Research article

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

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\textbf{ARTICLE INFO}

Keywords:
Type 2 diabetes mellitus
Cardiovascular disease
\textit{KCNJ11}
\textit{HHEX}
\textit{SLC30A8}
Polymorphism

\textbf{ABSTRACT}

\textbf{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 \textit{KCNJ11} rs5219, \textit{SLC30A8} rs13266634 and \textit{HHEX} rs1111875 polymorphisms with T2DM and related CVD.

\textbf{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.

\textbf{Results:} A significantly increased association of \textit{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 \textit{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 (\textit{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 \textit{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).

\textbf{Conclusions:} Our investigation indicates that \textit{KCNJ11} rs5219, \textit{SLC30A8} rs13266634 and \textit{HHEX} rs1111875 polymorphisms are associated with T2DM. Moreover, \textit{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 inefficac-}
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 NH2- terminal of Kir6.2 and 15th intron, respectively that attenuate the sensitivity of the channel to ATP [3, 22]. Previous studies have evaluated the role KCNJ11 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. Hae-matopoietically expressed homeobox or HHEX is one of the three genes of LD block, and it contains an insulin-degrading enzyme [23, 24, 25]. The eighth member of solute carrier family 30 (SLC30A8) is one of the three genes of KCNJ11 polymorphisms, the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) 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

### 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, Majidee, 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)-Na2 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 and 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).

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. 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         |
|------------|--------------------------|----------------|---------------|----------|-------------|---------------------|--------------------------|
| KCNJ1 rs5219 | F: AGCTTGCAAGTGGGCTTCT  | 95°C 30 s      | 62°C 30 s     | 72°C 30 s| 35          | 210                 | BamII (Takara, Japan)    | 37°C (incubated 4 h)      |
|            | R: GACTGCTGAGGATGGCACTTG |                |               |          |             |                     | AA: 28, 36, 146          |
| SLC30A8 rs1256634 | G: GAAGCTGGGAGCTTCTCTTCGAG | 94°C 30 s      | 59°C 27 s     | 72°C 40 s| 35          | 256                 | MspI (NEB, England)      | 37°C (incubated overnight) |
|            | R: TGCCTGCTCAACTTTGCAAGA |                |               |          |             |                     | AA: 46, 210              |
| HHEX rs1111875 | F: GCCTGCTATGGAAACTGTGATTG | 94°C 1 min     | 61°C 1 min    | 72°C 1 min| 35          | 161                 | BclI (Takara, Japan)     | 37°C (incubated overnight) |

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/m²) (±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 |
| 2h 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)                  | 56 (41.79)                    | <0.001  |
| Age (years) |                             |                               |         |
| Mean age, n (±SD) | 53.74 ± 11.42 | 53.92 ± 12.59 | 0.284   |
| Range     | 25–80                      | 25–80                         |         |
| BMI (kg/m²) (±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   |
| 2h 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 KCNJ1 rs5219, SLC30A8 rs1326634 and HHEX rs1111875.

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

| SNP       | rs5219 | rs1326634 | rs1111875 |
|-----------|--------|-----------|-----------|
| Chromosome | 11p15.1 | 8q24.11   | 10q23.33  |
| Position  | 17388025 | 117172544 | 92703125  |
| Gene      | KCNJ1  | SLC30A8   | HHEX      |
| N (Patient/Control) | 250/246 |  |
| Major/Minor allele | C/T | C/T | C/T |
| HWE p-value | 0.010 | 0.000 | 0.218 |
| 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 | (1.50–3.13) | 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.
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 factor 1a, 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 KCNJ11 polymorphism 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 factor1a, 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, recessive, heterozygote, and homozygote model) with T2DM patients of Bangladesh 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].

Moreover, zinc is essential for the process of insulin maturation to secrete 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 0.17; 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.

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

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

Md. Saddam Hussain, Md. Shahuhuddin Millat, Mohammad Sarowar Uddin: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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 | Controls (n = 246) (%) | HWE |
|-----------|---------------------|-----|------------------------|-----|
|           |                     | $\chi^2$ | P-value | $\chi^2$ | P-value |
| KCNJ11 rs5219 |                      |       |           |       |           |
| CL        | 64 (16.34)          | 102 (16.67) | 16.67 | 0.000 | 102 (16.67) | 16.67 | 0.000 |
| CT        | 104 (44.54)         | 76.67 | 0.010 | 96 (41.67) | 7.97 | 0.005 |
| TT        | 82 (41.12)          | 48 (41.67) | 41.67 | 0.000 | 48 (41.67) | 41.67 | 0.000 |
| SLC30A8 rs13266634 |            |       |           |       |           |
| CL        | 162 (82.73)         | 92 (77.78) | 77.78 | 0.55 | 92 (77.78) | 77.78 | 0.55 |
| CT        | 20 (8.33)           | 20 (8.33) | 8.33 | 0.009 | 20 (8.33) | 8.33 | 0.009 |
| TT        | 80 (82.73)          | 360 (73.17) | 73.17 | 0.001 | 360 (73.17) | 73.17 | 0.001 |
| T        | 268 (53.60)         | 192 (39.02) | 39.02 | 0.000 | 192 (39.02) | 39.02 | 0.000 |
| HHEX rs1111875 |                    |       |           |       |           |
| CC        | 48 (10.91)          | 78 (13.89) | 13.89 | 0.009 | 78 (13.89) | 13.89 | 0.009 |
| CT        | 112 (23.47)         | 138 (77.78) | 77.78 | 6.81 | 138 (77.78) | 77.78 | 6.81 |
| TT        | 90 (6.36)           | 30 (8.33) | 8.33 | 0.009 | 30 (8.33) | 8.33 | 0.009 |
| SLC30A8 rs13266634 |             |       |           |       |           |
| CC        | 208 (41.60)         | 294 (59.76) | 59.76 | 0.148 | 294 (59.76) | 59.76 | 0.148 |
| CT        | 112 (22.44)         | 198 (40.24) | 40.24 | 0.009 | 198 (40.24) | 40.24 | 0.009 |
| 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 | T2DM without CVD (n = 134) (%) | HWE |
|-----------|-------------------------------|-----|-------------------------------|-----|
|           |                               | $\chi^2$ | P-value | $\chi^2$ | P-value |
| KCNJ11 rs5219 |                      |       |           |       |           |
| CL        | 20 (9.17)                     | 134 (11.89) | 11.89 | 0.001 | 134 (11.89) | 11.89 | 0.001 |
| CT        | 60 (28.37)                    | 42 (77.78) | 77.78 | 0.000 | 42 (77.78) | 77.78 | 0.000 |
| TT        | 66 (29.82)                    | 58 (83.33) | 83.33 | 0.000 | 58 (83.33) | 83.33 | 0.000 |
| SLC30A8 rs13266634 |                |       |           |       |           |
| CL        | 30 (9.09)                     | 40 (13.89) | 13.89 | 0.000 | 40 (13.89) | 13.89 | 0.000 |
| CT        | 80 (25.64)                    | 82 (77.78) | 77.78 | 0.010 | 82 (77.78) | 77.78 | 0.010 |
| TT        | 6 (6.36)                      | 12 (8.33) | 8.33 | 0.001 | 12 (8.33) | 8.33 | 0.001 |
| 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.

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