Research Article

Association of Genetic Variants of KCNJ11 and KCNQ1 Genes with Risk of Type 2 Diabetes Mellitus (T2DM) in the Indian Population: A Case-Control Study

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Type 2 diabetes mellitus (T2DM) is a polygenic metabolic disease described by hyperglycemia, which is caused by insulin resistance or reduced insulin secretion. The interaction between various genetic variants and environmental factors triggers T2DM. The aim of this study was to find risk associated with genetic variants rs5210 and rs2237895 of KCNJ11 and KCNQ1 genes, respectively, in the development of T2DM in the Indian population. A total number of 300 cases of T2DM and 100 control samples were studied to find the polymorphism in KCNJ11 and KCNQ1 through PCR-RFLP. The genotype and allele frequencies in T2DM cases were significantly different compared to the control population. KCNJ11 rs5210 and KCNQ1 rs2237895 variants were found to be significantly associated with risk of T2DM in dominant (KCNJ11: OR, 2.07; 95% CI, 1.30–3.27; p = 0.001; KCNQ1: OR, 2.33; 95% CI, 1.46–3.70; p = 0.0003) and codominant models (KCNJ11: OR, 1.76; 95% CI, 1.09–2.84; p = 0.020; KCNQ1: OR, 1.85; 95% CI, 1.16–2.95; p = 0.009). We also compared clinicopathological characteristics between cases and control and observed a significant difference in all the parameters except HDL, gender, and family history. In this study, clinicopathological data with a carrier of a variant allele of both KCNJ11 and KCNQ1 genes were also analysed, and a significant association was found between the carrier of a variant allele with gender and PPG in KCNJ11 and with triglyceride in KCNQ1. We confirm the significant association of KCNJ11 (rs5210) and KCNQ1 (rs2237895) gene polymorphism with T2DM, indicating the role of these variants in developing risk for T2DM in Indian population.

1. Introduction

Type 2 Diabetes is a chronic metabolic disorder described by reduced insulin secretion and insulin action and hyperglycemia. Among different types of diabetes, T2DM is the most common wherein multiple environmental and genetic factors are associated with the development of this disease [1, 2]. As per the International Diabetes Federation’s (IDF) Diabetes Atlas, 8th edition, it has been estimated that the number of diabetes patients is expected to increase from 425 million in 2017 to 629 million by 2045. India is home to 74 million people with diabetes mellitus, with approximately 8.7% prevalence among the adult population, and considered as the “diabetes capital” of the world (IDF, 8th edition). Genome-wide association studies (GWAS) found various susceptibility genes for diabetes [3–5]. However, the
mechanisms these genes follow for the development of diabetes are still not clear. Many gene polymorphism findings demonstrated the association of various SNPs in the pathogenesis of T2DM, and in different populations, each SNP may exhibit dissimilar association with T2DM. Therefore, there is a necessity to discover various genetic risk markers of T2DM for its prevention and treatment.

KCNJ11 and KCNQ1 genes have gained significant attention as a potential candidate for T2DM susceptibility because of their position and function in regulating glucose-stimulated insulin secretion. The KCNJ11 gene is located at 11p15.1 on the human chromosome and lacks intronic sequences; this gene is a member of the potassium channel gene family [6]. The KCNJ11 gene encodes Kir6.2, an inward-rectifier potassium ion channel, which forms a KATP channel with sulfonylurea receptor 1 (SUR1). This KATP channel, through glucose metabolism, controls insulin secretion and production [7]. The KCNQ1 gene is located at the 11th chromosome 11p15.5, approximately 404 kb, and consists of 17 exons [8]. The potassium voltage-gated channel KQT-like subfamily, member 1 (KCNQ1), encodes the pore-forming subunit of KvLQT1, voltage-gated potassium channel, and inhibition of this channel by KCNQ1 inhibitors 293B increased insulin secretion [9], indicating the important role of the KCNQ1 channel in regulating insulin secretion. KCNJ11 and KCNQ1 are ATP-sensitive K+ channels and play crucial role in regulating insulin-secreting β cells making them potential susceptibility genes for T2DM. Therefore, the present case-control study was conducted to assess the association of KCNJ11 (rs5210) and KCNQ1 (rs2237895) gene variants with T2DM risk in Indian population and its association with various clinicopathological characteristics to understand the implications of ethnic diversity on the onset of T2DM.

