Saudi Journal of Biological Sciences 29 (2022) 2794–2799

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Independent case-control study in *KCNJ11* gene polymorphism with Type 2 diabetes Mellitus

Nada Alqadri

Department of Biology, Turabah University College, Taif University, PO Box-11099, Taif 21944, Saudi Arabia

ARTICLE INFO

Article history:
Received 28 November 2021
Revised 31 December 2021
Accepted 2 January 2022
Available online 6 January 2022

Keywords:
Type 2 Diabetes Mellitus
KCNJ11 gene
Rs5210 polymorphism
Saudi Population

ABSTRACT

Background: Type 2 Diabetes Mellitus (T2DM) is the most common form of diabetes in the aging population. This chronic metabolic disorder has discovered many candidate genes, and *KCNJ11* was one of the genes associated with insulin secretion pathways mediated by potassium channels. There have been limited studies on the rs5210 polymorphism in T2DM patients, and none of them have been conducted in Saudi Arabia.

Aim: The aim of this study is to investigate at genotyping levels of rs5210 polymorphism in the *KCNJ11* gene in older population with T2DM in the Saudi Population.

Methods: Based on the sample size design, this case-control study included 102 T2DM cases and 102 controls. Using the PCR-RFLP assay, 204 patients extracted DNA was genotyped for the rs5210 polymorphism. SPSS software was used for statistical analysis, including t-tests, HWE, genotyping, and multiple logistic regression analysis.

Results: The *t-tests* performed on T2DM cases and controls revealed a significant association in age, weight, BMI, FBG, Hb1Ac, SBP, DBP, HDLC, TC, and TG parameters ($p < 0.05$). HWE analysis found to be in consistent with rs5210 polymorphism. Allelic association was found in the rs5210 polymorphism (OR-1.64 [95 %CI: 1.08–2.49]; $p = 0.01$); however, no association ($p > 0.05$) was observed in the multivariate logistic regression assessment performed in this study.

Conclusion: These results indicate that the rs5210 polymorphism was primarily associated with allele frequencies, which could be attributable to the small sample size. Large sample size studies will be required to determine whether *KCNJ11* gene polymorphisms may be required as a risk marker for T2DM in the Saudi population.

1. Introduction

Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disorder characterized by low insulin secretion, low insulin action, and high blood glucose levels (Alharbi et al., 2016). Diabetes prevalence is expected to expand from 425 to 629 million between 2017 and 2045, according to the IDF. T2DM complications account for 8.5% of total deaths (Alharbi et al., 2021). Individual or combined genetic and environmental variables play a significant impact in the development and progression of T2DM. Advanced age, elevated body mass index (BMI), diet, lack of physical exercise, smoking, abnormal serum levels, and hypertension (HTN) were all considered to be risk factors for T2DM (Khan et al., 2015a). Obesity and Gestational Diabetes Mellitus (GDM) are risk factors for T2DM. Previous studies have confirmed that T2DM and GDM share a similar pathophysiology and genetic vulnerability, and documented studies with genetic polymorphism demonstrated that both T2DM and GDM have significantly correlated with genetic polymorphism (Khan et al., 2014).

The main function of genetic factors is to play a role in the development of T2DM, which results in reduced β-cell insulin action. Genetic factors also induce changes in Kir6.2 structure in pancreatic β-cells in *KCNJ11* gene polymorphisms, which impacts insulin secretion. Single nucleotide polymorphisms (SNPs) are more likely to be the source of these biological factors, and *KCNJ11* is an important candidate gene for the risk of T2DM due to its function in the control of glucose-induced insulin secretion. Chromosome 11p15.1 contains the *KCNJ11* gene, which plays an

E-mail address: naqadri@tu.edu.sa
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2022.01.008
1319-562X/

important role in insulin release from pancreatic β-cells that are activated by glucose. The variants in *KCNJ11* gene is correlated with lowered depolarization-evoked insulin exocytosis (Khan et al., 2019, Khan et al., 2015b). Numerous SNPs in the *KCNJ11* gene, notably rs757110, rs5215, rs5210, and rs5219, have been studied in many types of diabetes, including neonatal diabetes, type 1 diabetes mellitus (T1DM), T2DM, GDM, and post-transplant diabetes mellitus (Khan et al., 2019, Khan et al., 2015b, Ko et al., 2012, Abbasi et al., 2012). Previous studies documented all SNPs in the Saudi population (Alsmadi et al., 2008) except for rs5210 polymorphism in the *KCNJ11* gene, which is one of the reasons for designing this study with rs5210 polymorphism, and the importance of rs5210 polymorphism is currently being studied in worldwide studies. The aim of this study is to examine at the genotyping levels of rs5210 polymorphism in the *KCNJ11* gene in older population with T2DM in the Saudi population.

