Predominance of the A allele but no association of the \textit{KCNJ11} rs5219 E23K polymorphism with Type 2 Diabetes in a Nigerian population

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\textbf{ABSTRACT.} Though the rs5219 E23K variant of the \textit{KCNJ11} gene is commonly known to be associated with Type 2 diabetes (T2D) in Caucasian and Asian populations, little or none of such findings have been revealed in Nigeria. Hence, this study was aimed to assess the relationship between E23K polymorphic variant of the \textit{KCNJ11} gene and T2D in a Nigerian population. A case-control study involving 73 T2D patients and 75 non-diabetic (ND) patients aged above 30 years was conducted. Demographic, clinical, and anthropometric data was collected and the fasting blood glucose (FBG), total cholesterol (TC), triglyceride (TG), LDL-c and HDL-c were assayed. The \textit{KCNJ11} E23K polymorphism was genotyped by RFLP–PCR using BanII restriction enzyme. There was predominance of the mutant A allele as well as the homozygote AA genotype (92.5%) in both T2D and ND patients than the wild G allele and homozygote GG genotype (7.5%). The
heterozygote AG genotype was completely absent in the T2D and ND patients. The AA genotype showed no significant risk of T2D when compared to the GG genotype (OR: 1.183, 95% CI: 0.345-4.059, p=0.790) in. Genotype frequencies did not violate the Hardy-Weinberg equilibrium in the study population ($\chi^2$=0.071; p=0.790). HDL-c was significantly higher (p=0.002) in patients with the GG genotype compared to the patients with the AA genotype. In conclusion, the $KCNJ11$ E23K polymorphism was not associated with T2D though there was predominance of the mutant A allele in the study population.

**Key words**: Type 2 diabetes; $KCNJ11$ gene; E23K variant; HDL-c; genetic association; Nigeria

**INTRODUCTION**

Though infectious diseases and malnutrition continue to be the most predominant health related causes of morbidity and mortality in low-income countries (Mathers et al., 2009), chronic non-communicable diseases, notably type 2 diabetes (T2D) is fast becoming a major concern in these regions with its increasingly rising prevalence (WHO, 2016). The prevalence of diabetes in sub-Saharan Africa (SSA) alone is projected to double, with a rise from 15 to 28 million by the next decade (IDF, 2011).

Though the prevalence of T2D is greatest in the Western regions particularly in Caucasians, the differences in the prevalence of the disease across the globe could be accounted for by demographic, ethnic, socio-economic, behavioral, feeding habit etc. The contribution of demographic and ethnicity on the prevalence may suggest certain genetic influence on the disease. With such postulation, Genome wide Association studies (GWAS) have screened several genes with over 60 polymorphic variants identified to have increased susceptibility and associated with the disease (Voight et al., 2010; Dupuis et al., 2010).

One of such genes is the potassium inward rectifying channel subfamily J ($KCNJ11$). The $KCNJ11$ gene is a member of the potassium channel gene family located at 11p15 which encodes the islet ATP-sensitive potassium channel Kir6.2 (Haghvirdizadeh et al., 2015). The Kir6.2 protein, together with the high-affinity sulfonylurea receptor 1 (SUR1), forms the KATP channel which mediates insulin secretion. Mutations in the $KCNJ11$ gene can promote diabetes by altering the functioning of the KATP channel (Ashcroft, 2006). Several mutations of the $KCNJ11$ gene have been identified with about six of them receiving more attention for their association with diabetes (Gloyn et al., 2001).

Among these genetic variants, a common glutamate (E) → lysine (K) change at position 23 (E23K) has consistently been shown to be associated with T2D, with an overall allelic odd ratio (OR) close to 1.15 (Gloyn et al., 2001; Gloyn et al., 2003; Nielsen et al., 2003) when diabetic individuals were compared with non-diabetic control subjects. More so, other studies have shown normoglycaemic subjects with the lysine genotype to consistently demonstrate a defect in secretion of insulin. Also, this has been confirmed *In vitro*, where the lysine risk allele seems to affect potassium channel properties (Florez et al., 2004; van Dam et al., 2005).

The E23K variant has been reported to be associated with T2D in various ethnic populations, including European descent (Inoue et al., 1997), Asians (Koo et al., 2007; Zhou et al., 2009), Arab populations (Alsmaida et al., 2008) etc. In Africa, a few studies have been conducted on the E23K variants with inconsistent findings.

The polymorphic lysine variant was shown to be associated with T2D in Tunisia (Lasram et al., 2014) while the variant was almost completely present in Ghana with over 99.9% prevalence but showed no association with T2D (Danquah et al., 2013). None of such findings have been revealed in Nigeria thus, this study assessed the $KCNJ11$ E23K polymorphic variant and its association with T2D in a Nigerian population.