2. Materials and Methods

2.1. Ethical Approval and Study Subjects. Collection of clinical data in pretest Performa and sample collection of the diabetic patients and control/non-diabetic of North Indian origin from Ansari Health Center Jamia Millia Islamia New Delhi were performed with due consent, after getting Ethical clearance from the Institute Ethical Committee, Jamia Millia Islamia, New Delhi (Proposal No. 17/9/14/JMI/IEC/2015 dated 14/01/2016). The present cohort study was conducted in the Department of Biotechnology, Jamia Millia Islamia, New Delhi, and a total of 400 samples (300 cases and 100 controls) were collected, fulfilling all the relevant selection criteria.

All the study participants had undergone detailed clinical investigation, and written informed consent was collected from the patients before their participation. Various clinicopathological characteristics such as age, BMI, PPG, FPG, FPI, HbA1c, T-cholesterol, systolic BP, diastolic BP, triglycerides, LDL-C, HDL-C, gender, family history, smoking, and alcohol consumption were included in this study.

2.2. Single Nucleotide Polymorphism (SNP) Genotyping by PCR-RFLP. Genomic DNA was extracted from a fresh blood sample using the phenol chloroform method. The primers used for amplification were as follows: KCNJ11 (Forward: 5’- ATCCAGGGGTGGTTACAAGGCA-3’; reverse: 5’-TTTCAGGGACCAAGTAGCTG-3’) and KCNQ1 (Forward 5’-ATCCAGGGGTGGTTACAAGGCA-3’; reverse 5’-TTTACGAGCAACATGACGC -3’) in 20 ul of the reaction mixture volume using the Go TAQ Green Master Mix (Promega). For KCNJ11 rs5210, PCR conditions were as follows: an initial denaturation of 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec, and final extension of 72°C for 5 min, and for KCNQ1 rs2237895, an initial denaturation of 94°C for 5 minutes, 35 cycles (94°C for 30s, 58°C for 30s, and 72°C for 30s), and final extension of 72°C for 10 min in a PeqSTAR 96 universal gradient thermocycler (Peqlab, VWR). Amplified products were, then, electrophoresed on 2% agarose gel, and images were captured by using the gel documentation system (Biorad).

KCNJ11 (6956A>G) and KCNQ1 (395974 A>C) polymorphisms were detected by using a 6 ul PCR product with 2.5 units of restriction enzymes Hpy188III (NEB) and Ava I (NEB), respectively, incubated overnight at 37°C. Hpy188III digested the PCR product of the KCNJ11 gene; wild type allele (A/A) yielded 1 band of 316 bp; heterozygous (A/G) yielded 3 bands of 316 bp, 218 bp, and 98 bp, and risk allele (G/G) yielded two bands of 218 bp and 98 bp (Figure 1(a)), while Ava I digested the PCR product of the KCNJ11 gene; wild type allele (A/A) produced one band of 485 bp; heterozygous (A/C) produced three bands of 485 bp, 294 bp, and 191 bp, and risk allele (C/C) produced two bands of 294 bp and 191 bp (Figure 1(b)). The digested PCR products were subjected to electrophoresis in a 3% agarose gel, as shown in Figures 1(a) and 1(b).

2.3. Statistical Analysis. Statistical analysis was performed by SPSS Software (21.0 Version, IBM, United States). The chi-square test was applied for comparing genotype and allele frequencies for statistical significance between diabetic patients and controls. Data were presented as mean ± SD or as the number of cases. Comparative analysis of clinicopathological characteristics between cases and control was performed by Student’s t-test, while the chi-square test was used for gender, family history, smoking, and alcohol consumption. The level of significance was set at 95% (i.e., $p < 0.05$).

