

# Association of *KCNJ11* E23K/rs5219 Gene Polymorphism with Type 2 Diabetes and Diabetes-Related Cardiovascular Disease

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**Objective:** A potassium voltage-gated channel subfamily J member 11 (*KCNJ11*) is a candidate gene for diabetes and cardiovascular disease. We investigated the relationship of *KCNJ11* E23K gene polymorphism with type 2 diabetes (T2DM) and diabetes-related cardiovascular disease (CVD).

**Methods:** In this case-control study, the *KCNJ11* E23K (rs5219) single nucleotide polymorphism was evaluated using the PCR-RFLP method in 780 patients with T2DM and 425 healthy controls. The genotype distribution was compared between subgroups of patients with CVD (524) and without CVD (256).

**Results:** The genotyping results showed that the T allele and TT genotype were associated with the risk of T2DM (OR 1.26,  $p = 0.008$  and OR 1.55,  $p = 0.0019$ , respectively). The T2DM group was analyzed according to the presence or absence of CVD. The T allele frequency was significantly higher in CVD+ than CVD- patients (49% vs 28%,  $p = 0.0001$ ). The frequency of TT genotype in CVD+ subgroup was 20% compared to 8.5% in CVD-. This shows the significant correlation of the T allele with CVD in T2DM patients in all genetic association models. The OR for T allele was 2.44,  $p < 0.0001$  representing 2.5-fold higher odds of CVD. For TT genotype, the OR 5.61,  $p < 0.0001$  represents almost 6-fold higher risk of CVD development. The multiple logistic regression analysis showed that *KCNJ11* E23K polymorphism was a significant risk predictor for CVD development ( $p < 0.0001$ ).

**Conclusion:** This is the first study of the relationship between *KCNJ11* gene polymorphism and cardiovascular risk in T2DM patients in Polish population. The E23K (rs5219) polymorphism is associated with T2DM. It also increases the risk of cardiovascular disease in T2DM patients. If confirmed in other studies, it can be considered a potential marker for predicting the risk of CVD in T2DM patients.

**Keywords:** *KCNJ11* gene, type 2 diabetes, cardiovascular risk, single nucleotide polymorphism, genotyping

## Introduction

Type 2 diabetes mellitus (T2DM) is a polygenic disorder of glucose metabolism characterized by insulin resistance and/or pancreatic  $\beta$ -cell dysfunction.<sup>1</sup> It develops as a result of complex interactions between multiple genetic and environmental factors. T2DM accounts for close to 90% of all diabetes cases, affecting over 450 million people around the world.<sup>2</sup> Type 2 diabetes is associated with high morbidity and mortality due to macrovascular and microvascular complications.<sup>3,4</sup> The presence of cardiovascular disease (CVD) in T2DM patients is a serious clinical problem and one of the most frequent causes of death.<sup>5</sup> Genome-wide association studies (GWAS) have identified more than 100 loci for susceptibility to T2DM and different genetic variants significantly associated with diabetes and cardiovascular disease.<sup>6-8</sup> The identification of new genes predisposing to T2DM would expand our knowledge of this complex disease and its vascular complications and result in better preventing, diagnosis and treatment options.

One of the important candidate genes that may be associated with T2DM is the potassium inwardly rectifying channel subfamily J, member 11 (*KCNJ11*) involved in the insulin secretion pathway.<sup>9,10</sup> The *KCNJ11* gene is located on chromosome 11p15.1, it encodes Kir6.2 protein. The 390 amino acid protein is the internal fragment of the adenosine

triphosphate sensitive potassium ion channel (KATP) that plays an important role in glucose metabolism, pancreatic beta cell function and insulin action.<sup>11,12</sup> Several single nucleotide polymorphisms (SNP) have been described in the *KCNJ11* gene. One of them is E23K (rs5219) polymorphism caused by a change of guanine to adenine at codon 23, resulting in a glutamic acid to lysine substitution. It reduces sensitivity of potassium channel to ATP resulting in its over-activity and critically inhibits insulin secretion.<sup>13</sup> An association between *KCNJ11* rs5219 polymorphism and T2DM risk have been observed in several studies.<sup>14,15</sup> The *KCNJ11* gene guides the formation of ATP-sensitive potassium channel in cardiomyocytes. The genomic analysis studies have recently implicated the *KCNJ11* variants in pathological processes in a variety of cardiovascular diseases.<sup>16</sup>

We hypothesized that E23K polymorphism in *KCNJ11* gene is a risk factor of T2DM in a Caucasian population. The aim of the present study was to test this hypothesis by investigating the potential association of *KCNJ11* rs5219 polymorphism with T2DM and T2DM-related cardiovascular disease.