**MATERIALS AND METHODS**

**Study participants and ethical approval**

This is a continuation of an ongoing case–control study involving 73 T2D patients and 75 non-diabetic (ND) patients of Nigerian nationality at Enugu State University of Science and Technology Teaching Hospital (ESUTH) in Enugu Nigeria. Only outpatients with or without T2D above 30 years, without any critical or emergency health conditions or complications and not admitted at the hospital were recruited for the study.
Breastfeeding and/or pregnant women as well as HIV positive patients were excluded from the study. Patients considered as T2D patients had at least one-year history of the disease and were diagnosed according the IDF criteria (WHO-IDF, 2014).

The study was conducted in accordance with the Helsinki Declaration. Before commencement of study, ethical clearance was obtained from the ethical committee of ESUTH Enugu, Nigeria with approval no: ESUTHPC-MAC/RA/034/174 and written informed consent was obtained from all willing participants before enrolment.

Data collection

Patients’ data including age and sex was obtained and the height, weight, and waist circumference (WC) of the patients were measured. The Body Mass index (BMI) was calculated from the height (m) and weight (kg) and expressed as kg/m². The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using an automatic sphygmomanometer.

Biochemical assays

Fasting blood glucose (FBG) was measured from whole blood after an overnight fasting using an accuchek glucometer according to the glucose oxidase enzymatic method by Trinder (1969). Serum total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) were assayed using kits by Randox Laboratories Ltd, United Kingdom.

The TC was determined according to the enzymatic method of Allain and collaborators (1974), TG was determined by the enzymatic method of Esders and Michira (1997) and HDL-C by the precipitation method of Grove (1979). LDL-C was determined using the formula of Freidwald (1972): LDL-C=TC- (TG/5) - HDL-C

Molecular genotyping of KCNJ11 E23K variant

DNA was extracted using Thermo Scientific GeneJET Genomic DNA Purification kit (K0721) by Thermo Fisher Scientific. Inc. The E23K polymorphism of KCNJ11 gene was genotyped by the method of Jiang and collaborators (2014). PCR was performed with forward primer 5′-GACTCTGCAAGTGAGGCCCTA-3′ and reverse primer 5′-ACGTTGCCAGTTGCTTTCTT-3′. Each PCR had a final volume of 28 μL containing 8 µl -load 2x master mix with standard buffer from New England Biolab (NEB), USA.

The amplification started with a denaturing step at 95°C for 3 min followed by 35 cycles of 95°C for 30 s, annealing at 60°C for 30 s, and elongation at 72°C for 30 s with a final elongation step at 72°C for 9 min. The PCR product after electrophoresis on 2% agarose gel was 209 bp and was digested with 0.5 ul of BanII restriction enzyme (5U per amplicon) by New England Biolabs, Beverly, MA using 1x NEB Smartcut buffer as recommended by the manufacturer (New England Biolabs, Beverly, MA) and 8 ul of nuclease free water to a total reaction volume of 20 ul and separated on 3% agarose gels.

The substitution of G with A eliminated the BanII site. The expected product sizes were 150 bp and 59 bp for the normal homozygote GG genotype; 209 bp only for the mutant homozygote AA genotype and 209, 150 and 59 bp for the heterozygote GA genotype.

Statistical analysis

Data was analyzed using Statistical Package for Social Science (SPSS) version 16. Results were expressed as frequencies and mean ± Standard error of the mean (S.E.M) and presented in tables. Parametric independent sample t-test was used to compare mean differences of the lipid profile indices, demographic and clinical characteristics for the various KCNJ11 E23K genotypes of study participants.

Pearson chi-square (χ²) test was used to test for the Hardy–Weinberg equilibrium by comparing genotype and allele frequencies in the diabetic and non-diabetic subjects. Binary logistic regression was used to determine the odd ratio (OR) by comparing allele and genotype frequencies between diabetic and non-diabetic patients. A confidence interval (CI) of 95% was taken and a p-value less than 0.05 was considered statistically significant.

RESULTS

A total of 148 participants; 73 T2D patients and 75 ND patients were recruited for the study of which 54 were male and 94 were female. The proportion of male and female was not significantly different (p=0.229) between the T2D and ND patients. The demographic and clinical characteristics of participants are summarized in Table 1.
The *KCNJ11* E23K (G/A) gene fragment was successfully amplified for all study participants with a molecular size of 209 bp (Figure 1).

![Figure 1](image1.png)

The *KCNJ11* E23K (G/A) gene fragment was successfully amplified for all study participants with a molecular size of 209 bp (Figure 1).