3. Results

3.1. Clinicopathological Characteristics in T2DM Cases and Control. Various clinicopathological characteristics were compared between cases and control by using Student’s t-test and the chi-square test. The average age of T2DM cases was (40.33 ± 9.76) years, whereas it was (35.29 ± 7.96) years in controls. Compared to control, T2DM cases had significantly higher BMI, PPG, FPG, FPI, HbA1c, T-cholesterol, systolic BP, diastolic BP, triglycerides, and LDL-C. A significant difference was found in the smoking and alcohol consumption between cases and control, while no significant difference was found in HDL, gender, and family history, as depicted in Table 1 and Figure 2.
3.2. Genotype Distribution and Allelic Frequencies of KCNJ11 and KCNQ1 Genes among T2DM Cases and Controls. The genotype and allele distribution of the KCNJ11 gene is shown in Table 2. The distribution of the genotypes was significantly different between T2DM cases and controls. When the AA genotype was set as the reference, both AG and GG genotypes were found to be associated with increased risk for T2DM (OR, 2.05; 95% CI, 1.25–3.37; p = 0.004 for AG; and OR, 2.12; 95% CI, 0.99–4.5; p = 0.046 for GG, respectively, Table 2). The statistical analysis of the observed genotypic frequencies for KCNJ11 (rs5210) showed a significant association (p = 0.007). The frequency of KCNJ11 rs5210 G allele was significantly higher in cases than in controls (37% vs. 26%, respectively). When the A allele of rs5210 was set as reference, the G allele of KCNJ11 contributed to increased risk of T2DM (OR, 1.67; 95% CI, 1.16–2.38; p = 0.004). We found a significant relationship between KCNJ11 rs5210 A → G gene polymorphism and T2DM risk under the dominant (OR, 2.07; 95% CI, 1.30–3.27; p = 0.001) and codominant models (OR, 1.76; 95% CI, 1.09–2.84; p = 0.020), whereas no significant relationship was found under the recessive model (OR, 1.54; 95% CI, 0.74–3.20; p = 0.239).

The genotype and allele distribution of the KCNQ1 gene are shown in Table 3. The distribution of the genotypes was significantly different between T2DM cases and controls. When the AA genotype was set as the reference, both AC and CC genotypes were found to be associated with increased risk for T2DM (OR, 2.36; 95% CI, 1.43–3.89; p = 0.007 for AC; and OR, 2.26; 95% CI, 1.14–4.46; p = 0.017 for CC). The statistical analysis of the observed genotypic frequencies for KCNQ1 showed a significant association (p = 0.001). The frequency of KCNQ1 rs2237895 C allele was significantly higher in cases than in controls (44% vs. 32%, respectively). When the A allele of rs2237895 was set as reference, the C allele of KCNQ1 contributed to increased risk of T2DM (OR, 1.66; 95% CI, 1.19–2.34; p = 0.003). We found a significant relationship between KCNQ1 rs2237895 A → C gene polymorphism and T2DM risk under the dominant model (OR, 2.33; 95% CI, 1.46–3.70; p = 0.0003) and codominant model (OR, 1.85;
95% CI, 1.16–2.95; \( p = 0.009 \)), whereas no significant relationship was found under the recessive model (OR, 1.44; 95% CI, 0.76–2.71; \( p = 0.259 \)).

### 3.3. Observed and Expected Genotypes of KCNJ11 and KCNQ1 Polymorphism in Control and Cases

The observed and expected genotype frequencies of KCNJ11 and KCNQ1 gene polymorphism in controls and cases showed no deviation from the Hardy–Weinberg equilibrium. The chi-square test demonstrated that there was no significant deviation from the Hardy–Weinberg equilibrium for KCNJ11 and KCNQ1 SNP genotypes \( (p > 0.05) \), as shown in Table 4.

### 3.4. Association of the Clinicopathological Characteristic of Type 2 Diabetes Patients with the Carrier of a Variant Allele of KCNJ11 and KCNQ1 Genes

We analysed the clinicopathological data with the carrier of a variant allele (AG + GG) of the KCNJ11 gene and found out that there was no...
statistically significant association between the carrier of a variant allele with age, family history, smoking, alcohol consumption, BMI, FPG, HB1Ac, systolic BP, diastolic BP, cholesterol, FPI, triglyceride, HDL, and LDL. We observed a significant association of gender and PPG with the carrier of a variant allele (AG + GG), as shown in Table 5 and Figure 3 (the result is significant at $p < 0.05$).

Similarly, clinicopathological data with the carrier of a variant allele (AC + CC) of the KCNQ1 gene were analysed, and we found out that there was no statistically significant association between the carrier of a variant allele with age, gender, family history, smoking, alcohol consumption, BMI, FPG, PPG, HB1Ac, systolic BP, diastolic BP, cholesterol, FPI, triglyceride, HDL, and LDL. We observed a significant association of gender and PPG with the carrier of a variant allele (AG + GG), as shown in Table 5 and Figure 3 (the result is significant at $p < 0.05$).

