International Journal of Diabetes in Developing Countries (January–March 2024) 44(1):175–181
https://doi.org/10.1007/s13410-023-01213-4

ORIGINAL ARTICLE

Correlating the role of *KCNJ11* polymorphism (rs5219) and T2DM: A case control study

Archna Bhargave[1] · Imteyaz Ahmad[1] · Anita Yadav[2] · Ranjan Gupta[1]

Received: 22 February 2022 / Accepted: 21 May 2023 / Published online: 13 June 2023

Abstract

Background Diabetes is one of the four major types of non-communicable disease which has reached the epidemic proportions leading to major public health problems and concern. Several studies have shown the impact of genetic variations on diabetes pathogenesis. *KCNJ11* gene has been associated with T2DM. Any variation in this gene disrupts the insulin release from β cells ultimately causing diabetes.

Aim The present research aims to resolve whether genetic variants of KCNJ11 have association with susceptibility to T2DM in the North Indian population.

Method PCR-RFLP technique was used to genotype 200 subjects for rs5219 genetic variant of *KCNJ11* gene. Student's *t* test and chi square test (χ^2) were used to evaluate continuous and categorical variables. Association of *KCNJ11*genotypes with T2DM was done by odds ratio (OR) and confidence interval (CI). All statistical analyses were performed using IBM SPSS-21 software.

Results Environmental factors such as smoking and lack of exercise increase the risk for T2DM (OR>1). The genotype frequency distribution for *KCNJ11* rs5219 SNP was in Hardy-Weinberg equilibrium (HWE) for both control (*p*-value-0.96) and T2DM case group (*p*-value-0.685). rs5219 was associated with T2DM in dominant genetic model (*p*-value- <0.001; OR-3.781) and recessive genetic model (*p*-value- <0.001; OR- 3.740). It was found that T allele was a risk allele that increases susceptibility for T2DM.

Conclusion This study elucidated that rs5219 genetic variant of *KCNJ11* may increase the susceptibility for T2DM and TT genotype might be involved in predisposing individuals for development of disease.

Keywords Type 2 diabetes mellitus · *KCNJ11* · Single nucleotide polymorphism · Genetic model

Introduction

Diabetes mellitus, a lifelong chronic metabolic disease is characterized by hyperglycemia. It results from either defects in insulin secretion, its action or both [1]. Globally 463 million people were diagnosed with this disease in 2019. The number is predicted to rise up to 745 million in 2045 (Diabetes Atlas). In 2021, around 77 million people are affected by this disease in India which is predicted to rise up to 134 million people by 2045 [2].

Glucose-induced insulin secretion from β cells of pancreas is regulated by K_{ATP} channel. *KCNJ11* gene localized on chromosome 11 encodes for four pores forming inward rectifier subunit (Kir6.2) of K_{ATP} channel. During fed state, elevated level of blood glucose increases the ATP/ADP ratio, causing K_{ATP} channel to close. The closure causes the depolarization of membrane of pancreatic β cells and voltage-gated calcium channel to open. This leads to an increase in calcium concentration inside the cells which in turn triggers the release of insulin [3]. Mutation in the gene (*KCNJ11*) either impairs the ATP binding site of Kir6.2 or stabilizes the open state of channel. This decreases the sensitivity of channel for its inhibition by ATP [4].

✉ Ranjan Gupta
r.gupta@kuk.ac.in

Archna Bhargave
archna.bhargave90@gmail.com

Imteyaz Ahmad
ahmadimteyaz99@gmail.com

Anita Yadav
ayadav@kuk.ac.in

[1] Department of Biochemistry, Kurukshetra University, Kurukshetra 136119, Haryana, India

[2] Department of Biotechnology, Kurukshetra University, Kurukshetra, 136119, Haryana, India

Several association studies have reported various SNPs viz: rs5219, rs1800467, rs5215, rs241282930, rs2285676 of *KCNJ11* to be associated with T2DM [5–12], but rs5219 has received the most attention for its influence on glycemia [13]. In rs5219 SNP replacement of A to C alters the amino acid lysine to glutamine. This change reduces the ATP sensitivity of the channel and thus has its effect on secretion of insulin [14]. Though several studies have reported the association of rs5219 with disease [15, 16], the contradictory results in some population prevent to confirm it [17–19]. This implies that role of rs5219 in predisposing individual to T2DM is still divisive and warrants further investigation. Therefore the present study was undertaken to examine association of genetic variants of rs5219 with type 2 diabetes mellitus in North Indian population and to compare the levels of biochemical risk factor for susceptibility to diabetes with different genotypes.