2. Materials and methods

2.1. Study plan

This case-control study was carried out in a local community of 204 participants, who were classified into 102 T2DM patients and 102 controls. In this study, an equal number of T2DM cases and controls were chosen for each gender. The study participants with T2DM cases were between the age range of 50–85 years of old. T2DM cases and controls were collected from diverse regions within the Kingdom. The sample size for both T2DM cases and healthy controls was estimated using survey system criteria and an online tool, with 97 subjects in the T2DM cases and controls groups participating (Alfaifi, 2021). Based on American Diabetes Association criteria, 102 T2DM cases were recruited. The inclusion criteria for T2DM cases were confirmed, since the patients had fasting blood glucose (FBG) levels of 7.0mmol/l or higher. The exclusion criteria of T2DM was ruled out if the patient's FBG levels fell into the group of impaired fasting glucose or even normal glucose levels. All diabetic patients in this study were diagnosed with T2DM before or after an average of 5 years. Healthy controls (n=102) were selected based on normal glucose levels without any history of any form of diabetes. The exclusion criteria were categorized as patients had excessive glucose levels, were diagnosed with other metabolic disorders, or were on metformin medications. Ethical grant was obtained for this study. Signed informed consent form participants were involved in this study.

2.2. Measurements of participants and blood sampling

For T2DM cases and controls, age is recorded in years, gender is documented as male or female, height is measured in centimeters (cms), and weight is recorded in kilograms (kg). BMI was determined using weight and height using the formula BMI=kg/m^2 (Alharbi et al., 2020). Normal, overweight, and obese levels were classified based on BMI values. In this study, 5ml of venous blood was collected for biochemical and molecular analysis in an anticoagulant ethylenediaminetetraacetic acid (EDTA) vacutainer and also in coagulant tubes. Serum was separated for biochemical processes, and human DNA was isolated using EDTA blood. Systolic and diastolic blood pressure (SBP and DBP) readings were used to assess HTN (Alharbi et al., 2014).

2.3. Biochemical assays

Serum was extracted from the coagulant tubes and used for biochemical assays such as FBG and lipid profiles for total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDLC), and low-density lipoprotein cholesterol (LDLC). Furthermore, glycated hemoglobin (Hb1Ac) levels were determined from blood collected in an EDTA vacutainer. Biochemical analysis was performed with an automated assay (Alharbi et al., 2017).

2.4. Genotype determination

Two hundred and four EDTA blood samples were used to extract human DNA using a kit-based procedure that followed the manufacturer's recommended protocol (Qiagen, USA). The isolated DNA was diluted in 200μl of TE buffer before being placed onto a 1% agarose gel. The samples were afterwards maintained at −20°C until the polymerase chain reaction analysis process was completed. The PCR process is initiated with a genotyping examination of the rs5210 polymorphism in the *KCNJ11* gene. The target SNP locus rs5210 polymorphism was amplified in a single system utilizing a PCR machines thermal cycler. The PCR process used 50μl of reaction, which included 20μl of PCR master mix, 1μl of diluted forward/reverse primers, 23μl of purified water, and 5μl of genomic DNA. The primers were adapted from the previous studies (Khan et al., 2015b). The PCR program for the rs5210 polymorphism was as follows: initial denaturation at 95°C for 10 minutes, 40 cycles of total reaction with the remaining processes as denaturation at 94°C for 1 min, 64°C for 1 min as annealing temperature, 72°C for 1 min as extension, and 10 min to complete the final extension at 72°C. Following the end of the genotyping process, a 152bp PCR product was obtained, and a 2% agarose gel was utilized to check the PCR product using a 100bp DNA ladder. The amplified PCR products were digested using the HPY1888III restriction enzyme. The restriction digestion analysis lasted 18 h at 37°Celsius, and the digested products were 96/54 bp for the GG genotypes, 152 bp for the AA genotypes, and 152, 96, and 54 bp for the heterozygous genotypes. The digested PCR products were run on a 2% agarose gel stained with ethidium bromide once again. Validation was confirmed with Sanger sequencing analysis (Al-Otaiby et al., 2021).