**Table 3: Genotypic and allelic distribution and association analysis of KCNQ1 rs2237895 genes polymorphism and risk of T2DM under different genetic models.**

| Genotype | Cases ($n = 300$) | Control ($n = 100$) | Odds ratio (95% CI) | $p$ value |
|----------|------------------|-------------------|----------------------|-----------|
| AA       | 90 (30%)         | 50 (50%)          | Ref                  | Ref       |
| AC       | 153 (51%)        | 36 (36%)          | 2.36 (1.43–3.89)     | 0.0007*   |
| CC       | 57 (19%)         | 14 (14%)          | 2.26 (1.14–4.46)     | 0.017*    |
| Recessive model | CC | 57 | 14 | 1.44 (0.76-2.71) | 0.259 |
|          | AC + AA          | 243               | 86                   |           |
| Dominant model | AC + CC | 210 | 50 | 2.33 (1.46-3.70) | 0.0003* |
|          | AA               | 90                | 50                   |           |
| Codominant model | AC | 153 | 36 | 1.85 (1.16-2.95) | 0.009* |
|          | AA + CC          | 147               | 64                   |           |
| Allele   | A                | 336 (56%)         | 136 (68%)            | 1.66 (1.19 to 2.34) | 0.003* |
|          | C                | 264 (44%)         | 64 (32%)             |           |

**Table 4: Observed and expected genotypes of KCNJ11 and KCNQ1 polymorphism in control and cases.**

| Genotype | Observed genotype | Expected genotype | $p$ value | Chi square ($X^2$) |
|----------|-------------------|-------------------|-----------|-------------------|
| Control  | KCNJ11 rs5210     |                   |           |                   |
|          | AA                | 58                | 54.76     | 0.2422            | 2.836 |
|          | AG                | 32                | 38.48     | 0.8645            | 0.2912 |
|          | GG                | 10                | 6.76      | 0.2247            | 2.986 |
| KCNQ1 rs2237895 | AA                | 50                | 46.24     | 0.2247            | 2.986 |
|          | AC                | 36                | 43.52     | 0.2247            | 2.986 |
|          | CC                | 14                | 10.24     | 0.2247            | 2.986 |
| Cases    | KCNJ11 rs5210     |                   |           |                   |
|          | AA                | 120               | 117.8133  | 0.8645            | 0.2912 |
|          | AG                | 136               | 140.3733  | 0.8645            | 0.2912 |
|          | GG                | 44                | 41.8133   | 0.8645            | 0.2912 |
| KCNQ1 rs2237895 | AA                | 90                | 92.4075   | 0.8535            | 0.3167 |
|          | AC                | 153               | 148.185   | 0.8535            | 0.3167 |
|          | CC                | 57                | 59.4075   | 0.8535            | 0.3167 |

3.5. **Combined Effect of KCNJ11 + KCNQ1 Genotypes on Clinicopathological Parameters.** The effect of KCNJ11 + KCNQ1 genotypes together had a significant impact on the regulation of PPG, FPG, cholesterol, LDL, and diastolic BP (Table 7).

3.6. **Regression Analysis: Dependent Variable Cases and Control.** Regression analysis was performed to check the involvement of other variables with the occurrence of T2DM. It was observed that the variables such as age, BMI, PPG, FPG, FPI, HbA1C, cholesterol, LDL, systolic BP, diastolic BP, and triglycerides showed a significant association with the study subject and $R^2$ showed an association with T2DM (Table 8), while no association was observed with gender and HDL.

4. **Discussion**

In the present case-control study, we investigated the association of SNPs (rs5210) and (rs2237895) within the KCNJ11 and KCNQ1 genes, respectively, with the susceptibility to T2DM in Indian population. The KCNJ11 polymorphism association with T2DM risk has been extensively
studied among European population; however, their relationship in the Indian subcontinent is yet to be validated. Various GWAS also demonstrated that several KCNQ1 variants (i.e., rs2237892, rs2237895, and rs2237897) are associated with T2DM risk and reduced insulin secretion in Europeans, Asians, and American Indians [10–12]. However, studies in different races and regions have demonstrated different findings. It has also been stated that Indians might have different genetic predisposition to diabetes than Europeans [13]. In this study, we compared various clinicopathological characteristics between cases and control and observed a significant difference in all the parameters except HDL, gender, and family history. Dorman and Bunker suggested that people with a T2DM family