Materials and methods

Subject selection

A total of 200 subjects: 100 control (healthy) and 100 T2DM patients (who were on follow-up treatment) were enrolled for the present study. The subjects geographically belonged and lived in the northern part of India, particularly the state of Haryana. The T2DM case subjects considered for the study were according to the diagnostic criteria laid down by the Indian Council of Medical Research (ICMR). Controls were recruited from outpatient department (OPD) of hospitals who were on regular body check-ups. Subjects diagnosed with T1DM and other severe diseases of kidney, liver, and coronary artery disease were excluded from the study. Pregnant and lactating mothers were also excluded.

Demographic characteristics

Demographic characteristics such as age, gender, alcohol, and smoking consumption habits were analyzed using a questionnaire. Information regarding weight and height of individuals were collected during sample collection. Body mass index (BMI) was appraised by dividing the weight/height (kg/m^2). Venous blood samples were drawn early in the morning from subjects after 10–12 h of overnight fasting in both EDTA and non-EDTA coated vials.

Genomic DNA extraction

Genomic DNA was extracted from Miller et al. [20], method with minor modifications. Extracted DNA was visualized in UV transilluminator using 1% agarose gel. Purity of extracted DNA samples was ascertained using A260/A280 ratio. The samples having the ratio in the range of 1.6 to 2.0 were considered for PCR-based analysis. The isolated DNA was stored at −20°C.

Polymorphism chain reaction-restriction fragment length polymorphism (PCR-RFLP) of SNP rs5219

Amplification of desired sequence of *KCNJ11* gene was carried out. A set of primers verified through virtual amplification by using in silico PCR online software was used. The sequence of primers used is shown in Table 1. The targeted amplicon was amplified with reaction mixture (25 µl) using forward and reverse primer (0.5 µl each), $MgCl_2$ (2.5 µl), dNTPs (0.5 µl), nuclease-free water (15.5 µl), Taq buffer (2.5 µl), Taq DNA polymerase (1 µl) and DNA (2 µl) as template. The PCR profile included denaturation initially for 5 min at 94°C, followed by 35 cycles of (a) 45 s at 94°C of denaturation, (b) 45 s at 63.2°C of annealing, (c) 45 s at 72°C of extension; (d) 5 min at 72°C of final extension. Amplified PCR products of 220 bp were resolved by electrophoresis (5V/60 min) in Tris Borate-EDTA (TBE) buffer containing 0.5 µg/ml of ethidium bromide using 1.5% agarose gel. Molecular size ladder of 100 bp (Genei) was used to ascertain the size of the bands. UV Transilluminator was then used for viewing and photograph the gel (Fig. 1).

The amplified amplicon of *KCNJ11* gene was digested in 10 µl aliquot with 2–5 units of Ban II restriction

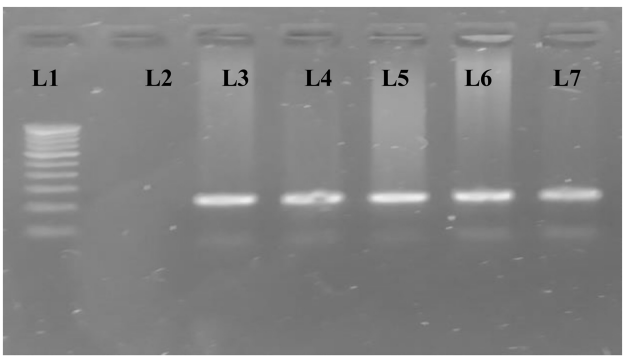

Fig. 1 PCR product of *KCNJ11* rs5219 SNP. Lane 1 shows 100 bp ladder, lane 2 shows blank, lanes 3, 4, 5 and 6 show 220 bp PCR product