2.5. Statistical analysis

SPSS software was used for the statistical analysis (25th version, USA). All clinical and genotype data were gathered in Excel and then converted to SPSS files for the convenience of performing the student *t*-test, Hardy Weinberg Equilibrium (HWE), and genotyping analysis for the rs5210 polymorphism between diabetic cases and controls. Furthermore, in diabetic cases, multiple nominal regression analysis was performed using FBG as the reference. Continuous variables are represented by the mean and standard deviation, while categorical variables are represented by percentages. When comparing two groups, a p value of <0.05 indicates a significant correlation (Khan et al., 2019).

3. Results

In this study, 204 Saudi participants were involved, with 102 experiencing T2DM and the remaining 102 as healthy controls. The analysis of this study was sub-divided into the following groups as follows

3.1. Basic characteristics of 204 Saudi subjects

The clinical details of T2DM cases and healthy controls were shown in Table 1. Both, T2DM and healthy controls had minimum and maximum ages of 50–85 years and 40–80 years, respectively. A substantial association was discovered based on the mean ages of both groups ($p < 0.0001$). The gender was selected equally for

Table 1
Clinical and demographical characteristics between control subjects and diabetic cases.

	Controls (n = 102)	Cases (n = 102)	P Value
Age (Years)	50.1 ± 7.63	62.1 ± 9.63	<0.0001
Gender (F:M)	51:51	51:51	1.00
Height (cms)	157.6 ± 6.96	158.4 ± 7.16	0.80
Weight (kg)	70.1 ± 10.63	75.4 ± 11.15	0.006
BMI (kg/m^2)	28.2 ± 3.13	30.1 ± 3.98	0.0001
FBG (mmol/l)	5.4 ± 1.03	10.4 ± 1.95	<0.0001
Hb1Ac	5.5 ± 0.62	7.7 ± 0.88	<0.0001
SBP (mmHg)	113.7 ± 10.54	119.4 ± 11.76	0.0003
DBP (mmHg)	73.1 ± 4.56	76.2 ± 6.56	0.0001
HDL-C (mmol/L)	1.1 ± 0.34	1.2 ± 0.41	<0.0001
LDL-C (mmol/L)	2.1 ± 0.81	3.5 ± 0.82	0.06
TG (mmol/L)	1.2 ± 0.57	1.7 ± 0.88	0.0001
TC (mmol/L)	4.7 ± 0.89	5.1 ± 1.07	0.004

both the subjects and showed no association (p=1.00). T2DM cases had a mean weight of 75.4 ± 11.15, whereas controls had a weight of 70.1+10.63, indicating a positive association (p = 0.006); it also shows in obese T2DM cases and overweight controls (p = 0.0001). The association between glucose levels in the blood and FBG and Hb1Ac levels was high. SBP and DBP levels were both found to be substantially associated. Among the lipid profiles, HDL-C, TC, and TG levels were greater in T2DM patients, with a significant association (p<0.05). Height and LDL-C levels were consistently similar in T2DM cases and controls and documents the negative association (p > 0.05).

3.2. HWE analysis

T2DM patients and healthy controls were genotyped for the rs5210 polymorphism. The call rate for the rs5210 polymorphism was found to be greater than 95%, indicating the importance of result dependability. The HWE analysis was performed on both controls and cases and revealed a significant association (p < 0.05).

3.3. Genotyping determination analysis

Table 2 summarizes the HWE analysis, genotype, and allele frequencies for T2DM patients and controls. The G allele frequency was found to be high in T2DM patients with 37.8% compared to 27% in controls. The controls data documents 37% of A allele when compared to 62.2% in T2DM. The significant association was found in allele frequencies (OR-1.64; 95% CI: 1.08–2.49, p=0.01). The presence of 45.1% AA, 34.3% AG, and 20.6% GG genotypes in T2DM patients and 58.8%, 28.4%, 12.8% in AA, AG, and GG genotypes in controls revealed a negative association (AG vs AA; OR-1.57; 95%CI:0.84–2.93; p=0.15 & GG vs AA; OR-2.11; 95%CI: 0.95–4.64; p=0.06). Genetic models also showed the negative associations (AA vs AG+GG; OR-1.73; 95%CI: 0.99–3.02; p=0.04, AA+GG vs AG; OR-0.76, 95%CI: 0.42–1.37; p=0.36 and AA+AG vs GG; OR-0.56; 95%CI: 1.08–2.49; p=0.01) which was described in Table 3.

Table 2
Genotype frequencies for controls subjects and diabetic cases.