| Characteristic | Total | Wild (AA) | Carrier (AG + GG) | p value |
|----------------|-------|-----------|-------------------|--------|
| Age ≤45        | 183   | 77        | 106               | 0.358  |
| Age >45        | 117   | 43        | 74                |        |
| Gender Male    | 177   | 84        | 93                | 0.0015*|
| Gender Female  | 123   | 36        | 87                |        |
| Family history Yes | 131   | 49        | 82                | 0.419  |
| Family history No  | 169   | 71        | 98                |        |
| Smoking Yes    | 139   | 59        | 80                | 0.421  |
| Smoking No     | 161   | 68        | 100               |        |
| Alcohol consumption Yes | 140   | 62        | 78                | 0.156  |
| Alcohol consumption No  | 160   | 5         | 102               |        |
| BMI ≤25        | 86    | 34        | 52                | 0.916  |
| BMI >25        | 214   | 83        | 128               |        |
| FPG ≤140       | 214   | 87        | 127               | 0.715  |
| FPG >140       | 86    | 3         | 53                |        |
| PPG ≤208       | 171   | 77        | 94                | 0.040* |
| PPG >208       | 129   | 43        | 86                |        |
| HBA1C ≤7       | 192   | 75        | 117               | 0.658  |
| HBA1C >7       | 108   | 45        | 63                |        |
| Systolic BP ≤146| 128   | 46        | 82                | 0.215  |
| Systolic BP >146| 172   | 74        | 98                |        |
| Diastolic BP ≤102| 206   | 90        | 116               | 0.053  |
| Diastolic BP >102| 94    | 30        | 64                |        |
| Cholesterol ≤245| 192   | 84        | 108               | 0.077  |
| Cholesterol >245| 108   | 36        | 72                |        |
| FPI ≤9         | 135   | 52        | 83                | 0.635  |
| FPI >9         | 165   | 68        | 97                |        |
| Triglyceride ≤355| 156   | 66        | 90                | 0.395  |
| Triglyceride >355| 144   | 54        | 90                |        |
| HDL-C ≤45      | 189   | 77        | 112               | 0.732  |
| HDL-C >45      | 111   | 43        | 68                |        |
| LDL-C ≤194     | 93    | 39        | 54                | 0.646  |
| LDL-C >194     | 207   | 81        | 126               |        |

*p values were determined by the chi-square test. *Significant association at p > 0.05.
Table 6: Clinicopathological characteristics of T2DM patients between wild and carrier of a variant allele of the KCNQ1 gene.

| Characteristic       | Total | Wild (AA) | Carrier (AC + CC) | p value |
|----------------------|-------|-----------|------------------|---------|
| Age                  |       |           |                  |         |
| ≤45                  | 183   | 56        | 127              | 0.77    |
| >45                  | 117   | 34        | 83               |         |
| Gender               |       |           |                  |         |
| Male                 | 177   | 59        | 118              | 0.130   |
| Female               | 123   | 31        | 92               |         |
| Family history       |       |           |                  |         |
| Yes                  | 131   | 39        | 92               | 0.939   |
| No                   | 169   | 51        | 118              |         |
| Smoking              |       |           |                  |         |
| Yes                  | 139   | 42        | 97               | 0.939   |
| No                   | 161   | 48        | 113              |         |
| Alcohol consumption  |       |           |                  |         |
| Yes                  | 140   | 45        | 95               | 0.448   |
| No                   | 160   | 45        | 115              |         |
| BMI                  |       |           |                  |         |
| ≤25                  | 86    | 27        | 59               | 0.738   |
| >25                  | 214   | 63        | 151              |         |
| FPG                  |       |           |                  |         |
| ≤140                 | 214   | 64        | 150              | 0.955   |
| >140                 | 86    | 26        | 60               |         |
| PPG                  |       |           |                  |         |
| ≤208                 | 171   | 50        | 121              | 0.740   |
| >208                 | 129   | 40        | 89               |         |

Table 6: Continued.

| Characteristic       | Total | Wild (AA) | Carrier (AC + CC) | p value |
|----------------------|-------|-----------|------------------|---------|
| HBA1C                |       |           |                  |         |
| ≤7                   | 192   | 62        | 130              | 0.248   |
| >7                   | 108   | 28        | 80               |         |
| Systolic BP          |       |           |                  |         |
| ≤146                 | 128   | 33        | 95               | 0.168   |
| >146                 | 172   | 57        | 115              |         |
| Diastolic BP         |       |           |                  |         |
| ≤102                 | 206   | 62        | 144              | 0.956   |
| >102                 | 94    | 28        | 66               |         |
| Cholesterol          |       |           |                  |         |
| ≤245                 | 192   | 63        | 129              | 0.156   |
| >245                 | 108   | 27        | 81               |         |
| FPI                  |       |           |                  |         |
| ≤9                   | 135   | 42        | 93               | 0.704   |
| >9                   | 165   | 48        | 117              |         |
| Triglyceride         |       |           |                  |         |
| ≤355                 | 156   | 36        | 120              | 0.006*  |
| >355                 | 144   | 54        | 90               |         |
| HDL-C                |       |           |                  |         |
| ≤45                  | 189   | 57        | 132              | 0.937   |
| >45                  | 111   | 33        | 78               |         |
| LDL-C                |       |           |                  |         |
| ≤194                 | 93    | 29        | 64               | 0.764   |
| >194                 | 207   | 61        | 146              |         |

p values were determined by the chi-square test. *Significant association at p > 0.05.