## Materials and Methods

### Study Subjects

The patients enrolled in this preliminary case-control study were recruited from the University Hospital, Medical University of Lublin. The patient group included 780 unrelated subjects (422 males and 358 females) with type 2 diabetes of at least 10-year duration (mean age  $62.9 \pm 9.1$  years). All patients were Caucasians of Polish origin. The majority of patients and control subjects were included in our earlier study.<sup>17</sup> In the current investigation, patients were genotyped with another SNP, as a new candidate gene, to evaluate the effect of the *KCNJ11* gene polymorphism on the development of diabetes-related cardiovascular disease.

The diagnosis of type 2 diabetes was based on the current American Diabetes Association criteria.<sup>18</sup> The disease onset was at  $>30$  years of age in all subjects. A complete physical examination included plasma fasting glucose, glycated hemoglobin (HbA1c), full lipid profile, and body mass index (BMI). Patients with the following conditions were excluded: type 1 and other types of diabetes, liver dysfunction, acute or chronic inflammatory conditions, autoimmune diseases and malignancies. The T2DM duration was defined as the period between the first diagnosis of diabetes and the time of enrollment in the study.

T2DM-related cardiovascular disease was diagnosed in 524 patients (67%) with at least one of the following pathological conditions: congestive heart failure, left ventricular hypertrophy, angina pectoris, ischemic heart disease, myocardial infarction and ischemic cerebral stroke. To verify the clinical presentation of CVD, the relevant biochemical tests were performed and the radiographic, echocardiographic and vascular diagnostic criteria applied.

The healthy control group of randomly selected 425 individuals (mean age  $57.5 \pm 8.1$  years) consisted of unrelated volunteers with fasting plasma glucose less than 100 mg/dl, HbA1c  $<5.7\%$  and no known personal or family history of diabetes of any type and cardiovascular disease.

Participation in the study was voluntary and before being included all T2DM patients and healthy controls provided a written informed consent. All procedures and experiments in this study were carried out in accordance with principles of the Declaration of Helsinki (version 2013). The ethical approval of detailed research protocol for the study was provided by the Bioethics Committee of Medical University of Lublin (KE-0254/26/01/2023).

### Determination of *KCNJ11* E23K (rs5219) Genotype

Genomic DNA was extracted from venous blood using a Qiagen DNeasy Blood & Tissue Kit (Qiagen Poland, Warsaw, Poland). DNA purity and concentration were determined in Nano Drop 2000 (Thermo Fisher Scientific, Waltham, MA, USA). The E23K variant in the *KCNJ11* gene was detected by polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) assay using the Thermal Cycler 9700 (Applied Biosystems, Foster City, CA., USA). The primer sequences were as follows: forward 5'-GACTCTGCAGTGAGGCCCTA-3' and reverse 5'-ACGTTGCAGTTGCCTTCTT-3'. The PCR program was carried out in the following steps: after initial denaturation at 95°C for 6 min DNA was subjected to 35 amplification cycles of denaturation at 94°C for 30s, annealing at 62°C for 30s and extension at 72°C for 1 min, with a final extension at 72°C for 10 min. The PCR product of 210 bp was

incubated with Ban II restriction endonuclease (Thermo Fisher Scientific, Waltham, MA, USA) at 37°C for 12 h. The products of digestion were separated by electrophoresis in 2.5% agarose gel. DNA fragments of 146 bp, 36 bp and 28 bp corresponded to the minor C allele (E in E23K) and two fragments, 174 bp and 36 bp represented the T allele (K in E23K). The genotyping was validated by repeating PCR reactions for 10% of samples. In addition, randomly selected samples (20 for each genotype) were directly sequenced in CEQ 8000 Genetic Analysis System (Beckman Coulter, High Wycombe, UK) to confirm the genotype reading in agarose gel. The genotype concordance rate between assays was 100%.