Genotype	Controls (n = 102)	Diabetic Cases (n = 102)
AA	60 (58.8%)	46 (45.1%)
AG	29 (28.4%)	35 (34.3%)
GG	13 (12.8%)	21 (20.6%)
A allele	149 (73%)	127 (62.2%)
G allele	55 (27%)	77 (37.8%)
HWE	0.27	0.38
X^2	7.8	7.4
P values	0.004	0.006

Table 3
Statistical association between controls and diabetic cases.

	P value	95 %CI	ORs
1/2 vs 1/1	0.15	0.84–2.93	1.57
2/2 vs 1/1	0.06	0.95–4.64	2.11
1/1 vs 1/2 + 2/2	0.04	0.99–3.02	1.73
1/1 + 2/2 vs 1/2	0.36	0.42–1.37	0.76
1/1 + 1/2 vs 2/2	0.13	0.26–1.19	0.56
2 vs 1	0.01	1.08–2.49	1.64

3.4. Multiple logistic regression analysis

Multiple logistic regression analysis was used in this study with anthropometric and biochemical variables considering FBG as dependent factors and Age, Weight, BMI, Hb1Ac, SBP, DBP, HDLC, LDLC, TG, and TC as independent variables. When genotyping values were eliminated, none of the factors demonstrated a positive association (Hb1Ac; OR-0.10;95%CI:0.19–0.67; p=0.28 and TC; OR-0.10; 95%CI: 0.16–0.56; p=0.28). The multiple logistic regression analysis is described in Table 4. Furthermore, Fig. 1 illustrates FBG levels in the form of histograms, while Fig. 2 represents a scatterplot with FBG as the dependent variable, indicating that FBG levels were found to be higher in T2DM patients.

4. Discussion

Diabetes has become a prevalent condition in the modern era, and according to the 8th edition of the Diabetes Atlas, around 425 million individuals aged 20–79 suffer from all forms of diabetic diseases. T2DM is a chronic metabolic disorder which is very common in the 20th century due to the uncontrolled diet, lack of physical activity and with the sedentary life style. Additionally, genetics also plays a role in every inherited families (Khan, 2021). According to the International Diabetes Federation, if preventive measures are not adopted, half of the Saudi population would be diabetes by 2030. Diabetes has reached epidemic proportions in Saudi Arabia (Robert and Al Dawish, 2020). Approximately 1.1 million children and adolescents under the age of 20 are expected to be affected by type 1 diabetes in Saudi Arabia, which ranks eighth internationally in terms of total cases and fourth in terms of incidence as it has 33.5/0.1 million cases (AlHaidar et al., 2020).

The current study results concur a positive association in the form of allelic association (OR-1.64; 95% CI: 1.08–2.49, p=0.01), with no genotype (AG vs AA; OR-1.57; 95%CI:0.84–2.93; p=0.15 & GG vs AA; OR-2.11; 95%CI: 0.95–4.64; p=0.06) or genetic models (AA vs AG+GG; OR-1.73; 95%CI: 0.99–3.02; p=0.04, AA+GG vs AG; OR-0.76, 95%CI: 0.42–1.37; p=0.36 and AA+AG vs GG; OR-0.56; 95%CI: 1.08–2.49; p=0.01) showing a positive association. Prior to this, HWE analysis showed positive association with rs5210 polymorphism. When compared to controls, anthropometric data such

Table 4
Multiple logistic regression analysis.

Characteristics	OR	95 %CI	P value
Age (Years)	0.153	0.38–0.44	0.87
Weight (CMS)	0.032	0.51–0.62	0.84
BMI (Kg/m^2)	0.048	0.13–0.18	0.77
Hb1AC	0.107	0.19–0.67	0.28
SBP (mmHg)	0.027	0.29–0.38	0.79
DBP (mmHg)	0.240	0.01–0.13	0.02
HDLC (mmol/l)	0.142	0.30–1.62	0.17
LDLC (mmol/l)	0.098	0.23–0.71	0.32
TC (mmol/l)	0.108	0.16–0.56	0.28
TG (mmol/l)	0.231	0.06–0.95	0.02

