

Association of KCNJ11 E23K (rs5219) polymorphism to Type 2 diabetes mellitus: a case-control study in Indian population of Eastern Uttar Pradesh

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Abstract

Aim: Potassium inwardly rectifying channel, subfamily J, member 11 (KCNJ11) gene has a key role in insulin secretion and is of substantial interest as a candidate gene for Type 2 Diabetes (T2D). The current work was performed to delineate the genetic influence of the most associated SNP of KCNJ11 gene E23K (rs5219) polymorphism on risk of T2D in Indian population of eastern Uttar Pradesh through case-control association study.

Method: A case-control study of 240 T2D cases and 229 controls was performed using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) approach to analyze the association of KCNJ11 E23K (rs5219) polymorphism on the risk of T2D. Odds ratio with 95% Confidence Interval (CI) was used to assess the association strength.

Result: Type 2 diabetes patients studied for the gene had significantly higher levels of Fasting Plasma Glucose (FPG) and 2 hr PPPG ($P < 5 \times 10^{-2}$) than healthy controls. The genetic variants of loci show Hardy-Weinberg distributions in our study. The genotype and allele distributions of polymorphism are significantly different between the T2D patients and healthy control groups. Our data show weak association to T2D with odds ratio 1.086 (95% CI 0.832-1.416; $P = 0.544$).

Conclusion: Our data provides valuable information for comparison with other ethnic groups as well as in determining disease susceptibility in Indian population of eastern Uttar Pradesh. However, in view of the genetic diversity of Indians, the result needs to be replicated in other groups. Interestingly, our data for the SNP show small effect size than those reported in European and East Asian populations; and North-Western Indian populations.

Keywords: Association; Body Mass Index; KCNJ11; Ethnicity; SNP; Type 2 Diabetes; KATP Channel; E23K Polymorphism; Caucasian Population

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Introduction

Type 2 Diabetes mellitus (T2D) is a public health problem, which affects a millions worldwide. T2D is considered a multifactorial disorder, with both environmental and genetic factors contributing to its development. Impaired insulin secretion and insulin resistance both contribute to the pathogenesis of type 2 diabetes. The recently discovered genes by Genome-wide Association Studies (GWAS) suggest a shift from genes involved in insulin action to those involved in insulin secretion, indicating pivotal role of beta-cell dysfunction in the pathogenesis of T2D (reviewed in Singh, 2015) [1]. An important issue linked with diabetes development is the failure of the insulin releasing mechanism in pancreatic beta cells. Therefore, genes encoding proteins critical in pancreatic beta-cell functions including those associated with insulin secretion are particularly good candidates for susceptibility to T2D.

ATP-sensitive potassium (KATP) channels in beta-cells regulate insulin secretion through coupling the metabolic state of the cell to membrane potential. Serum blood glucose levels influence activity of KATP channels [2, 3]. This channel is a trans-membrane protein encoded by the KCNJ11 and ABCC8 genes. Increased glucose concentrations reduce permeability of KATP channels that leads to increase in Ca^{2+} influx into the cell

and hence stimulates insulin secretion [4]. KATP channels are normally formed as an octamer, consisting of four K⁺ inward rectifier Kir6.2 (KCNJ11) subunits that generate the pore, and four regulatory sulfonylurea receptor SUR1 (ABCC8) subunits, which are a target of sulfonylurea drugs [5]. Mutations in both KCNJ11 and ABCC8 cause neonatal diabetes [6] and congenital hyperinsulinaemia in humans, a rare genetic disorder characterized by higher insulin levels in parallel with low blood glucose [7].

As a candidate gene for T2D in humans, a non synonymous E23K variant (rs5219) which results from a G - A transition in codon 23 in the NH2-terminal tail of Kir6.2 was identified [8]. Polymorphisms in this gene have been linked to T2D because of the role of KATP in insulin release. While several genetic variations have been reported to be associated with this disease, the E23K (rs5219) polymorphism is most commonly associated with this pathology.

The frequent KCNJ11 E23K polymorphism has been analyzed in several studies, most of which have reported a positive association between the minor K allele and type 2 diabetes [9-15]. A recent meta analysis of 11 published association studies adding up to a total of 5083 type 2 diabetic patients and 4747 control subjects showed a significant association between the K allele of the KCNJ11 E23K polymorphism and type 2 diabetes [odds ratio (OR), 1.15; $P < 10^{-5}$] [15]. Furthermore, a functional effect of this polymorphism, leading to an overactive KIR6.2 channel with a decreased sensitivity toward ATP, has been reported [16]. Thus, the association between the KCNJ11 E23K polymorphism and type 2 diabetes may be explained in part by a decreased insulin release caused by the polymorphism.