Figure 3: Graphical representation of clinicopathological characteristics of T2DM patients between wild and carrier of a variant allele of the KCNJ11 gene.
Table 7: Combined effect of KCNJ11 + KCNQ1 genotypes on clinicopathological parameters.

| Parameters | Genotype variables KCNJ11 + KCNQ1 | Mean ± SD | p value |
|------------|----------------------------------|-----------|---------|
| BMI        | (AA + AA)                        | 27.79 ± 4.47 |         |
|            | (AG + AC)                        | 29.24 ± 5.07 |         |
|            | (GG + CC)                        | 25.99 ± 3.37 |         |
|            | (AG + CC)                        | 27.88 ± 5.49 |         |
|            | (AG + AA)                        | 27.45 ± 4.8 |         |
|            | (GG + AA)                        | 27.47 ± 5.00 |         |
| PPG        | (AA + AA)                        | 177.41 ± 53.14 |         |
|            | (AG + AC)                        | 193.88 ± 39.85 |         |
|            | (GG + CC)                        | 177.59 ± 47.63 |         |
|            | (AG + CC)                        | 210.09 ± 68.61 |         |
|            | (AG + AA)                        | 184.07 ± 46.72 |         |
|            | (GG + AA)                        | 196.50 ± 62.67 |         |
| FPG        | (AA + AA)                        | 121.12 ± 40.06 |         |
|            | (AG + AC)                        | 132.55 ± 38.45 |         |
|            | (GG + CC)                        | 120.45 ± 26.15 |         |
|            | (AG + CC)                        | 132.07 ± 43.79 |         |
|            | (AG + AA)                        | 122.18 ± 30.65 |         |
|            | (GG + AA)                        | 134.38 ± 42.72 |         |
| FPI        | (AA + AA)                        | 9.04 ± 1.20 |         |
|            | (AG + AC)                        | 9.54 ± 1.41 |         |
|            | (GG + CC)                        | 8.99 ± 1.14 |         |
|            | (AG + CC)                        | 9.42 ± 1.24 |         |
|            | (AG + AA)                        | 9.39 ± 1.23 |         |
|            | (GG + AA)                        | 9.42 ± 1.40 |         |
| HbA1C      | (AA + AA)                        | 6.57 ± 1.10 |         |
|            | (AG + AC)                        | 6.94 ± 1.12 |         |
|            | (GG + CC)                        | 6.59 ± 0.93 |         |
|            | (AG + CC)                        | 6.79 ± 1.08 |         |
|            | (AG + AA)                        | 6.74 ± 1.08 |         |
|            | (GG + AA)                        | 6.92 ± 1.24 |         |

Figure 4: Graphical representation of clinicopathological characteristics of T2DM patients between wild and carrier of a variant allele of the KCNQ1 gene.
However, family history is considered as a strong and independent risk factor for diabetes [15–19].

In humans, the significance of KCNJ11 in insulin secretion was suggested by its function in permanent neonatal diabetes [20] and familial persistent hyperinsulinaemic hypoglycaemia of infancy [21]. KCNJ11 is considered as a promising candidate susceptibility gene for T2DM due to the protein encoded by KCNJ11, which is crucial for pancreatic beta-cell function. In the present study, we observed a significant difference in the distribution of KCNJ11 genotypes among T2DM cases and healthy controls and higher risk allele distribution was observed among cases compared to healthy controls. Also, the KCNJ11 rs5210 variant demonstrated a significant association between T2DM cases and controls under dominant and codominant models. Our findings indicated an association of the KCNJ11 rs5210 polymorphism with T2DM in Indian patients. Our results were found to be similar to other studies which confirmed the association of KCNJ11 variant 3p+215 (rs5210) with T2DM [22, 23]. Similarly, meta-analysis in the East Asian population and genotypic and allelic contrast also suggested