After restriction enzyme digestion, the product sizes were 150 bp and 59 bp for the normal homozygous GG genotype and 209 bp only for the mutant homozygous AA genotype (Figure 2).

![Figure 2](image2.png)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Diabetic</th>
<th>Non-diabetic</th>
<th>Minimum</th>
<th>Maximum</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>56.87 ± 1.19</td>
<td>49.03 ± 1.90</td>
<td>30</td>
<td>92</td>
<td>0.001</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.59 ± 0.01</td>
<td>1.61 ± 0.01</td>
<td>1.37</td>
<td>1.90</td>
<td>0.218</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.16 ± 3.38</td>
<td>71.52 ± 1.95</td>
<td>35.00</td>
<td>190.00</td>
<td>0.052</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>31.42 ± 1.38</td>
<td>27.81 ± 0.76</td>
<td>18.20</td>
<td>85.13</td>
<td>0.023</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>100.25 ± 1.68</td>
<td>89.37 ± 2.25</td>
<td>36.00</td>
<td>149.00</td>
<td>0.000</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>132.95 ± 2.62</td>
<td>132.86 ± 3.23</td>
<td>100</td>
<td>213</td>
<td>0.982</td>
</tr>
<tr>
<td>DSP (mmHg)</td>
<td>79.12 ± 1.38</td>
<td>81.76 ± 2.24</td>
<td>58</td>
<td>151</td>
<td>0.297</td>
</tr>
<tr>
<td>FBG (mg/dl)</td>
<td>166.38 ± 11.23</td>
<td>65.75 ± 3.79</td>
<td>11.00</td>
<td>520.00</td>
<td>0.000</td>
</tr>
</tbody>
</table>

**Note:** Results are presented as Mean ± SEM; SBP: Systolic Blood Pressure; DSP: Diastolic Blood Pressure; FBG: Fasting Blood Glucose; BMI: Body Mass Index; WC: Waist Circumference
The heterozygous GA genotype was absent and the 59 bp was not visualized. There was predominance of the A allele as well as the homozygous AA genotype (92.5%) in both T2D and ND patients than the G allele and homozygote GG genotype (7.5%). The heterozygous GA genotype was completely absent in the T2D and ND patients. Because of the absence of the heterozygote AG genotype, the dominant and recessive models could not be tested for risk of T2D. For the codominant model (GG vs AA), there was no significant risk (OR: 1.183, 95% CI: 0.435-4.059) of the AA genotype with T2D compared to the GG genotype as there was no significant difference (p = 0.790) of the polymorphic variant (AA genotype) between T2D patients (45.9%) and ND patients (46.6%). Genotype frequencies did not violate the Hardy-Weinberg equilibrium in the study population ($\chi^2 = 0.071; p = 0.790$). Results are summarized in Table 2.

<table>
<thead>
<tr>
<th>KCNJ11 G/A variant</th>
<th>T2D (%)</th>
<th>ND (%)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
<th>$\chi^2$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Allele</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>10 (3.4)</td>
<td>12 (4.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>136 (45.9)</td>
<td>138 (46.6)</td>
<td>1.183 (0.494-2.828)</td>
<td>0.706</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>146 (49.3)</td>
<td>150 (50.7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Genotype</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>5 (3.4)</td>
<td>6 (4.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>68 (45.9)</td>
<td>69 (46.6)</td>
<td>1.183 (0.345-4.059)</td>
<td>0.790</td>
<td>*1.421 (0.394-5.119)</td>
<td>0.071</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>73 (49.3)</td>
<td>75 (50.7)</td>
<td></td>
<td></td>
<td>*0.591</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** OR: Odd Ratio, * Indicates age adjusted OR, CI: Confidence Interval, $\chi^2$: Chi-square

Comparison of study parameters of participants with the various genotypes showed the ages of patients with the GG genotype to be significantly higher (p = 0.004) than those of the AA genotype. Also, the HDL was significantly higher (p = 0.002) in patients with the GG genotype compared to the patients with the AA genotype. However, the anthropometric measures (WC and BMI), FBG and most of the lipids parameters (TC, TG and LDL-c) did not show any significant differences (p < 0.05) between the GG and AA genotypes (Table 3).