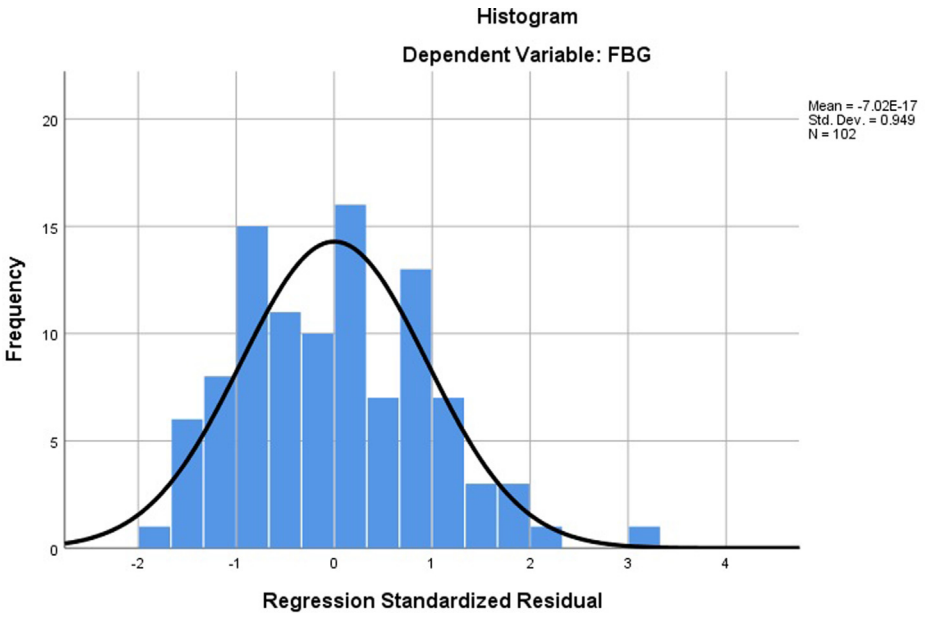

Fig. 1. Representation of Histogram for assessing FBG levels in T2DM patients.

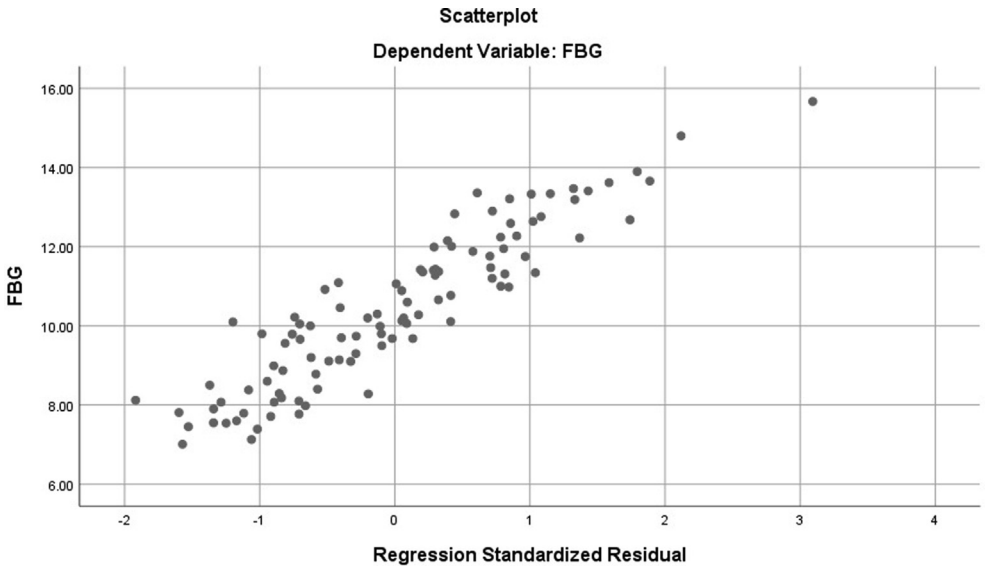

Fig. 2. Illustration of FBG levels using Scatterplot analysis in T2DM patients.

as age, weight, and BMI, as well as biochemical analysis FBG, Hb1Ac, and lipid profile parameters such as HDLC, TC, and TG, revealed elevated levels with a statistical association among T2DM cases ($p < 0.05$). None of the variables exhibited a positive correlation using multiple logistic regression analysis in T2DM cases when FBG was assessed as the dependent parameter ($p > 0.05$).

The rs5210 polymorphism was found to be significantly associated with Khan et al studies (Khan et al., 2015b) in an Indian population, which was confirmed by VKhan et al (Khan et al., 2020) and Liu et al (Liu et al., 2006) studies. In an Indian population with GDM and PTDM, this rs5210 polymorphism was found to be associated (Khan et al., 2019, Khan et al., 2015b). Sakamoto et al reported rs5210 polymorphism to be associated with T2DM in a previous Japanese study (Sakamoto et al., 2007). A previous *meta*-analysis studies was conducted with a group of SNPs in which E23K and rs5210 polymorphisms were often observed, and it was established that the rs5210 polymorphism was associated with T2DM (Qin et al., 2013, Yang et al., 2012). However, the current study was not in accordance with previously documented studies, and the current study was supported by a group of previously recorded global studies (Khan et al., 2020, Sakamoto et al., 2007, Al Hussieny and Alsahlawi, 2021, Koo et al., 2007, Malekizadeh et al., 2021, Willer et al., 2007, Cruz et al., 2010, Čejková et al., 2007). The rs5210 polymorphism was also studied in children with T1DM (Blasetti et al., 2020). Xu et al studies confirmed rs5210 polymorphism was associated with improved clinical efficacy of gliclazide (Xu et al., 2009).

