170 (5) (2009) 537–545.
[36] S. Rizvi, S.T. Raza, F. Mahdi, S.P. Singh, M. Rajput, Q. Rahman, Genetic polymorphisms in KCNJ11 (E23K, rs5219) and SDF-1β (G801A, rs1801157) genes are associated with the risk of type 2 diabetes mellitus, Br. J. Biomed. Sci. 75 (3) (2018) 139–144.
[37] N.M. Phani, V. Guddattu, R. Bellampalli, et al., Population specific impact of genetic variants in KCNJ11 gene to type 2 diabetes: a case-control and meta-analysis study, PLoS One 9 (9) (2014), e107021.
[38] H. Tanaka, T. Yamamoto, T. Ban, et al., Hex stimulates the hepatocyte nuclear factor 1 alpha-mediated activation of transcription, Arch. Biochem. Biophys. 442 (1) (2005) 117–124.
[39] A.T. Hattersly, R.M. Freathy, A.I. Bennett, et al., Direct evidence to support the fetal insulin hypothesis as the type 2 diabetes risk alleles at the CDKAL1 and HHEX-IDE gene loci reduce birth weight, Diabetologia 51 (2008) S127–S128.
[40] N.M. Phani, P. Adhikari, S.K. Nagri, S.C. D'Souza, K. Satyamoorthy, P.S. Rai, Replication and relevance of multiple susceptibility loci discovered from genome wide association studies for type 2 diabetes in an Indian population, PLoS One 11 (6) (2016), e0157364.
[41] J.P. Lewis, N.D. Palmer, P.J. Hicks, et al., Association analysis in african americans of European-derived type 2 diabetes single nucleotide polymorphisms from whole-genome association studies, Diabetes 57 (8) (2008) 2220–2225.
[42] L. Cheng, D. Zhang, L. Zhou, J. Zhao, B. Chen, Association between SLC30A8 rs13266634 polymorphism and type 2 diabetes risk: a meta-analysis, Med. Sci. Monit. 21 (2015) 2178–2189.
[43] M. Fan, W. Li, L. Wang, et al., Association of SLC30A8 gene polymorphism with type 2 diabetes, evidence from 46 studies: a meta-analysis, Endocrine 53 (2016) 381–394.
[44] I.M. Stratton, A.I. Adler, H.A. Neil, et al., Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study, BMJ 321 (7258) (2000) 405–412.