Table 1 Primers, product size and restriction enzyme used

Gene	Variant	Primers	Product size	Restriction enzyme (RE)
KCNJ11	**rs5219**	**F-GAATACGTGCTGACACGCCT R-GCCAGCTGCACAGGAAGGACAT**	**220**	**Ban II**

endonuclease at 37°C for 2 h [3]. Restriction enzyme was verified using an online tool Restriction Mapper. The 10 µl reaction mixture consisted of: PCR product (8.5 µl), Ban II (0.5 µl), 10× buffer solution (1 µl). The digested fragments were resolved on 3% agarose gel stained with ethidium bromide and was observed on UV transilluminator. Wild type DNA shows two bands with 151, 69 bp, whereas mutated DNA shows three bands of 28, 41 and 151 bp, heterozygous mutated show four bands of 28, 41, 69 and 151 bp (Fig. 2).

Statistical analysis

The sample size of the present study was calculated by using online software OSSE (online sample size estimator). Sample size at 90% power came out to be 95 for control and T2DM case group respectively.The Hardy-Weinberg Equilibrium (HWE) ($p > 0.05$) for genotype and allele distribution was ascertained by employing $\chi 2$ goodness-of-fit test. Student's *t*-test was used to compare demographic and clinical characteristics of T2DM case and control groups. Chi-square ($\chi 2$) test was applied for comparison of allelic frequencies and genotype distribution. Association of *KCNJ11*genotypes with T2DM was done by odds ratio (OR) and confidence interval (CI) calculation. Data analyzed were defined statistically significant at *p*-value <0.05. All statistical analyses were carried out using IBM SPSS Statistics 21software.

Results

Analysis of demographic characteristics

Analysis of demographic characteristics revealed significant difference among two groups when their smoking status was considered. Smokers are highly susceptible to T2DM (OR-2.95; 95% CI-1.586-5.508, *p*- <0.001). The risk by demographic characteristics have been highlighted in Table 2. Vegetarian diet prevents the risk for diabetes with non-vegetarians being highly susceptible towards T2DM.

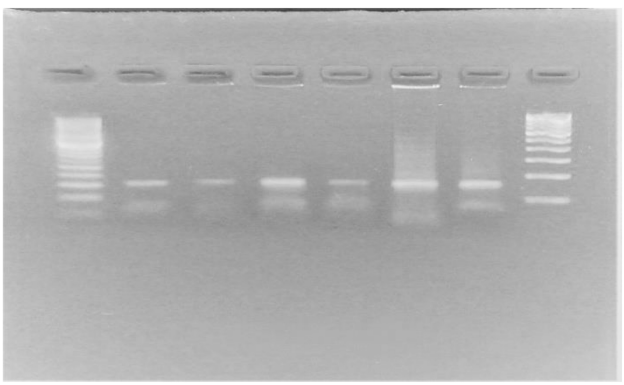

Fig. 2 Restriction digestion of *KCNJ11* rs5219 SNP products. Lane 1 shows 50 bp ladder, lane 8 shows 100 bp ladder, lanes 2, 3, 4, 5 and 7 show 151 and 69 bp band, lane 6 shows 151 and 41 bp band (28 bp band not visible)

Analysis of genotype

The genotype distribution of rs5219 for both control and case were in agreement with Hardy-Weinberg equilibrium (HWE) as calculated by χ^2 goodness of fit test (Table 3).

The genotype distribution frequency of CC, CT, and TT of rs5219 SNP of *KCNJ11* in T2DM group 19%, 54%, 27%, and for the T2DM cases 47%, 44%, and 9% respectively differs substantially with *p* value-0.000 (Table 4). It was observed that CC genotype was significantly higher in the control group whereas TT genotype was considerably higher in case group. Allelic frequency of T was found to be higher in T2DM cases (OR-2.613; 95% CI- 1.736–3.932; *p*- <0.001). Significant association was observed between SNP rs5219 and T2DM in recessive model: OR (3.740); 95% CI (1.656–8.447), *p*- value (< 0.001), and dominant model: OR (3.781); 95% CI (2.003–7.137), *p*- value (<0.000) (Table 4).

