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

Godwill Azeh Engwa[1*], Friday Nweke Nwalo[2], Chosen E. Obi[3], Christie Onyia[3], Opeolu Oyejide Ojo4 Wilfred Fon Mbacham[5], Benjamin Ewa Ubi[6]

[1]Biochemistry programme, Department of Chemical Sciences, Godfrey Okoye University, P.M.B 01014, Thinkers Corner, Enugu Nigeria

[2]Department of Biotechnology, Federal University Ndufu-Alike Ikwo (FUNAI), P.M.B. 1010 Abakaliki, Nigeria

[3]Department of Biotechnology, Godfrey Okoye University, P.M.B 01014, Thinkers Corner, Enugu Nigeria

[4]Department of Biology, Chemistry and Forensic Science, School of Sciences, University of Wolverhampton, Wolverhampton, WV1 1LY, UK

[5]Laboratory for Public Health Research Biotechnologies, The Biotechnology Centre, University of Yaounde I, BP 8094, Yaounde, Cameroon

[6]Biotechnology Programme, Department of Biological Sciences, Ebonyi State University, P.M.B 53 Abakaliki, Nigeria

Corresponding author: Godwill Azeh Engwa

E-mail: engwagodwill@gmail.com

Genet. Mol. Res. 17 (1): gmr16039889

Received January 14, 2018

Accepted February 26, 2018

Published March 01, 2018

DOI http://dx.doi.org/10.4238/gmr16039889

ABSTRACT. Though the rs5219 E23K variant of the *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 *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 *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 (Alsmadi 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: ESUTHP/C-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 accucheck 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′-GACTCTGCAGTGAGGCCCTA-3′ and reverse primer 5′-ACGTTGCAGTTG CCTTTCTT-3′. Each PCR had a final volume of 28 µL containing 8 µl (□10 ng) of genomic DNA, 4 µl of 50 µM of each primer, and 12 µl of tag quick-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 ($\chi 2$) 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.

Table 1: Demographic and clinical characteristics of participants

Characteristics	Diabetic	Non-diabetic	Minimum	Maximum	*p*-value
Age (years)	56.87 ± 1.19	49.03 ± 1.90	30	92	0.001
Height (m)	1.59 ± 0.01	1.61 ± 0.01	1.37	1.90	0.218
Weight (kg)	79.16 ± 3.38	71.52 ± 1.95	35.00	190.00	0.052
BMI (Kg/m^2)	31.42 ± 1.38	27.81 ± 0.76	18.20	85.13	0.023
WC (cm)	100.25 ± 1.68	89.37 ± 2.25	36.00	149.00	0.000
SBP (mmHg)	132.95 ± 2.62	132.86 ± 3.23	100	213	0.982
DSP (mmHg)	79.12 ± 1.38	81.76 ± 2.24	58	151	0.297
FBG (mg/dl)	166.38 ± 11.23	65.75 ± 3.79	11.00	520.00	0.000

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

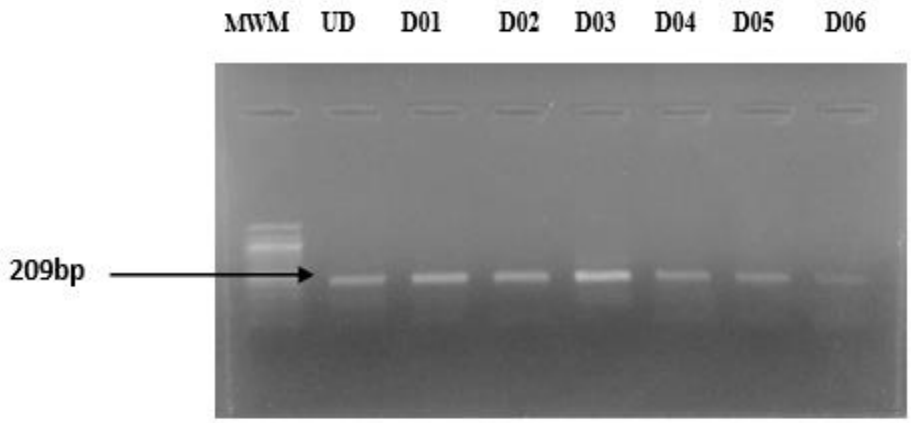

Figure 1. RFLP-PCR of *KCNJ11* E23K Polymorphism. **MWM:** molecular weight marker. **UD** indicates undigested amplified sample. D01 to D06 are digested samples and were all the mutant homozygote AA genotype.