### Table 7: Continued.

| Variables         | (AA + AA) | (AG + AC) | (GG + CC) | (AG + CC) | (AG + AA) | (GG + AA) |
|-------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| Cholesterol       | 211.97 ± 46.99 | 230.80 ± 38.24 | 218.63 ± 52.46 | 234.34 ± 36.31 | 216.69 ± 44.70 | 223.84 ± 45.10 |
| HDL               | 46.69 ± 9.18  | 45.03 ± 10.48  | 47.72 ± 9.52  | 45.39 ± 12.97  | 46.80 ± 10.20  | 44.23 ± 12.98  |
| LDL               | 159.88 ± 48.49 | 182.14 ± 41.31 | 170.90 ± 53.74 | 183.42 ± 41.27 | 166.94 ± 47.61 | 176.92 ± 45.11  |
| Cholesterol       | 277.84 ± 133.61 | 317.14 ± 125.36 | 274.09 ± 109.46 | 336.88 ± 123.27 | 289.20 ± 128.33 | 313.30 ± 120.98  |
| Systolic BP       | 132.08 ± 24.79 | 138.63 ± 21.56 | 133.36 ± 25.96 | 141.96 ± 22.52 | 134.87 ± 24.31 | 138.53 ± 23.17  |
| Diastolic BP      | 93.84 ± 22.38 | 100.10 ± 17.54 | 95.45 ± 21.38 | 100.39 ± 16.99 | 93.22 ± 19.21 | 94.87 ± 15.87  |

*Significant at p > 0.05.

### Table 8: Regression analysis: dependent variable cases and control.

| Variables | R² | p value |
|-----------|----|---------|
| Age       | 0.05 | <0.0001 |
| Gender    | 0.006 | 0.11 |
| BMI       | 0.119 | <0.0001 |
| PPG       | 0.360 | <0.0001 |
| FPG       | 0.317 | <0.0001 |
| FPI       | 0.099 | <0.0001 |
| HbA1C     | 0.290 | <0.0001 |
| Cholesterol | 0.862 | <0.0001 |
| HDL       | 0.00 | 0.80 |
| LDL       | 0.689 | <0.0001 |
| Systolic BP | 0.560 | <0.0001 |
| Diastolic BP | 0.378 | <0.0001 |
| Triglycerides | 0.535 | <0.0001 |

*Significant at p > 0.05.

history are three times more susceptible to this disease [14]. However, in our study, we have not observed any association of family history with T2DM risk between cases and control.
a significant association of KCNJ11 and T2DM for rs5210 [24]. Furthermore, the rs5210 variant of the KCNJ11 gene was indicated to be related with T2DM risk in meta-analysis of 5 studies, and it was significantly heterogeneous ($p = 0.02$) [25]. Similar results were also observed among the Mexican, Finnish, and Korean populations [26–28]. Our results were found to be inconsistent with other studies in which no association was found between KCNJ11 E23K polymorphism and T2DM in Iranian [29], Czech [30], Moroccan [31], and Mongolian population [32].

KCNQ1 comprises of 676 amino acids with 1 ion-selective P loop and 6 transmembrane regions. The P loop is composed of 4 identical $\alpha$ subunits, which is an ion-filter duct. It consists of a porous structure which is highly conservative leading to higher selectivity to potassium. The KCNQ1 knockout mouse showed prominent increase in insulin sensitivity, suggesting that KCNQ1 may act as a novel element affecting insulin sensitivity through glucose metabolism [33]. In our study, the genotype and allele distribution of KCNQ1 rs2237895 showed a significant difference between T2DM cases and controls and higher risk allele distribution was observed among cases as compared to healthy controls. Also, the KCNQ1 rs2237895 variant showed a significant association between T2DM cases and controls under dominant and codominant models. Our findings indicated an association of the KCNQ1 rs2237895 polymorphism with T2DM in Indian patients. Our findings were found to be consistent with the previous study in which KCNQ1 gene polymorphism (rs2074196, rs2237892, rs2237895, rs2283228, and rs2237897) was linked with T2DM risk among Japanese populations in 2 independent genome-wide association studies [3, 10]. Furthermore, in European population, according to GWA scans and replication studies, SNPs (rs151290, rs2237892, and rs2237895) of the KCNQ1 were indicated to be a genetic risk factor for T2DM [3, 10, 34–36]. In addition, many previous studies proposed that various variants of KCNQ1 rs2237895, rs2283228, and rs2237897) was linked with T2DM risk among Japanese populations in 2 independent genome-wide association studies [3, 10, 34–36]. In addition, many previous studies proposed that various variants of KCNQ1 rs2237895, rs2237892, rs2237897, and rs151290 showed an association with T2DM in China [37], Germany [35], Pakistan, and the Netherlands [38, 39].