## Statistical Analysis

The statistical management and analysis of collected data were conducted using a statistical software SPSS version 18.0 for Windows (SPSS, Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was used for normality. The values of normally distributed variables for the baseline characteristics are shown as mean  $\pm$  standard deviation (SD) or numbers and percentages where appropriate. Differences in discrete and continuous variables between groups were evaluated by an unpaired Student's *t*-test and Mann–Whitney test. Potential deviation from Hardy–Weinberg balance in the genotype distribution was assessed in a Fisher's exact test. The *KCNJ11* rs5219 polymorphism distributions in patients with and without CVD were compared using a Pearson chi-square test of independence. The strength of the observed associations of studied polymorphism with clinical phenotype was estimated by computing the odds ratios (OR) with corresponding 95% confidence intervals (CI), with a non-risk allele/genotype considered a reference. Variables showing significant associations were analyzed in multivariate logistic regression analysis. It was used to find independent predictors of CVD risk in T2DM patients. ORs were adjusted based on age, gender, BMI and the presence of hypertension. The level of statistical significance for all tests was set at  $p < 0.05$ .

## Results

### Characteristics of the Study Population

The study cohort consisted of 780 T2DM patients and 425 control individuals. T2DM patients were divided into two phenotype subgroups, patients with CVD ( $n = 524$ ) and those without CVD ( $n = 256$ ) for the purpose of comparison. The clinical and laboratory parameters of the study subjects are summarized in Table 1. Patients with CVD were older (mean age  $64.6 \pm 11.3$  years) than those without CVD (mean age  $61.3 \pm 9.2$  years). The male/female ratio was similar in CVD+ and CVD– subgroups ( $p = 0.087$ ). The CVD+ patients were younger at the time of diabetes diagnosis ( $p = 0.001$ ). There were no statistically significant differences between patients with and without CVD with respect to BMI, triglyceride

**Table 1** Basic Characteristics of T2DM Patients and Controls According to the Presence or Absence of CVD

| Parameters                     | T2DM CVD+       | T2DM CVD–       | Controls       | <i>p</i> * | <i>p</i> ** |
|--------------------------------|-----------------|-----------------|----------------|------------|-------------|
| N                              | 524             | 256             | 425            |            |             |
| Males / females (n)            | 293 / 231       | 129 / 127       | 223 / 202      | 0.087      | 0.296       |
| Age (yrs)                      | $64.6 \pm 11.3$ | $61.3 \pm 9.2$  | $58.7 \pm 8.4$ | <0.001     | <0.001      |
| Age at diagnosis (yrs)         | $38.6 \pm 8.3$  | $40.7 \pm 9.6$  | NA             | 0.001      |             |
| T2DM duration (yrs)            | $14.7 \pm 8.1$  | $16.9 \pm 10.2$ | NA             | 0.001      |             |
| BMI ( $\text{kg}/\text{m}^2$ ) | $27.8 \pm 5.2$  | $28.1 \pm 5.9$  | $27.4 \pm 4.2$ | 0.469      | 0.203       |
| TC (mmol/l)                    | $4.9 \pm 1.6$   | $4.5 \pm 1.3$   | $4.4 \pm 0.8$  | <0.001     | <0.001      |
| HDL C (mmol/l)                 | $1.2 \pm 0.4$   | $1.3 \pm 0.5$   | ND             | 0.002      |             |
| TG (mmol/l)                    | $1.8 \pm 1.2$   | $1.7 \pm 1.3$   | ND             | 0.288      |             |
| HbA1c (%)                      | $8.2 \pm 2.1$   | $8.1 \pm 1.6$   | ND             | 0.501      |             |

**Notes:** Variables presented as means  $\pm$  SD or numbers (%). Variable values determined by Student's *t*-test for continuous and Mann–Whitney test for discrete variables. \**p* calculated for T2DM CVD+ vs T2DM CVD–. \*\**p* calculated for T2DM CVD+ vs controls.

**Abbreviations:** T2DM, type 2 diabetes; CVD, cardiovascular disease; BMI, body mass index; TC, total cholesterol; HDL C, HDL cholesterol; TG, triglyceride; HbA1c, glycated hemoglobin A1c; NA, not applicable; ND, not determined.

levels and HbA1c values. The levels of total cholesterol were higher ( $p < 0.001$ ) and of HDL cholesterol were lower ( $p = 0.002$ ) in the CVD+ subgroup.