Effects of genotypes on clinical characteristics

Comparative analysis of rs5219 SNP genotypes with clinical parameters in cases and control was performed by using the dominant (CT+TT vs.CC) (Table 5) and recessive (TT vs. CC+CT) (Table 6) genetic model. In the recessive genetic model (TT vs. CC+CT), statistical significant

Table 2 Demographic characteristics

Demographic data	OR (95% CI)	*p* value
Gender (F/M)	0.961 (.551–1.675)	0.443
Smoking (Y/N)	2.95 (1.586–5.508)	<0.001*
Alcohol (Y/N)	1.084 (0.622–1.888)	0.198
Exercise (Y/N)	0.295 (0.160–0.544)	<0.001*
Diet (NonVeg/Veg)	2.364 (1.272–4.392)	0.003*

**p* value <0.05 considered as statistically significant; OR, odds ratio; CI, confidence interval

Table 3 Hardy-Weinberg equilibrium test

Genotype	Control	*p*-value	Case	*p*-value
CC	47	0.960	19	0.685
CT	44		54	
TT	9		27	

Table 4 *KCNJ11* rs5219 SNP genotype and allelic distribution frequencies

SNP(rs5219)		Cases (%)	Control (%)	OR(95% CI)	*p*-value
Genotype	CC	19	47		<0.001*
	CT	54	44		
	TT	27	9		
Alleles	T	108 (54)	62 (31)	2.613 (1.736–3.932)	<0.001*
	C	92 (46)	138 (69)		
Recessive model	TT	27	9	3.740 (1.656–8.447)	<0.001*
	CC+CT	73	91		
Dominant model	CT+TT	81	53	3.781 (2.003–7.137)	<0.001*
	CC	19	47		

*p-value <0.05 considered as statistically significant; OR, odds ratio; CI, confidence interval

Table 5 Analysis *of KCNJ11* rs5219 genotype distribution with clinical parameters under dominant model

Parameters	Case			Control		
	CT+TT	CC	*p*-value	CT+TT	CC	*p*-value
BMI (kg/m^2)	27.44 ± 3.30	27.85 ± 3.46	0.313	24.04 ± 3.68	24.47 ± 2.95	0.242
DBP (mmHg)	82.54 ± 9.48	84.89 ± 10.22	0.170	81.00 ± 9.96	81.28 ± 9.17	0.443
SBP (mmHg)	150.17 ± 14.71	141.36 ± 22.08	0.018*	124.7 ± 11.44	122.34 ± 11.87	0.157
PSF (mg/dl)	187.98 ± 61.17	185.26 ± 44.85	0.427	86.18 ± 7.45	87.78 ± 8.5	0.160
HbA1c (%)	7.50 ± 1.76	7.60 ± 1.51	0.405	5.43 ± 0.511	5.51 ± 0.50	0.228
TC (mg/dl)	189.74 ± 47.54	188.67 ± 39.89.74	0.464	154.69 ± 28.70	146.00 ± 36.90	0.094
TG (mg/dl)	193.45 ± 44.28	192.711 ± 49.21	0.474	110.24 ± 21.18	110.59 ± 21.86	0.467
HDL-C (mg/dl)	47.11 ± 17.22	44.01 ± 10.75	0.227	46.88 ± 9.30	49.37 ± 10.44	0.105
LDL-C (mg/dl)	104.57 ± 41.69	110.17 ± 35.87	0.295	57.72 ± 18.48	59.64 ± 22.07	0.319
VLDL-C (mg/dl)	35.93 ± 20.15	37.94 ± 19.11	0.347	24.21 ± 9.97	22.04 ± 9.00	0.129

Data shown as mean ± standard deviation, *p-value <0.05 considered as statistically significant