In East Asian origin patients, the KCNQ1 gene has been found to play a significant role in contributing to T2D susceptibility [3, 10, 40]. Some studies were contradictory to our finding which did not find any association of KCNQ1 variants (rs2237892 and rs2237895) and T2DM risk [41, 42]. A study on the South Indian population from Hyderabad also showed a significant association of KCNJ11 (rs5210) and KCNQ1 (rs2283228) with T2DM risk, which corroborates with our results [43]. However, we did not come across any such study investigating the association of KCNJ11 (rs5210) and KCNQ1 (rs2237895) gene polymorphism with T2DM risk in North Indian origin patients.

Additionally, we analysed clinicopathological data with the carrier of a variant allele of KCNJ11 and KCNQ1. We found an association between the carrier of a variant allele with gender and PPG in KCNJ11 and with triglyceride in KCNQ1. Chen et al. also suggested that KCNQ1 was associated with triglyceride levels in the Chinese Han population [44]. This study will help in understanding the genetic background of T2DM, which is very essential for the identification of high-risk individuals and to include prevention plan in these high-risk individuals and general population.

5. Conclusions
In the present study, we identify a significant association of KCNJ11 (rs5210) and KCNQ1 (rs2237895) gene polymorphism with T2DM risk, suggesting the role of these variants in the increased risk of developing T2DM in the Indian population. We also found that the G allele of rs5210 and C allele of rs2237895 polymorphism of KCNJ11 and KCNQ1, respectively, are the major risk factors that confer susceptibility to T2DM. Furthermore, these genetic variants will enable us to find the risk prediction of T2DM development and its underline pathogenesis, which will be applicable for individualized treatment of T2DM.

Abbreviations

| Acronym | Description |
|---------|-------------|
| IDF     | International Diabetes Federation |
| KCNJ11  | Potassium inwardly rectifying channel, subfamily J, member 11 |
| KCNQ1   | Potassium voltage-gated channel KQT-like subfamily, member 1 |
| GWAS    | Genome-wide association studies |
| PCR     | Polymerase chain reaction-restriction fragment |
| RFLP    | length polymorphism |
| SUR1    | Sulfonylurea receptor 1 |
| KATP    | ATP-sensitive K channel |
| OR      | Odds ratio |
| CI      | Confidence interval |
| HWE     | Hardy–Weinberg equilibrium |
| SNP     | Single nucleotide polymorphism |

Data Availability

We confirm that the data used during the research will not be shared with anybody or broadcasted in any public domain, since it is impermissible as per the policy instructions of JMI. The metadata, supporting the study outcomes, can be accessed from JMI through proper consent; however, privileged data with restricted open accessibility, cedes institutional authorization, and discretion and other related information and data can also be retrieved from the authors with permission of JMI.

Additional Points

**Highlights.** (i) The genotype and allele frequencies in type 2 diabetes cases were significantly different compared to the control population. (ii) Clinicopathological parameters were analysed, and a significant association was found between the carrier of a variant allele with gender and PPG in KCNJ11 and with triglyceride in KCNQ1. (iii) KCNJ11 (rs5210) and KCNQ1 (rs2237895) gene variants are significantly associated with type 2 diabetes in the Indian population.
Ethical Approval

Institutional Ethics Committee of Jamia Millia Islamia (Proposal No. 17/9/14/JMI/IEC/2015 dated 14/01/2016) approved this study.

Consent

Written informed consent was obtained before inclusion in the study.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors’ Contributions

Amit Kumar Verma, Vasiuddin Khan, and Deepti Bhatt equally contributed to this work. Conceptualization was carried out by KD, AKV and MYS; VK, AKV, and SK were responsible for the methodology; DB, AKV, and AHR were responsible for the software; validation was performed by AKV, YG, RH, and MAA; formal analysis was performed by BM, AKV, and KD; visualization was performed by BM, AKV, and KD; review and editing were performed by YG, SR, AA, BM, AKV, and DB; VK, DB, and AKV wrote the original draft; VK, SK, and AKV; data curation was performed by VK, SK, and AKV; supervision was performed by KD; validation was performed by DB, AKV, and AHR; and funding acquisition was performed by AKV, and KD; and supervision was performed by KD.

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