Recently, GWAS studies have confirmed the association of KCNJ11 polymorphism (rs5219) with T2D in Caucasians with an overall risk allele (OR=1.14, 95% CI=1.10–1.19) [17-19]. Several association studies and a recent meta-analysis showed a strong relationship between the rs5219 polymorphism and susceptibility to T2DM [13, 20-34], whereas 11 studies did not confirm this finding [35-45]. Another meta-analysis showed that the rs5219 polymorphism is a risk factor for developing T2DM in Caucasians and in East Asian populations. Populations from east Asia were more prone to this disease, where the K allele

frequency in most patients was more common than in controls indicating genetic background can affect susceptibility to T2DM [46]. However, studies on the association between KCNJ11 polymorphism (rs5219) and T2D gave contradictory results in south Asian populations [47].

In the past decade, a number of case-control studies have been conducted to investigate the relationship between the KCNJ11 polymorphism (rs5219) and T2D in different Indian populations. However, these studies have yielded contradictory results. In the present study, we have examined the association of the most significant genetic variant of loci KCNJ11 E23K (rs5219) with type 2 diabetes in the population of Eastern Uttar Pradesh, India. Considering the functional importance of the gene, we have performed a case-control association study with 240 T2D cases and 229 healthy controls to provide an assessment of the risk association between KCNJ11 polymorphism (rs5219) and T2D in Indian population of Eastern Uttar Pradesh.

Material and Methods

Sample Collection

Samples were collected from Eastern Uttar Pradesh in this case-control study. Blood from diabetic patients and normal healthy controls (>35 years) was collected after informed consent according to the approved protocol by the Institutional Ethical Committee of Banaras Hindu University from the patients attending out-patient departments of Institute of Medical Sciences, Banaras Hindu University, Heritage Hospital and Prakash Pathology, Varanasi.

Screening of the study subjects

We have genotyped single nucleotide polymorphism of KCNJ11 gene E23K (rs5219) in 469 unrelated individuals from eastern Uttar Pradesh, India, including 240 type 2 diabetic patients and 229 ethnically matched control subjects. Subjects were diagnosed diabetic according to WHO criteria [48]. Subjects were included in the diabetes group if they had fasting glucose concentrations ≥ 126 mg/dl or 2-hour glucose concentrations ≥ 200 mg/dl after a 75 g Oral Glucose Tolerance Test (OGTT). Clinical history of diabetes and associated complications as well as the family history were recorded. Non-diabetic control subjects were chosen based on the absence of a history of diabetes in the subject and among first-degree relatives, as well as normal glucose tolerance, confirmed by a 75 g Oral Glucose Tolerance

Test (OGTT). To avoid interferences with biological variables, people with previous diagnosis of type 1 diabetes or those, receiving treatment for hypercholesterolaemia, hypertension or T2D, were excluded from the study. After screening with standard OGTT, age, gender and Body Mass Index (BMI) matched 229 normal healthy controls were enrolled from the population undergoing routine health check-up.

Anthropometric and biochemical evaluation

Anthropometric measurements including weight, height, and waist were obtained using standard protocol. The BMI was calculated as the weight in kilograms divided by the square of the height in meters. Clinical and biochemical data (Fasting Plasma Glucose (FPG) and 2 hour Postprandial Plasma Glucose (PPPG)) were obtained as part of our study protocol.

DNA analysis and genotyping

Blood sample (4-5 ml) was taken in 0.5 M EDTA (Sigma, USA) vials. Genomic DNA was extracted from peripheral blood leukocytes using the standard salting-out method.

KCNJ11 E23K (rs5219) - We have genotyped 240 diabetic subjects and 229 healthy controls. Polymorphic region of KCNJ11 E23K was PCR amplified using a – forward primer: F-5'GACTCTGCAGTGAGGCCCTA 3' and reverse R-5'ACGTTGCAGTTGCCTTTCTT 3'. The reaction was carried out in a total volume of 15µl, containing genomic DNA (50ng), 20pmol of each primer, 1X Taq polymerase buffer and 1U of Taq DNA polymerase (NEB, USA). PCR amplification was performed in Veriti 96 thermo cycler gradient (ABI, USA). The cycling conditions were 94°C for 5 minutes, followed by 32 cycles of 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds and final extension at 72°C for 7 minutes. The PCR products of 281 bp were digested with BanII for the E23K (rs5219) polymorphism. The resulting products were electrophoresed on a 3 % agarose gel. RFLP shows a 249 & 32 bp fragment for KK; 221, 32 & 28 bp for EE; 249, 221, 32 & 28 bp for EK.

Statistical Analysis

Clinical characteristics of the studied patients & controls were recorded. Data on quantitative characteristics are expressed as mean ± SD. Comparison of allele and genotype frequencies

between T2D patients and healthy controls were performed using 2x2 contingency table/2x3 contingency table respectively, with χ^2 analysis. P values of less than 0.05 were considered statistically significant. Odds Ratio (OR) at 95% Confidence Interval (CI) was determined to describe the strength of association. Models for the effect of the E23K variant were tested against the general model with separate effects of genotypes. A recessive and a dominant model for the effect of the E23K variant were examined.