Diabetes can be caused by both pathogenic and non-pathogenic genetic mutations. SNPs, which are non-pathogenic mutations, have been associated to several types of diabetes susceptibilities. The decrease in ATP ability to inhibit K_{ATP} channel activity, along with an increase in MgATP's ability to simultaneously stimulate this channel function via the voltage-dependent potassium chan-

nel, results in the closure of voltage-dependent calcium channels. Diabetes can be induced by mutations in the KCNJ11 gene, which reduces the capacity of ATP to block K_{ATP} channel activation. The rs5210 polymorphism is present on 3′UTR region located at chromosome location 17,386,704 with documented 0.46 as minor allele frequency. Insulin exocytosis is important for blood glucose regulation, and miRNAs have been found to regulate insulin exocytosis in pancreatic β-cells (Haghvirdizadeh et al., 2015). SNPs in KCNJ11 gene has been studied in various forms of diabetes such as NDM, T1DM, T2DM, GDM and PTDM (Abbasi et al., 2012, Koo et al., 2007, Khan et al., 2019, Khan et al., 2015b).

Glycated hemoglobin (Hb1Ac) is produced by the irreversible post-translational non-enzymatic interaction of the aldehyde group of glucose and other hexoses with the amino terminal valine of the hemoglobin β-chain (Chandalia and Krishnaswamy, 2002). Hb1Ac has been shown to be an everlasting approach for evaluating glycemic management and monitoring blood glucose levels. The majority of global studies found a significant strong correlation between Hb1Ac and mean glucose levels, and some studies confirmed that Hb1Ac can be utilized as a diagnostic marker in diabetes patients (Kostov and Blazhev, 2020). In the current study, the mean levels of Hb1Ac was found to be 7.7±0.88 and 5.5±0.62 in controls.

This study confirms both the studies strengths and limitations. The strength of this study was participants were recruited from Saudi community, followed diabetic subjects based on ADA criteria and reconfirmed T2DM patients based on FBG and Hb1Ac levels. The limitation of this study can be confirmed as low sample size and studies only rs5210 polymorphism without documenting any medication history in T2DM patients.

5. Conclusion

The *KCNJ11* gene polymorphism rs5210 polymorphism was shown to be the sole source of allelic association in this study. There may be many explanations for this, but this study proposes that future studies be conducted in large cohorts and that rs5210 polymorphism should be explored globally to rule out the diagnostic marker for various types of diabetes from infants to adults. There should be regular updates to the *meta*-analysis in global studies with all the SNPs involved in *KCNJ11* gene.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abbasi, F., Saba, S., Ebrahim-Habibi, A., Sayahpour, F.A., Amiri, P., Larijani, B., Amoli, M.M., 2012. Detection of KCNJ11 gene mutations in a family with neonatal diabetes mellitus. Mol. Diagnosis Ther. 16, 109–114.

Al-Otaiby, M., Althnayan, R., Binmethem, A., Alenezy, R.B., Alhadlg, M.A., Alaqeel, A., Alqahtani, S.H., Ghufran, N., Alotaibi, A.A., Alayed, N., 2021. The prevalence of Factor V Leiden (Arg506Gln) mutation in King Khalid University Hospital patients, 2017–2019. Nagoya J. Med. Sci. 83, 407.

Al Hussieny, B.A., Alsahlawi, M.M., 2021. The Association Between genetic variations of KCNj11 gene and type 2 diabetes mellitus in a sample of Iraqi population. Al-Kufa Univ. J. Biol. 13.

Alfaifi, M., 2021. Contribution of genetic variant identified in HHEX gene in the Overweight Saudi patients confirmed with Type 2 Diabetes Mellitus. Saudi J. Biol. Sci.

Alhaidar, A.M., Alshehri, N.A., Alhussaini, M.A., 2020. Family support and its association with glycemic control in adolescents with type 1 diabetes mellitus in Riyadh, Saudi Arabia. J. Diabetes Res.