Table 6 Analysis of *KCNJ11* rs5219 SNP genotypes with clinical parameters under recessive model

Parameters	Case			Control		
	CC+CT	TT	*p*-value	CC+CT	TT	*p*-value
BMI(kg/m2)	27.52 ± 3.27	27.52 ± 3.50	0.499	24.34 ± 3.42	23.01 ± 2.36	0.129
DBP(mmHg)	82.56 ± 9.47	84.14 ± 10.10	0.233	81.21 ± 9.76	80.33 ± 6.98	0.397
SBP(mmHg)	142.30 ±17.46	141.37 ± 11.87	0.399	123.32 ± 11.73	126.33 ± 10.943	0.231
PSF(mg/dl)	180.42 ± 53.78	206.51 ± 66.23	0.023*	87.16 ± 7.94	84.66 ± 8.63	0.187
HbA1c(%)	7.45 ± 1.76	7.68 ± 1.56	0.276	5.47 ± 0.51	5.47 ± 0.40	0.497
TC(mg/dl)	187.03 ± 45.03	214.50 ± 55.22	0.006*	150.47 ± 32.54	152.0 ± 38.75	0.447
TG(mg/dl)	193.86 ±47.73	200.52 ± 38.85	0.274	109.52 ± 21.46	119.33 ± 19.62	0.095
HDL-C(mg/dl)	46.48 ± 11.79	46.63 ± 24.77	0.484	47.75 ± 10.15	43.47 ± 5.77	0.108
LDL-C(mg/dl)	101.89 ± 39.05	102.75 ± 39.31	0.461	58.34 ± 20.866	61.52 ± 11.22	0.327
VLDL-C(mg/dl)	36.35 ± 17.83	36.22 ± 24.97	0.488	22.95 ± 9.29	25.64 ± 12.14	0.216

Data shown as mean ± standard deviation, *p-value <0.05 considered as statistically significant

association was observed with regards to PSF,TC and LDL in TT genotype in T2DM patients with p value 0.023,0.006 and 0.017 respectively (Table 6). The levels of $HbA1_C$ and TG were found to be higher in TT genotype than the CC+CT genotype in the T2DM group but the difference was not statistically different (Table 6). Regarding dominant genetic model except SBP no dissimilarity was observed in clinical parameters among CC and CT+TT genotypes in both control group and case group (p-value <0.05) (Table 5).

Discussion

T2DM is a polygenic disorder that involves contribution of several genetic and environmental factors for its development due to dysfunctional insulin secretion resulting in impaired glucose metabolism. The K_{ATP} channel is involved in glucose-stimulated insulin secretion from pancreatic beta cells. K_{ATP} channel subunit Kir6.2 protein is encoded by *KCNJ11* gene. The activity of K_{ATP} channel is inhibited by ATP and stimulated by MgATP or Mg ADP. Any genetic variation in the *KCNJ11* gene can either reduce ATP's ability to inhibit the K_{ATP} channel's activity or increase MgATP ability to simultaneously stimulate the channel's function [4].

KCNJ11 gene variants at various loci have been demonstrated to be associated with diabetes in number of previous studies [8–12, 21, 22]. The present study investigated and elucidated the association of *KCNJ11* gene variant at rs5219 with T2DM (p value-<0.001). The frequency of the minor allele T (54%) is significantly higher in T2DM subjects (OR-2.613, p value- <0.001) than control subjects in this investigation, indicating that people with this genotype or allele are more likely to develop T2DM. The results of present study corroborated with findings of previous study conducted on Syrian [23] , Chinese Han [24], Iranian [25] and Indian population [9, 17, 26]. A meta-analysis by Wang et al. [27] and Gong et al. [13] also showed rs5219 SNP as risk factor for type 2 diabetes. Due to extensive diversity in the genetic and environmental makeup of the worldwide population, studies carried on Euro-Brazilian [28], South Asian [17], Iranian population [29, 30] Czech [31] and Moroccan population [18] were not able to replicate the result of associating rs5219 genetic variant with T2DM. In these studies mutated allele did not confer risk of T2DM. The current study also revealed relationship of TT genotype with increased risk of T2DM under both dominant (OR-3.781, p value- <0.001) and recessive (OR-3.740, p value-<0.001) genetic model. Similar to our results, Rizvi et al. [32] and Makhzoom et al. [23] also confirmed the association of rs5219 SNP under dominant (p value-0.022) and recessive genetic model (p value-0.035) respectively.