Results

Clinical characteristics of the studied patients & controls are shown in Table 1. Type 2 diabetes patients studied for the gene had significantly higher levels of Fasting Plasma Glucose (FPG) and 2 hr PPPG ($P < 5 \times 10^{-2}$) than healthy controls. A comparison between diabetic patients and normal healthy controls showed significantly higher age in diabetics ($P < 0.003$). The Waist Circumference (WC) and BMI were also significantly different between diabetic patients and normal healthy controls ($P < 0.001$; $P < 0.015$, respectively) (Table 1). The genetic variants of loci show Hardy-Weinberg distributions in our study. The genotype and allele distributions of polymorphism are significantly different between the type 2 diabetes patients and healthy control groups (table 2; figure 1). Our data show weak association to T2D with OR 1.086 (95% CI: 0.832-1.416; $P = 0.544$) (Table 2). In the present study, the association of the E23K polymorphism with type 2 diabetes did not attain statistical significance may be because of smaller sample size. In this study, there is no significant association between the KCNJ11 codon E23K marker and glucose intolerance (Table 3). Then, we examined the association between the polymorphism and T2D risk using dominant and recessive genetic models. Although, the data showed weak association under both dominant (OR- 1.12, (95% CI) 0.768-1.626; $P = 0.563$) and recessive (OR- 1.15, (95% CI) 0.630-2.106; $P = 0.650$) models, but did not attain statistical significance (Table 4).

Table 1: Clinical characteristics of the study population for KCNJ11 E23K (rs5219) polymorphism

Variables	Cases	Control	P- value
Number	240	229	-
Male: Female	165: 75	153:76	-
Age (Yrs)	56.84±12.54	50.10 ±12.17	<0.003
BMI (kg/m ²)	25.53±5.85	20.15 ±3.67	<0.015
Waist Circumference (cm)	90.30±10.96	82.85 ±12.53	<0.001
FPG (mg/dL)	154.27 ± 42.83	82.26 ±8.87	<0.0001
2hrs PPPG (mg/dL)	229.46 ± 38.82	117.24±14.48	<0.0001

Mean±S.D.

Table 2: Genotype /Allele frequency distribution of variant KCNJ11 E23K (rs5219) polymorphism among normal healthy control subjects and type 2 diabetes patients and their Odds Ratio (OR)

Genotype/ Allele	T2DM frequency (%)	Control Frequency (%)	χ ²	P-value	OR	95% CI
KCNJ11 E23K (rs5219)	n = 240	n =229				
E/E	85 (0.35)	87 (0.38)				
E/K	130 (0.54)	121 (0.53)	0.230	0.631	1.100	0.746 – 1.620
K/K	25 (0.11)	21 (0.09)	0.353	0.553	1.218	0.638 – 2.326
E	300 (0.63)	295 (0.64)				
K	180 (0.37)	163 (0.36)	0.369	0.544	1.086	0.832 – 1.416

Figure 1: Histogram showing allele frequency and genotype frequency distribution between case and control group for KCNJ11 E23K (rs5219) polymorphism

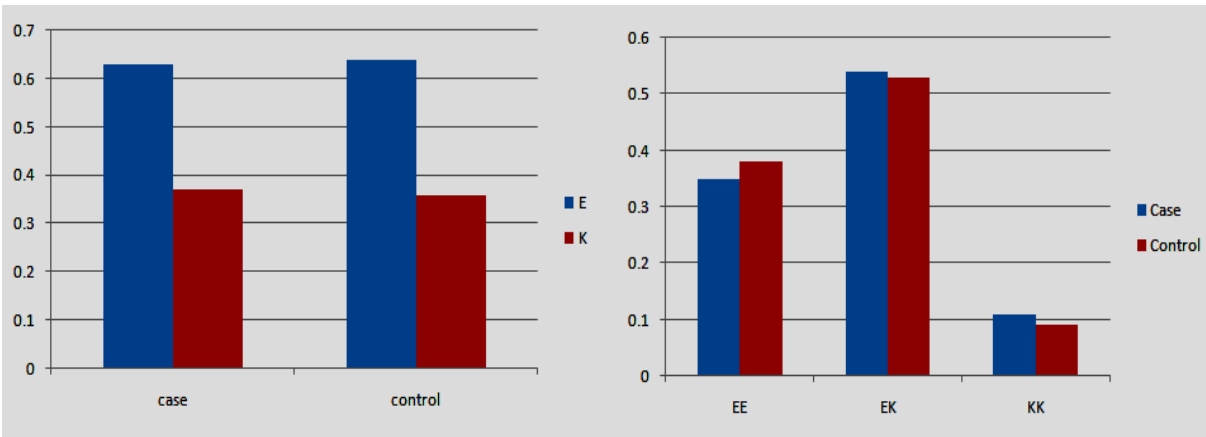


Table 3: Association of the **KCNJ11E23K (rs5219)** variant with glucose tolerance