## KCNJ11 E23K Genotyping

All subjects were successfully genotyped with the E23K (rs5219) SNP in the *KCNJ11* gene. The frequencies of the genotypes (CC, CT and TT) among T2DM patients and healthy controls are compared in Table 2. The minor allele (T) frequency in control group was similar to those reported for other European populations.<sup>19,20</sup> The allele and genotype proportions in control group are consistent with Hardy–Weinberg equilibrium ( $p = 0.532$ ). In the polymorphism distribution comparison of T2DM and control groups, we observed a statistically significant differences in the codominant and dominant genetic models. The T allele and TT genotype were associated with the risk of T2DM (OR 1.26, 95% CI 1.06–1.49,  $p = 0.008$  and OR 1.55, 95% CI 1.07–2.26,  $p = 0.019$ , respectively). The frequency of the minor allele in healthy individuals involved in this study was 37% compared to 43% in T2DM group ( $p = 0.043$ ).

## Association Between KCNJ11 E23K Polymorphism and CVD

The group of T2DM patients was further analyzed according to the presence or absence of CVD. The distribution of the E23K (rs5219) polymorphism was compared in subgroups of CVD+ ( $n = 524$ ) and CVD– ( $n = 256$ ) patients. The results presented in Table 3 show that genotype and allele frequencies differed significantly between these subgroups. The frequency of the minor T allele in the CVD+ subgroup was significantly higher than in patients without CVD (49% and 28%, respectively,  $p < 0.0001$ ). The TT genotype was also more frequent in the subgroup with CVD compared to CVD– patients (20% and 8.5%, respectively,  $p < 0.0001$ ). These results show the significant correlation of the T allele carriership with a development of CVD in T2DM patients in all genetic association models. The OR for the T allele was 2.44 (1.94–3.08),  $p < 0.0001$ , representing 2.5-fold higher odds of CVD. For the TT genotype, the OR 5.61 (3.32–9.50),  $p < 0.0001$  represents almost 6-fold higher odds of CVD compared to CC homozygotes. The T allele was also more frequent in both CVD+ and CVD– subgroups when compared to healthy controls ( $p = 0.0002$  for CVD+ and  $p = 0.016$  for CVD–). The adjusted OR for the T allele was 2.12 (1.86–2.95),  $p < 0.0001$  for CVD+ and 1.12 (0.73–1.36),  $p = 0.001$  for CVD–. The clinical and biochemical characteristics of CVD+ and CVD– patients were further analyzed with respect to genotype (Table 4).

**Table 2** Distribution of *KCNJ11* E23K Genotypes and Alleles in T2DM Patients and Healthy Controls

|               | N             | Genotypes |          |                  | MAF  | H-W<br>Equilibrium              | OR (95% CI) <sup>b</sup>        |                                 |
|---------------|---------------|-----------|----------|------------------|------|---------------------------------|---------------------------------|---------------------------------|
|               |               | CC (%)    | CT (%)   | TT (%)           |      |                                 | T Allele                        | TT Genotype <sup>a</sup>        |
| T2DM patients | 780           | 242 (31)  | 413 (53) | 125 (16)         | 0.43 | $\chi^2 = 5.418$<br>$p = 0.019$ | 1.26 (1.06–1.49)<br>$p = 0.008$ | 1.55 (1.07–2.26)<br>$p = 0.019$ |
| Controls      | 425           | 166 (39)  | 204 (48) | 55 (13)          | 0.37 | $\chi^2 = 0.389$<br>$p = 0.532$ | ref.                            | ref.                            |
| Model         | T2DM patients |           | Controls | OR (95% CI)      |      | p value                         | OR (95% CI)                     | p value                         |
| Codominant    |               |           |          |                  |      |                                 |                                 |                                 |
| CC            | 242           |           | 166      | Ref.             |      |                                 | Ref.                            |                                 |
| CT            | 413           |           | 204      | 1.38 (1.07–1.79) |      | 0.013                           | 1.74 (1.22–2.14)                | 0.037                           |
| TT            | 125           |           | 55       | 1.55 (1.07–2.26) |      | 0.019                           | 1.67 (1.09–2.34)                | 0.009                           |
| Dominant      |               |           |          |                  |      |                                 |                                 |                                 |
| CC            | 242           |           | 166      | Ref.             |      |                                 | Ref.                            |                                 |
| TT + CT       | 538           |           | 259      | 1.42 (1.11–1.82) |      | 0.005                           | 1.51 (1.04–2.33)                | 0.018                           |
| Recessive     |               |           |          |                  |      |                                 |                                 |                                 |
| CT + CC       | 655           |           | 370      | Ref.             |      |                                 | Ref.                            |                                 |
| TT            | 125           |           | 55       | 1.26 (0.89–1.77) |      | 0.178                           | 1.13 (0.92–1.86)                | 0.053                           |

**Notes:** Genotype distribution is shown as numbers (%). <sup>a</sup>Calculated vs CC genotype. <sup>b</sup>ORs adjusted for age, gender and BMI H-W equilibrium was assessed by a Fisher exact test. Pearson's Chi-square test of independence was used for comparing polymorphism distribution between groups.