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).

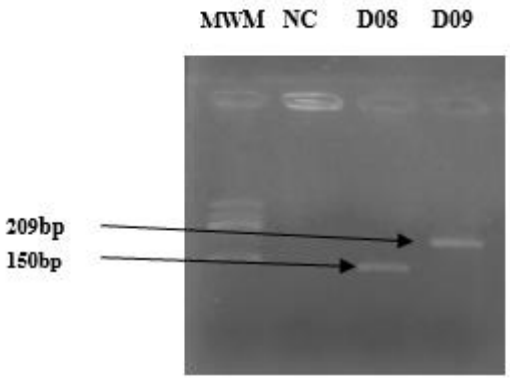

Figure 2. Digested samples of the *KCNJ11* gene. MWM**:** Molecular Weight Marker. NC indicates the negative control. D08 sample indicates the homozygote GG genotype and D09 is the mutant homozygote AA genotype.

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: 0.345-4.059) 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 ($\chi2$=0.071; p=0.790). Results are summarized in Table 2.

Table 2: Association between the *KCNJ11* E23K (G/A) polymorphism and T2D

***KCNJ11* G/A variant**	**T2D (%)**	**ND (%)**	**OD (95% CI)**	***p-value***	**$\chi2$**	***p-value***
Allele						
G	10 (3.4)	12 (4.1)	-------			
A	136 (45.9)	138 (46.6)	1.183 (0.494-2.828)	0.706		
Total	146 (49.3)	150 (50.7)				
Genotype						
GG	5 (3.4)	6 (4.1)	------			
AG	0 (0.0)	0 (0.0)	------			
AA	68 (45.9)	69 (46.6)	1.183 (0.345-4.059)	0.790	0.071	0.790
			∗1.421 (0.394-5.119	∗0.591		
Total	73 (49.3)	75 (50.7)				

Note: OR: Odd Ratio, ∗ Indicates age adjusted OR, CI: Confidence Interval, $\chi2$: 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)	64.45 ± 5.37	51.92 ± 1.16	0.004
WC (cm)	94.27 ± 2.73	94.94 ± 1.58	0.902
BMI (Kg/m^2)	32.57 ± 5.99	29.36 ± 0.733	0.299
FBG (mg/dl)	112.67 ± 20.88	115.92 ± 7.61	0.914
TC (mg/dl)	194.02 ± 14.52	226.22 ± 16.78	0.587
TG (mg/dl)	169.42 ± 63.54	196.13 ± 8.74	0.451
LDL-c (mg/dl)	103.37 ± 22.29	147.15 ± 16.99	0.467
HDL-c (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.

ACKNOWLEDGMENT

This study was partly supported by the competitive research grant of Godfrey Okoye University (GOU). The authors are thankful to the students of medical laboratory sciences and medical students of ESUTH Enugu for their assistance in data collection and blood collection. Also, the authors are grateful to Mrs. Ngozika Mariagoretti Ukwueze of the Biotechnology laboratory, GOU Enugu for her assistance throughout the study

REFERENCES

Abdelhamid I, Lasram K, Meiloud G et al. (2014). E23K variant in *KCNJ11* gene is associated with susceptibility to type 2 diabetes in the Mauritanian population. *Primary Care Diabetes* 8 (2): 171-175. https://doi.org/10.1016/j.pcd.2013.10.006

Allain CC, Poon LS, Chan CS, Richmond W. (1974). Total cholesterol assay. *Clin. Chem*. 20: 470-471.