Alharbi, K.K., Abudawood, M., Khan, I.A., 2021. Amino-acid amendment of arginine-325-tryptophan in rs13266634 genetic polymorphism studies of the SLC30A8 gene with type 2 diabetes-mellitus patients featuring a positive family history in the Saudi population. J. King Saud Univ.-Sci. 33, 101258.

Alharbi, K.K., Al-Sheikh, Y.A., Alsaadi, M.M., Mani, B., Udayaraja, G.K., Kohailan, M., Ali Khan, I., 2020. Screening for obesity in the offspring of first-cousin consanguineous couples: A Phase-I study in Saudi Arabia. Saudi J. Biol. Sci. 27, 242–246.

Alharbi, K.K., Alharbi, F.K., Ghneim, H., Al-Sulaiman, A., Alodhayani, A., Tabassum, S., Khan, I., 2016. Amendment of amino acid in Q192R genetic polymorphism of paraoxonase 1 is a conventional risk factor for type 2 diabetes mellitus in the Saudi population. Int. J. Clin. Exp. Med. 9, 16605–16612.

Alharbi, K.K., Alnbaheen, M.S., Alharbi, F.K., Hasanato, R.M., Khan, I.A., 2017. Q192R polymorphism in the PON1 gene and familial hypercholesterolemia in a Saudi population. Ann. Saudi Med. 37, 425–432.

Alharbi, K.K., Khan, I.A., Al-Sheikh, Y.A., Alharbi, F.K., Alharbi, F.K., Al-Nbaheen, M.S., 2014. Lack of association between UBE2E2 gene polymorphism (rs7612463) and type 2 diabetes mellitus in a Saudi population. Acta Biochim. Pol. 61, 769–772.

Alsmadi, O., Al-Rubeaan, K., Wakil, S.M., Imtiaz, F., Mohamed, G., Al-Saud, H., Al-Saud, N.A., Aldaghri, N., Mohammad, S., Meyer, B.F., 2008. Genetic study of Saudi diabetes (GSSD): significant association of the KCNJ11 E23K polymorphism with type 2 diabetes. Diabetes/metabolism Res. Rev. 24, 137–140.

Blasetti, A., Castorani, V., Comegna, L., Franchini, S., Prezioso, G., Provenzano, M., di Giulio, C., Iannucci, D., Matonti, L., Tumini, S., 2020. Role of the KCNJ Gene Variants in the Clinical Outcome of Type 1 Diabetes. Horm. Metab. Res. 52, 856–860.

Čejková, P., Novota, P., Černá, M., Kološtová, K., Nováková, D., Kučera, P., Novak, J., Anděl, M., Weber, P., Ždárský, E., 2007. KCNJ11 E23K polymorphism and diabetes mellitus with adult onset in Czech patients. Folia Biol. (Praha) 53, 173–175.

Chandalia, H., Krishnaswamy, P., 2002. Glycated hemoglobin. Curr. Sci., 1522–1532

Cruz, M., Valladares-Salgado, A., Garcia-Mena, J., Ross, K., Edwards, M., Angeles-Martinez, J., Ortega-Camarillo, C., Escobedo De La Peña, J., Burguete-Garcia, A., Wacher-Rodarte, N., 2010. Candidate gene association study conditioning on individual ancestry in patients with type 2 diabetes and metabolic syndrome from Mexico City. Diabetes/metabolism Res. Rev. 26, 261–270.

Haghvirdizadeh, P., Mohamed, Z., Abdullah, N.A., Haghvirdizadeh, P., Haerian, M.S., Haerian, B.S., 2015. KCNJ11: genetic polymorphisms and risk of diabetes mellitus. J Diabetes Res.

Khan, I.A., 2021. Do second generation sequencing techniques identify documented genetic markers for neonatal diabetes mellitus? Heliyon 7.

Khan, I.A., Jahan, P., Hasan, Q., Rao, P., 2019. Genetic confirmation of T2DM meta-analysis variants studied in gestational diabetes mellitus in an Indian population. Diabetes Metab. Syndr. 13, 688–694.

Khan, I.A., Movva, S., Shaik, N.A., Chava, S., Jahan, P., Mukkavali, K.K., Kamineni, V., Hasan, Q., Rao, P., 2014. Investigation of Calpain 10 (rs2975760) gene polymorphism in Asian Indians with Gestational Diabetes Mellitus. Meta Gene 2, 299–306.

Khan, I.A., Poornima, S., Jahan, P., Rao, P., Hasan, Q., 2015a. Type 2 Diabetes Mellitus and the Association of Candidate Genes in Asian Indian Population from Hyderabad, India. J. Clin. Diagn. Res. 9, Gc01-5.