| **Table 3:** Relationship between KCNJ11 (G/A) polymorphism and some study |
|-----------------------------|-----------------------------|-----------------------------|
| **GG (E)**                  | **AA (K)**                  | **p-value** |
| Age (yr)                    | Age (yr)                    | 64.45 ± 5.37                | 51.92 ± 1.16 | 0.004 |
| WC (cm)                     | WC (cm)                     | 94.27 ± 2.73                | 94.94 ± 1.58 | 0.902 |
| BMI (Kg/m²)                 | BMI (Kg/m²)                 | 32.57 ± 5.99                | 29.36 ± 0.73 | 0.299 |
| FBG (mg/dl)                 | FBG (mg/dl)                 | 112.67 ± 20.88              | 115.92 ± 7.61| 0.914 |
| TC (mg/dl)                  | TC (mg/dl)                  | 194.02 ± 14.52              | 202.22 ± 16.78| 0.587 |
| TG (mg/dl)                  | TG (mg/dl)                  | 103.37 ± 22.29              | 147.15 ± 16.99| 0.467 |
| HDL (mg/dl)                 | HDL (mg/dl)                 | 81.16 ± 20.30               | 45.80 ± 2.80 | 0.002 |

**Note:** Results are expressed as mean ± S.E.M; S.E.M: Standard error of the mean
DISCUSSION

T2D is a complex metabolic disease caused by multiple environmental and genetic factors whereby the level of heritability from twin and family studies is estimated at 22% - 73% (Kaprio et al., 1992; Poulsen et al., 1999). After the identification of over 40 T2D-associated genetic loci primarily from studies involving mainly individuals of European ancestry, other candidate-gene association studies have discovered association between T2D and a few missense variants including KCNJ11 which is an antidiabetic drug target site (Gloyn et al., 2001). It was proposed that this missense mutation may alter the charge of the ATP-binding region and decrease channel sensitivity to ATP. Among the variants of KCNJ11, the rs5219 E23K polymorphism due to a change from G to A has been shown to be associated with T2D in diverse ethnic populations in several studies (Abdelhamid et al., 2014). Several meta-analysis and association studies have showed a strong association between the E23K polymorphism and susceptibility to T2D mostly in Caucasians and in some Asian populations (Gonen et al., 2012; He et al., 2008; Chistiakov et al., 2003). However, some other association studies did not show any relationship between this polymorphism and susceptibility to T2D (Keshavarz et al., 2014; Gamboa-Mel’endez et al., 2012; Souza et al., 2016). More so, some populations notably from East Asia showed the mutant A allele to be more frequent in the non-diabetic controls than the diabetic patients (Qiu et al., 2014). In Africa, just a few studies have assessed this polymorphism notably in Tunisia and Ghana. In the Tunisia study, this polymorphism was shown to be T2D-associated with a frequency of the A allele above 35% in both the diabetic patients and control subjects (Lasram et al., 2014). On the other hand, this polymorphic variant did not show any association with T2D in a Ghanaian population as there was over 99.9% predominance of the mutant A allele as well as the AA (K) genotype (Danquah et al., 2013). In this present study, there was a 92.5% predominance of the mutant A allele as well as the AA genotype in both the T2D and the ND patients and thus, the E23K polymorphism was not associated with T2D as the difference between this polymorphic variant in T2D patients (45.9%) and the ND patients (46.9%) was not significant (OR: 1.183, 95% CI: 0.345-4.059, p=0.790). The genotype frequencies did not violate the Hardy-Weinberg equilibrium in this population ($\chi^2$=0.071; p=0.790). Since the mutant AA genotype frequency was very high in a Nigerian population in this study and in a Ghana population in a previous study; both countries of the sub-Saharan region, it is suggested that the A allele may seem to be the wild allele in this region of Africa.

Owing to the fact that insulin plays multiple roles in the body including carbohydrate and lipid metabolism, the relationship between the KCNJ11 E23K polymorphism with blood sugar, obesity, cholesterol, triglyceride, and lipoproteins was assessed. Findings from this study did not show any significant differences in the anthropometric or obesity parameters (BMI, WC), FBG and some serum lipids (TC, TG and LDL-c) between the GG and AA genotypes of patients. This confirms the findings of a previous study which also showed no association of this polymorphism with anthropometric parameter, obesity, and body fat (Lasram et al., 2014). However, HDL level was significantly lower ($p < 0.002$) in patients with the AA genotype compared to the GG genotype. This may suggest that the GG genotype can improve the HDL level in patients with T2D which has an essential role to remove free cholesterol in blood thus having a protective effect against T2D as high HDL-c level is known to reduce susceptibility to T2D.

CONCLUSION

In conclusion, this study showed no associated risk of the KCNJ11 E23K polymorphism with T2D though there was predominance of the mutant A allele and AA genotype in both T2D patients and the non-diabetic control patients. The GG genotype was associated with high level of HDL-c suggesting a possible protective effect of reducing susceptibility to T2D.

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