Alsmadi O, Al-Rubeaan K, Wakil SM et al. (2008). Genetic study of Saudi diabetes (GSSD): significant association of the *KCNJ11* E23K polymorphism with type 2 diabetes. *Diabetes/Met. Res. Rev.* 24 (2): 137-140. https://doi.org/10.1002/dmrr.777

Ashcroft FM (2006). KATP channels and insulin secretion: a key role in health and disease. *Biochem. Soc. Transac.* 34 (2): 243-246. https://doi.org/10.1042/bst20060243

Chistiakov DA, Potapov VA, Khodirev DC, Shamkhalova MS et al. (2009). Genetic variations in the pancreatic ATP-sensitive potassium channel, *β*-cell dysfunction, and susceptibility to type 2 diabetes. *Acta Diabetologica* 46 (1): 43-49. https://doi.org/10.1007/s00592-008-0056-5

Dam RM, Hoebee B, Seidell JC, Schaap MM et al. (2005). Common variants in the ATP-sensitive K+ channel genes *KCNJ11* (Kir6.2) and *ABCC8* (SUR1) in relation to glucose intolerance: population-based studies and meta-analyses. *Diabet. Med*. 22:590-598. https://doi.org/10.1111/j.1464-5491.2005.01465.x

Danquah I, Othmer T, Frank LK, Bedu-Addo G et al. (2013). The TCF7L2 rs7903146 (T) allele is associated with type 2 diabetes in urban Ghana: a hospital-based case–control study. *BMC Med. Gen.* 14:96. https://doi.org/10.1186/1471-2350-14-96

Dupuis J, Langenberg C, Prokopenko I, Saxena R et al. (2010). New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. *Nat. Genet*. 42(2):105–116.

Esders TN, Michira CA (1997). Triglyceride estimation. *J. Biol. Chem.* 254: 2710-2712.

Florez JC, Burtt N, de Bakker PIW, Almgren P et al. (2004). Haplotype structure and genotype-phenotype correlations of the sulfonylurea receptor and the islet ATP-sensitive potassium channel gene region. *Diabetes* 53:1360-1368. https://doi.org/10.2337/diabetes.53.5.1360

Friedwald WT, Levy RI, Fredrickson DS. (1972). Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma without use of Preparative Ultracenrifugation. *Clin. Chem.* 18: 499-502.

Gamboa-Mel´endez MA, Huerta-Chagoya A, Moreno-Mac´ıas H et al. (2012). Contribution of common genetic variation to the risk of type 2 diabetes in the Mexican Mestizo population. *Diabetes* 61 (12): 3314-3321. https://doi.org/10.2337/db11-0550

Gloyn AL, Hashim Y, Ashcroft SJ, Ashfield R et al. (2001). Association studies of variants in promoter and coding regions of β-cell ATP-sensitive K-channel genes SUR1 and Kir6.2 with type 2 diabetes mellitus (UKPDS 53). *Diabet. Med*. 18:206-212. https://doi.org/10.1046/j.1464-5491.2001.00449.x

Gloyn AL, Weedon MN, Owen KR, Turner MJ et al. (2003). Large-scale association studies of variants in genes encoding the pancreatic beta-cell KATP channel subunits Kir6.2 (*KCNJ11*) and SUR1 (*ABCC8*) confirm that the *KCNJ11* E23K variant is associated with type 2 diabetes. *Diabetes* 52: 568-572. https://doi.org/10.2337/diabetes.52.2.568

Gonen MS, Arikoglu H, Kaya D et al. (2012). Effects of single nucleotide polymorphisms in KATP channel genes on type 2 diabetes in a Turkish population. *Arch. Med. Res.* 43 (4): 317–323. https://doi.org/10.1016/j.arcmed.2012.06.001

Grove TH. (1979). Grove's method of high density lipoproteins estimation. *Clin. Chem.* 25: 560-562.

Haghvirdizadeh P, Mohamed Z, Abdullah NA, Haghvirdizadeh P et al. (2015). *KCNJ11*: Genetic Polymorphisms and Risk of Diabetes Mellitus. *J. Diabetes Res.* 2015:1-9.

He YY, Zhang R, Shao XY et al. (2008). Association of *KCNJ11* and ABCC8 genetic polymorphisms with response to repaglinide in Chinese diabetic patients. *Acta Pharmacologica Sinica* 29 (8): 983-989. https://doi.org/10.1111/j.1745-7254.2008.00840.x

Inoue H, Ferrer J, Warren-Perry M, Zhang Y et al. (1997). Sequence variants in the pancreatic islet β-cell inwardly rectifying K+ channel Kir6.2 (*Bir*) gene: identification and lack of role in Caucasian patients with NIDDM. *Diabetes* 46:502-507. https://doi.org/10.2337/diabetes.46.3.502

International Diabetes Federation (2011). Diabetes Atlas. 5th edition. Brussels: International Diabetes Federation.