**Abbreviations:** T2DM, type 2 diabetes mellitus; MAF, minor allele frequency.

**Table 3** Distribution of *KCNJ11* E23K Polymorphism in T2DM Patients with and without CVD

|              | N                    | Genotypes            |                    |                | MAF                            | OR (95% CI) <sup>b</sup> |                          |
|--------------|----------------------|----------------------|--------------------|----------------|--------------------------------|--------------------------|--------------------------|
|              |                      | CC (%)               | CT (%)             | TT (%)         |                                | T allele                 | TT genotype <sup>a</sup> |
| T2DM CVD+    | 524                  | 110 (21)             | 311 (59)           | 103 (20)       | 0.49                           | 2.44 (1.94–3.08)         | 5.61 (3.32–9.50)         |
| T2DM CVD–    | 256                  | 132 (51.5)           | 102 (40)           | 22 (8.5)       | 0.28                           | ref.                     | ref.                     |
| <b>Model</b> | <b>CVD+ patients</b> | <b>CVD– patients</b> | <b>OR (95% CI)</b> | <b>p value</b> | <b>OR (95% CI)<sup>b</sup></b> | <b>p value</b>           |                          |
| Codominant   |                      |                      |                    |                |                                |                          |                          |
| CC           | 110                  | 132                  | Ref.               |                |                                |                          |                          |
| CT           | 311                  | 102                  | 3.65 (2.61–5.12)   | < 0.0001       | 3.06 (2.72–531)                | < 0.0001                 |                          |
| TT           | 103                  | 22                   | 5.61 (3.32–9.50)   | < 0.0001       | 6.02 (2.97–9.13)               | < 0.0001                 |                          |
| Dominant     |                      |                      |                    |                |                                |                          |                          |
| CC           | 110                  | 132                  | Ref.               |                |                                |                          |                          |
| TT + CT      | 414                  | 124                  | 4.0 (2.90–5.53)    | < 0.0001       | 3.46 (2.83–4.96)               | < 0.0001                 |                          |
| Recessive    |                      |                      |                    |                |                                |                          |                          |
| CT + CC      | 421                  | 234                  | Ref.               |                |                                |                          |                          |
| TT           | 103                  | 22                   | 2.60 (1.59–4.23)   | 0.0001         | 2.73 (2.16–4.33)               | < 0.0001                 |                          |

**Notes:** Genotype distribution data are expressed as numbers (%). <sup>a</sup>Calculated versus CC genotype. <sup>b</sup>ORs adjusted for age, gender and BMI: CVD+ vs CVD– were: T allele 2.19 (1.86–2.57),  $p < 0.0001$ ; TT genotype 5.82 (3.24–8.51),  $p < 0.0001$ . Hereditary model. Pearson's Chi-square test of independence was used for comparing polymorphism distribution between groups.