For assessing the presentation and progression of T2DM genotype distributions were correlated with clinical parameters under both dominant (Table 5) and recessive genetic model (Table 6). SBP (Table 5), PSF and TC (Table 6) clinical parameters were observed to be significantly higher in risk genotype. The T2DM subjects with TT genotype exhibited significantly higher levels of PSF in the studied population. These results are consistent with the reports of Bankura et al. [8] who have cited the association of *KCNJ11* rs5219 genetic variant with reduced secretion of insulin. The risk genotype TT genotype is associated with significantly higher levels of cholesterol in the present study (Table 6) which may be due to low cholesterol absorption efficiency associated with insulin resistance [33].The current study also showed association of rs5219 variant with SBP (Table 5) which is in coherence with study of Koo et al. [5].

Apart from genetic factors, plethora of lifestyle factors also contributes towards development of T2DM. The present study concluded the association of diet with risk of T2DM, with non-vegetarian diet increasing the risk. The study corroborates with findings of Chiu et al. [34], who associated lifelong vegetarians with 35% reduced risk for developing T2DM. The present study also observes the association of smoking with T2DM. Maddatu et al. [35] also confirm that smoking increases the risk of development of T2DM. Another lifestyle factor, exercise is also associated with T2DM, conferring decreased risk of T2DM in the current case-control study.

In summary, genetic variation in SNP rs5219 of *KCNJ11* gene is linked with increased risk for predisposing individual to T2DM with the CC genotype being protective and TT genotype conferring the risk for T2DM. This study also observes that controlling the lifestyle/environmental factors can control the risk of developing diabetes. Considering the sample size of present study, further studies with large sample size are needed. The results may be important in the ongoing efforts to identify individuals at increased risk of developing type 2 diabetes mellitus.

Conclusion

The results of the present study indicate that individuals with TT genotype of *KCNJ11* SNP rs5219 are more susceptible for T2DM than individuals with CC genotype in Indian population. T allele was more common in T2DM patients, thus it may be a risk factor in predisposing individual for type 2 diabetes mellitus. However, further studies with large sample size are required to evaluate the association of rs5219 genetic variant of *KCNJ11* as predisposing risk factor for T2DM.

Acknowledgment We would like to acknowledge the contribution of blood donors in this study.

Authors contribution Archna Bhargave — Conceptualization, Methodology, Data collection and analysis, Writing of original draft for publication

Imteyaz Ahmed — Methodology, Data analysis

Anita Yadav — Conceptualization

Ranjan Gupta — Conceptualization, Methodology, Resources, Data analysis and editing of original draft

All authors have read and approved the final draft.

Declarations

Ethics approval The study protocol was approved by the Institutional Human Ethical Committee Kurukshetra University, Kurukshetra Haryana.

Consent to participate Informed written consent was obtained from all the participating individuals.

Consent for publication The participants provided the written consent to publish their data.

Competing interests The authors declare no competing interests.