Jiang Y, Chuang L, Pei D, Lee Y et al. (2014). Genetic Variations in the Kir6.2 Subunit *(KCNJ11)* of Pancreatic ATP-Sensitive Potassium Channel Gene Are Associated with Insulin Response to Glucose Loading and Early Onset of Type 2 Diabetes in Childhood and Adolescence in Taiwan. *Int. J. Endocrinol.* 2014: 1-7. https://doi.org/10.1155/2014/983016

Kaprio J, Tuomilehto J, Koskenvuo M, Romanov K, et al. (1992). Concordance for type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes mellitus in a population- based cohort of twins in Finland. *Diabetologia* 35: 1060-1067. https://doi.org/10.1007/bf02221682

Keshavarz P, Habibipour R, Ghasemi M, Kazemnezhad E et al. (2014). Lack of genetic susceptibility of *KCNJ11* E23K polymorphism with risk of type 2 diabetes in an Iranian population. *Endocrine Res.* 39 (3): 120-125. https://doi.org/10.3109/07435800.2013.860607

Koo BK, Cho YM, Park BL et al. (2007). Polymorphisms of *KCNJ11* (Kir6.2 gene) are associated with type 2 diabetes and hypertension in the Korean population. *Diabetic Med.* 24 (2): 178–186. https://doi.org/10.14341/probl201561526-29

Lasram K, Halim NB, Hsouna S, Kefi R et al. (2014). Evidence for Association of the E23K Variant of *KCNJ11* Gene with Type 2 Diabetes in Tunisian Population: Population-Based Study and Meta-Analysis. *BioMed Res. Int.* 2014: 1-9. https://doi.org/10.1155/2014/265274

Mathers CD, Boerma T, Ma Fat D. (2009). Global and regional causes of death. *Br. Med. Bull.* 92:7-32. https://doi.org/10.1093/bmb/ldp028

Nielsen E-MD, Hansen L, Carstensen B, Echwald SM et al. (2003). The E23K variant of Kir6.2 associates with impaired post- OGTT serum insulin response and increased risk of type 2 diabetes. *Diabetes* 52: 573-577. https://doi.org/10.2337/diabetes.52.2.573

Poulsen P, Kyvik KO, Vaag A, Beck-Nielsen H (1999). Heritability of type II (non-insulin-dependent) diabetes mellitus and abnormal glucose tolerance—A population-based twin study. *Diabetologia* 42: 139-145. https://doi.org/10.1007/s001250051131

Qiu L, Na R, Xu R et al. (2014). Quantitative assessment of the effect of *KCNJ11* gene polymorphism on the risk of type 2 diabetes. *PLoS ONE* 9 (4): 1-8. https://doi.org/10.1371/journal.pone.0093961

Souza SW, Alcazar LP, Arakaki PA, Santos-Weiss ICR et al. (2017). Polymorphism E23K (rs5219) in the *KCNJ11* gene in Euro-Brazilian subjects with type 1 and 2 diabetes. *Gen. Mol. Res.* 16 (2):1-9. https://doi.org/10.4238/gmr16029543

Trinder P. (1969). Determination of blood glucose using 4-aminophenazone as oxygen acceptor. *J. Clin. Pathol*. 22 (246): 1-6. https://doi.org/10.1136/jcp.22.2.246-b

Voight BF, Scott LJ, Steinthorsdottir V, Morris AP et al. (2010). Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. *Nat. Genet.* 42(7): 579–589.

WHO (2016). Global report on diabetes. WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland.

WHO-IDF. (2014). Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation. http://www.idf.org/webdata/docs/WHO_IDF_definition_diagnosis_of_diabetes.pdf.

Zhou D, Zhang D, Liu Y et al. (2009). The E23K variation in the *KCNJ11* gene is associated with type 2 diabetes in Chinese and East Asian population. *J. Human Gen.* 54 (7): 433-435. https://doi.org/10.1038/jhg.2009.54