Khan, I.A., Vattam, K.K., Jahan, P., Mukkavali, K.K., Hasan, Q., Rao, P., 2015b. Correlation between KCNQ1 and KCNJ11 gene polymorphisms and type 2 and post-transplant diabetes mellitus in the Asian Indian population. Genes Dis. 2 (3), 276–282.

Khan, V., Verma, A.K., Bhatt, D., Khan, S., Hasan, R., Goyal, Y., Ramachandran, S., Alsahli, M.A., Rahmani, A.H., Almatroudi, A., Shareef, M.Y., Meena, B., Dev, K., 2020. 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. Int. J. Endocrinol. 2020, 1–12.

Ko, J.M., Yang, S., Kim, S.Y., Lee, H.S., Hwang, J.S., Hwang, I.T., 2012. E23K polymorphism of the KCNJ11 gene in Korean children with type 1 diabetes. World J. Pediatrics 8, 169–172.

Koo, B., Cho, Y., Park, B., Cheong, H., Shin, H., Jang, H., Kim, S., Lee, H., Park, K., 2007. Polymorphisms of KCNJ11 (Kir6. 2 gene) are associated with Type 2 diabetes and hypertension in the Korean population. Diabet. Med. 24, 178–186.

Kostov, K., Blazhev, A., 2020. Use of glycated hemoglobin (A1c) as a biomarker for vascular risk in type 2 diabetes: Its relationship with matrix metalloproteinases-2,-9 and the metabolism of collagen IV and elastin. Medicina 56 (5), 231. https://doi.org/10.3390/medicina56050231.

Liu, Z., Zhang, Y., Feng, Q., Li, Y., Wu, G., Zuo, J., Xiao, X., Fang, F., 2006. Association analysis of 30 type 2 diabetes candidate genes in Chinese Han population. *Zhongguo yi xue ke xue Yuan xue bao*. Acta Academiae Medicinae Sinicae 28, 124–128.

Malekizadeh, A., Rahbaran, M., Afshari, M., Abbasi, D., Aghaei Meybodi, H.R., Hasanzad, M., 2021. Association of common genetic variants of KCNJ11 gene with the risk of type 2 diabetes mellitus. Nucleosides, Nucleotides Nucleic Acids 40, 530–541.

Qin, L., Lv, Y., Huang, Q., 2013. Meta-analysis of association of common variants in the KCNJ11-ABCC8 region with type 2 diabetes. Genet Mol. Res. 12, 2990–3002.

Robert, A.A., Al Dawish, M.A., 2020. The worrying trend of diabetes mellitus in Saudi Arabia: an urgent call to action. Curr. Diabetes Rev. 16, 204–210.

Sakamoto, Y., Inoue, H., Keshavarz, P., Miyawaki, K., Yamaguchi, Y., Moritani, M., Kunika, K., Nakamura, N., Yoshikawa, T., Yasui, N., 2007. SNPs in the KCNJ11-ABCC8 gene locus are associated with type 2 diabetes and blood pressure levels in the Japanese population. J. Hum. Genet. 52, 781–793.

Willer, C.J., Bonnycastle, L.L., Conneely, K.N., Duren, W.L., Jackson, A.U., Scott, L.J., Narisu, N., Chines, P.S., Skol, A., Stringham, H.M., Petrie, J., Erdos, M.R., Swift, A.J., Enloe, S.T., Sprau, A.G., Smith, E., Tong, M., Doheny, K.F., Pugh, E.W., Watanabe, R.M., Buchanan, T.A., Valle, T.T., Bergman, R.N., Tuomilehto, J., Mohlke, K.L., Collins, F.S., Boehnke, M., 2007. Screening of 134 single nucleotide polymorphisms (SNPs) previously associated with type 2 diabetes replicates association with 12 SNPs in nine genes. Diabetes 56 (1), 256–264.

Xu, H., Murray, M., McLachlan, A.J., 2009. Influence of genetic polymorphisms on the pharmacokinetics and pharmacodynamics of sulfonylurea drugs. Curr. Drug Metab. 10, 643–658.

Yang, L., Zhou, X., Luo, Y., Sun, X., Tang, Y., Guo, W., Han, X., Ji, L., 2012. Association between KCNJ11 gene polymorphisms and risk of type 2 diabetes mellitus in East Asian populations: a meta-analysis in 42,573 individuals. Mol. Biol. Rep. 39, 645–659.