References

1. Kharroubi AT. Diabetes mellitus: the epidemic of the century. World J Diabetes. 2015;6:850–67.
2. Luhar S, Kondal D, Jones R, Anjana RM, Patel SA, Kinra S, et al. Lifetime risk of diabetes in metropolitan cities in India. Diabetologia. 2021;64:521–9.
3. Abed AA, Ayesh BM, Hamdona OM. Single nucleotide polymorphism E23K of KCNJ11 gene and other risk factors associated with type-2 diabetes mellitus in Gaza. Int J Chem Life Sci. 2013;2:1146–52.
4. Ashcroft FM. KATP channels and insulin secretion: a key role in health and disease. Biochem Soc Trans. 2006;34(2):243–46.
5. Koo BK, Cho YM, Park BL, Cheong HS, Shin HD, Jang HC, et al. Polymorphisms of KCNJ11 (Kir6.2 gene) are associated with Type 2 diabetes and hypertension in the Korean population. Diabet Med. 2007;24:178–86.
6. Han YY, Wang LJ, Zhang L, Zhang WW, Ma KT, Li L, et al. Association between potassium channel SNPs and essential hypertension in Xinjiang Kazak Chinese patients. Exp Ther Med. 2017;14:1999–2006.
7. Odgerel Z, Lee HS, Erdenebileg N, Gandbold S, Luvsanjamba M, Sambuughin N, et al. Genetic variants in potassium channels are associated with type 2 diabetes in a Mongolian population. J Diabetes. 2012;4:238–42.
8. Bankura B, Pattanayak AK, Ghosh S, Guria S, Sinha A, Das M. Implication of KCNJ11 and TCF7L2 gene variants for the predisposition of type 2 diabetes mellitus in West Bengal. India. Diabetes Epidemiol Manag. 2022;6:100066.
9. Aswathi R, Viji D, Charmine PSP, Husain RSRA, Ameen SHN, Ahmed SSSJ, et al. Influence of KCNJ11 gene polymorphism in T2DM of south Indian population. Front Biosci - Elit. 2020;12:199–222.
10. Alqadri N. Independent case-control study in KCNJ11 gene polymorphism with Type 2 diabetes Mellitus. Saudi J Biol Sci. 2022;29:2794–9.
11. Malekizadeh A, Rahbaran M, Afshari M, Abbasi D, Aghaei Meybodi HR, Hasanzad M. Association of common genetic variants of KCNJ11 gene with the risk of type 2 diabetes mellitus. Nucleosides, Nucleotides and Nucleic Acids. 2021;40:530–41.
12. Sachse G, Haythorne E, Hill T, Proks P, Joynson R, Terrón-Expósito R, et al. The kcnj11-e23k gene variant hastens diabetes progression by impairing glucose-induced insulin secretion. Diabetes. 2021;70:1145–56.
13. Gong B, Yu J, Li H, Li W, Tong X. The effect of KCNJ11 polymorphism on the risk of type 2 diabetes: a global meta-analysis based on 49 case-control studies. DNA Cell Biol. 2012;31:801–10.
14. Haghvirdizadeh P, Mohamed Z, Abdullah NA, Haghvirdizadeh P, Haerian MS, Haerian BS. KCNJ11: genetic polymorphisms and risk of diabetes mellitus. J Diabetes Res. 2015;2015.
15. Li YY. The KCNJ11 E23K gene polymorphism and type 2 diabetes mellitus in the Chinese Han population: a meta-analysis of 6,109 subjects. Mol Biol Rep. 2013;40:141–6.
16. Song J, Yang Y, Mauvais-Jarvis F, Wang YP, Niu T. KCNJ11, ABCC8 and TCF7L2 polymorphisms and the response to sulfonylurea treatment in patients with type 2 diabetes: a bioinformatics assessment. BMC Med Genet. BMC Medical Genetics. 2017;18:1–17.
17. Phani NM, Guddattu V, Bellampalli R, Seenappa V, Adhikari P, Nagri SK, et al. Population specific impact of genetic variants in KCNJ11 gene to type 2 diabetes: a case-control and meta-analysis study. PLoS One. 2014;99:e107021.
18. Benrahma H, Charoute H, Lasram K, Boulouiz R, Ben ARK, Fakiri M, et al. Association analysis of IGF2BP2, KCNJ11, and CDKAL1 polymorphisms with type 2 diabetes mellitus in a Moroccan population: a case–control study and meta-analysis. Biochem Genet. 2014;52:430–42.