**Abbreviations:** T2DM, type 2 diabetes mellitus; CVD, cardiovascular disease; MAF, minor allele frequency.

**Table 4** Correlation Between rs5219 Genotype and Basic Characteristics of T2DM Patients with and without CVD

| Characteristics            | T2DM CVD+ (n = 524) |            |            |         | T2DM CVD– (n = 256) |             |             |         |
|----------------------------|---------------------|------------|------------|---------|---------------------|-------------|-------------|---------|
|                            | CC                  | CT         | TT         | p value | CC                  | CT          | TT          | p value |
| Age at diagnosis (yrs.)    | 38.5 ± 8.6          | 38.5 ± 8.2 | 38.7 ± 8.1 | 0.861   | 38.6 ± 9.2          | 41.7 ± 9.7  | 41.6 ± 9.9  | 0.163   |
| T2DM duration (yrs.)       | 14.3 ± 7.9          | 14.8 ± 8.3 | 14.7 ± 7.9 | 0.582   | 17.2 ± 10.3         | 16.7 ± 10.1 | 16.8 ± 10.2 | 0.866   |
| BMI (kg/m <sup>2</sup> )   | 27.6 ± 5.4          | 27.8 ± 5.4 | 28.0 ± 5.2 | 0.583   | 28.1 ± 5.6          | 27.7 ± 5.8  | 28.5 ± 6.0  | 0.560   |
| Total cholesterol (mmol/l) | 4.7 ± 1.5           | 5.0 ± 1.6  | 5.0 ± 1.6  | 0.159   | 4.7 ± 1.3           | 4.5 ± 1.3   | 4.3 ± 1.2   | 0.179   |
| HDL cholesterol (mmol/l)   | 1.2 ± 0.4           | 1.2 ± 0.3  | 1.3 ± 0.3  | 0.041   | 1.3 ± 0.8           | 1.3 ± 0.3   | 1.3 ± 0.4   | 1.000   |
| Triglycerides (mmol/l)     | 1.8 ± 1.3           | 1.7 ± 1.2  | 1.9 ± 1.2  | 0.561   | 1.6 ± 1.5           | 1.7 ± 0.9   | 1.8 ± 1.4   | 0.559   |
| HbA1c (%)                  | 8.3 ± 2.5           | 8.4 ± 2.6  | 7.9 ± 1.6  | 0.168   | 8.1 ± 1.5           | 8.5 ± 1.7   | 7.7 ± 1.6   | 0.087   |

**Note:** Comparisons were performed by ANOVA.

**Abbreviations:** T2DM, type 2 diabetes; CVD, cardiovascular disease; BMI, body mass index; HbA1c, glycated hemoglobin A1c.

A statistically significant difference was found in CVD+ patients between genotypes and HDL cholesterol levels ( $p = 0.041$ ). There were no other differences related to genotypes in CVD+ or CVD– subjects.

## Multivariate Logistic Regression Analysis

Multiple logistic regression analysis was applied to confirm the possible independent risk factors for CVD in T2DM patients (Table 5). The results showed that *KCNJ11* E23K polymorphism was a significant risk predictor for CVD development ( $p < 0.0001$ ). In addition, other variables, T2DM duration age at T2DM onset and HbA1c were significantly correlated with cardiovascular disease phenotype ( $p = 0.022$ ,  $p = 0.034$  and  $p = 0.005$ , respectively).

**Table 5** Multivariate Logistic Regression Analysis

| Variable          | Adjusted OR | 95% CI    | p value  |
|-------------------|-------------|-----------|----------|
| Age at study      | 1.17        | 0.61–1.24 | 0.072    |
| Gender            | 1.21        | 0.87–1.65 | 0.086    |
| T2DM duration     | 1.32        | 0.95–1.91 | 0.022    |
| Age at T2DM onset | 0.97        | 0.86–1.67 | 0.034    |
| BMI               | 1.19        | 0.68–1.32 | 0.116    |
| HbA1c             | 1.43        | 1.27–1.75 | 0.005    |
| T allele*         | 2.63        | 1.16–3.24 | < 0.0001 |

**Notes:** \**KCNJ11* E23K minor allele. An unconditional model of multiple logistic regression analysis was carried out for interaction between CVD as a response variable and other variables.

**Abbreviation:** OR, odds ratio.

## Discussion

In this study, we evaluated the potential association between the E23K polymorphism (rs5219) in the *KCNJ11* gene and the susceptibility to type 2 diabetes and diabetes-related cardiovascular disease in T2DM patients. The *KCNJ11* is considered a promising candidate sensibility gene for T2DM due to the Kir6.2 protein crucial for pancreatic beta-cell function.<sup>12</sup> The previous reports supported the association of the E23K polymorphism with T2DM<sup>20–24</sup> and its microvascular complications.<sup>25,26</sup> The prevalence of the T allele in our study was 37%, comparable to other European studies.<sup>19,20,27,28</sup> The minor T allele carriership was significantly associated with type 2 diabetes. The number of the T allele carriers (CT + TT genotypes) was much higher in the T2DM patient group than in the control group ( $p < 0.005$ ). The higher risk of developing T2DM was associated with both T allele and TT genotype (OR 1.26,  $p = 0.008$  and OR 1.55,  $p = 0.019$ , respectively). In a meta-analysis study, the *KCNJ11* E23K polymorphism increased the risk of T2DM by 1.25-fold.<sup>14</sup> In the comparison of the polymorphism distribution in T2DM and control groups in different genetic models we observed statistically significant differences under codominant and dominant models ( $p = 0.019$  and  $p = 0.005$ , respectively). This result is similar to the study of Aka et al of 250 T2DM subjects, in which the association of the E23K polymorphism with diabetes was observed in all models, including dominant and co-dominant models.<sup>22</sup> In contrast to these results, some authors reported the association of E23K polymorphism with T2DM only in the recessive model.<sup>21,24</sup> The *KCNJ11* E23K polymorphism might be associated with T2DM due to suppressed insulin secretion caused by decreased ATP sensitivity of the KATP channel.<sup>29</sup>