19. Ezenwaka CE, Kalloo R, Uhlig M, Schwenk R, Eckel J. The E23K variant in the Kir6.2 subunit of the ATP-sensitive K+ channel does not augment impaired glucose tolerance in Caribbean subjects with a family history of type 2 diabetes. J Endocrinol. 2005;185:439–44.
20. Mwer S, Dykes D, Polesky H. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16:1215.
21. Reddy S, Maddhuri S, Nallari P, Ananthapur V, Kalyani S, Krishna M, et al. Association of ABCC8 and KCNJ11 gene variants with type 1 diabetes in south Indians. Egypt J Med Hum Genet. Egypt J Med Hum Genet; 2021;22(1):1–11
22. Isakova J, Talaibekova E, Vinnikov D, Saadanov I, Aldasheva N. ADIPOQ, KCNJ11 and TCF7L2 polymorphisms in type 2 diabetes in Kyrgyz population: a case-control study. J Cell Mol Med. 2019;23:1628–31.
23. Makhzoom O, Kabalan Y, Al-Quobaili F. Association of KCNJ11 rs5219 gene polymorphism with type 2 diabetes mellitus in a population of Syria: a case-control study. BMC Med Genet. 2019;20:1–6.
24. Zhou D, Zhang D, Liu Y, Zhao T, Chen Z, Liu Z, et al. The E23K variation in the KCNJ11 gene is associated with type 2 diabetes in Chinese and East Asian population. J Hum Genet. 2009;54:433–5.
25. Rabbani M, Sadeghi H, Moazen F, Hasanzadeh A, Imani E, Rastegari A. Association of KCNJ11 (E23K) gene polymorphism with susceptibility to type 2 diabetes in Iranian patients. Adv Biomed Res. 2015;4:1.
26. Rizvi S, Raza ST, Mahdi F, Singh SP, Rajput M, Rahman Q. Genetic polymorphisms in KCNJ11 (E23K, rs5219) and SDF-1β (G801A, rs1801157) genes are associated with the risk of type 2 diabetes mellitus. Br J Biomed Sci. 2018;75:139–44.
27. Wang D, dong CX, Yang Y, Xu LC. Association of K ir 6.2 gene rs5219 variation with type 2 diabetes: a meta-analysis of 21,464 individuals. Prim Care Diabetes. 2018;12:345–53.
28. Souza SW, Alcazar LP, Arakaki PA, Santos-Weiss ICR, Alberton D, Picheth G, et al. Polymorphism E23K (rs5219) in the KCNJ11 gene in Euro-Brazilian subjects with type 1 and 2 diabetes. Genet Mol Res. 2017;16:1–9.
29. Maay JKR, Hastuti P, Sadewa AH. Correlation of E23K gena KCNJ11 polymorphism as a risk factor for obesity with type 2 diabetes mellitus in Jayapura. Papua. Int J Res Med Sci. 2021;9:2201.
30. Keshavarz P, Habibipour R, Ghasemi M, Kazemnezhad E, Alizadeh M. Lack of genetic susceptibility of KCNJ11 E23K polymorphism with risk of type 2 diabetes in an Iranian population. Endoc Res. 2014;39(3):120–125.
31. Čejková P, Novota P, Černá M, Kološtová K, Nováková D, Kučera P, et al. KCNJ11 E23K polymorphism and diabetes mellitus with adult onset in Czech patients. Folia Biol. (Praha). 2007:173–5.
32. Rizvi S, Raza ST, Mehdi SR, Siddiqi Z, Eba A, Mahdi F. The relationship between Multidrug Resistance Protein 1(rs1045642)

and Cholesterol 24-hydroxylase (rs754203) genes polymorphism with type 2 diabetes mellitus. Br J Biomed Sci. 2017;74:30–5.
33. Iettinen TATUAM, Simonen PP, Gylling HK, Miettinen TA. Diabetes contributes to cholesterol metabolism. Medscape. 2002:25.
34. Chiu THT, Pan WH, Lin MN, Lin CL. Vegetarian diet, change in dietary patterns, and diabetes risk: a prospective study. Nutr Diab 2018;81:12.
35. Maddatu J, Anderson-Baucum E, Evans-Molina C. Smoking and the risk of type 2 diabetes. Transl Res. 2017;184:101–7.