The presence of cardiovascular disease in type 2 diabetes patients is a serious clinical problem.<sup>5</sup> The ATP-sensitive potassium channels are implicated in physiological and pathological processes in a variety of cardiovascular diseases.<sup>16,30,31</sup> It was reported that *KCNJ11* contributes to CVD in individuals with diabetes.<sup>22,32,33</sup>

For our analysis of an association between the *KCNJ11* E23K variant and cardiovascular disease, the distribution of the polymorphism was compared in the CVD+ and CVD– subgroups of T2DM patients. In this analysis, the T allele and homozygous TT genotype were strongly associated with the occurrence of cardiovascular disease in all genetic models. The increased frequency of the T allele carriers in T2DM CVD+ subgroup suggests that E23K polymorphism might be a risk factor for CVD in type 2 diabetes patients. Similarly, the evidence that the T allele of E23K polymorphism is associated with coronary heart disease was presented in the study of Raza et al. The frequency of the TT genotype was much higher in CAD patients than in those without CAD, resulting in 25-fold higher risk of CAD.<sup>31</sup> The results of Chinese study of CHD patients also confirmed the association of the E23K variant with increased risk of CHD.<sup>34</sup> In the study of Aka et al, the authors investigated the *KCNJ11* E23K gene polymorphism for the risk of cardiovascular complications in T2DM patients. They found a significant association between E23K and cardiovascular disease under the allele and recessive models.<sup>22</sup> There are also reports suggesting that the E23K polymorphism can identify subjects with low risk of CAD. In a Spanish study of 318 patients with T2DM and diabetic nephropathy, the E23K SNP was associated with a lower risk of atherosclerotic plaques.<sup>35</sup> The results of an Italian study suggested an important role of rs5219 SNP in the *KCNJ11* gene in the susceptibility to ischemic heart disease (IHD) and coronary microvascular

dysfunction. In this study, a trend was observed for the TT (K23K) genotype of rs5219 to be associated with protection against coronary microvascular dysfunction.<sup>36</sup> This protective effect might be due to a modest increase in  $K_{ATP}$  channel activity by the E23 variant. In any case, the *KCNJ11* E23K polymorphism modifies the  $K_{ATP}$  channel current and/or activity that correlates with cardiovascular disease. The observed phenotypic effects of the K23 allele might be modified by several factors such as hypertension or stress.<sup>37</sup>

Our study has some limitations. Although the strict selection criteria for the patient and control groups were applied, there could be a potential selection bias due to the design of the study as retrospective. Patients were enrolled regardless of the time of CVD diagnosis. It is possible that some T2DM patients classified as CVD– could have undetected clinically silent atherosclerosis, which would affect the results. Furthermore, our preliminary study was restricted to one SNP in the *KCNJ11* gene, so some other polymorphisms at this locus could contribute to the development of CVD.

## Conclusion

This is the first study in the Polish population on the association of the *KCNJ11* gene polymorphism with T2DM and cardiovascular risk in T2DM patients. Our results confirmed the role of the E23K polymorphism in the pathogenesis of type 2 diabetes. Moreover, they demonstrated a strong association of the E23K polymorphism with increased risk of cardiovascular disease in T2DM patients. If this effect is replicated in other studies, the *KCNJ11* E23K polymorphism could be considered a potential clinically relevant marker for predicting risk of CVD in T2DM patients.

## Data Sharing Statement

The datasets used and analyzed during this study are available from the corresponding author on reasonable request.

## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas, took part in drafting, revising or critically reviewing the article, gave final approval of the version to be published, have agreed on the journal to which the article has been submitted, and agree to be accountable for all aspects of the work.

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## Disclosure

The authors declare no conflict of interest.

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