Characteristics	Type 2 diabetes (n=240)			Control (n=229)		
	EE (n=85)	EK+KK (n=155)	P-value	EE (n=87)	EK+KK (n=142)	P-value
	(Non-risk group)	(Risk group)		(Non-risk group)	(Risk group)	
Fasting plasma glucose	136.33±38.52	143.65±54.11	0.556	88.48±12.68	86.34±10.36	0.614
2h postprandial Plasma glucose	195.68±54.78	218.06±84.79	0.254	133.13±20.22	125.16±20.54	0.136

Mean±S.D.

Table 4: Comparison of Dominant and Recessive model for association of KCNJ11 E23K (rs5219) polymorphism to T2D

Dominant model	Recessive model
EE vs EK+KK	KK vs EE+EK
OR (95% CI)	OR (95% CI)
1.12 (0.768 – 1.626)	1.15 (0.630 – 2.106)
P-value = 0.563	P-value = 0.650

Discussion

The KCNJ11 gene on chromosome 11p15.1 has attracted considerable attention as a promising candidate for T2D because of its function as a key factor in the regulation of glucose-induced insulin secretion. Although inconsistent results have been obtained in some earlier studies by Hani et al. [9] and Yamada et al. [49], but Gloyn et al. [11] and Love-Gregory et al. [12] confirmed the association of KCNJ11 rs5219 polymorphism and the susceptibility to T2D in Caucasian subjects. Later studies in Caucasians have shown that normoglycemic lysine carriers show consistently a defect in insulin secretion [13, 15, 44]. Functional studies suggested that the KK genotype might induce a critical inhibition of glucose-induced insulin release from pancreatic b-cells [16]. Furthermore, the KCNJ11 E23K variant was found to be associated with glucose intolerance and conversion from impaired glucose tolerance to T2D among Caucasians [42, 50]. Previous studies indicated that the E23K variant is functional by affecting in vitro properties of KATP channel via increasing the threshold of ATP concentration for insulin secretion [16, 51].

The results of several large population studies [11, 15, 52, 53] and meta-analyses [8, 11, 17, 49, 54-56] suggest that this genetic variant contributes to T2D risk in Caucasians. The population- attributable risk for clinical T2D in Caucasians is 6.2% for the KCNJ11 KK genotype alone and 10.1% for the KCNJ11 EK and KK genotype combined based on meta- analysis done by Yokoi et al. [56]. Chistiakov et al. [31] also found similar results for Russian population (P = 0.023). In a study by Asaf et al. [57] in Gaza population, it has been found that the inheritance of the K allele predisposes to T2D. Both case-control and meta-analyses results revealed significant association between the E23K variant of KCNJ11 and Type 2 diabetes among Tunisians and Arabs [58]. However, in Han people in Qingdao area no association was found between the E23K and T2D [59].

The comprehensive meta-analysis of KCNJ11 and T2D carried out by Qui et al. [46], which included a total of 48 studies suggest a modest but statistically significant effect of the 23K allele of rs5219 in susceptibility to T2D, particularly in East Asians and Caucasians with per allele OR of 1.13 (95% CI: 1.08-1.17, $P < 10^{-5}$) and OR of 1.12 (95% CI: 1.08-1.16, $P < 10^{-5}$) respectively. However, no such association was detected in Indian and other ethnic populations in all genetic models in stratified analysis for ethnic populations. Wang et al. [27] has reported influence of differences in ethnicity on predisposition to human diseases. South Asian populations differ in their ethnic background, and are quite different from East Asian populations. A systematic review and meta-analysis of KCNJ11 rs5219 gene polymorphism on all available studies from South Asian populations also showed no significant association to T2D. Also, susceptibility of this variant to T2D was compared to results with the meta-analysis of East Asian population and global population by Yang et al. [60] and Gong et al. [61]. The overall population analysis with South Asians, East Asians and global population combined showed a significant association of KCNJ11 polymorphism (rs5219) and T2D risk, but the positive result was not replicated in South Asian sub-group analysis.

In a case-control study performed in South Indian population, it was concluded that KCNJ11(rs5219) gene polymorphism is not an independent risk factor for T2D but in combination with rs1800467 exhibits a risk to the development of T2D in South Indians [47]. However, in North-Western Indian populations KCNJ11 (rs5219) gene polymorphism was reported to be significantly associated with T2D with an OR- 1.39 (95% CI: 1.26-1.54; $P = 6.7 \times 10^{-11}$) [62]. Therefore, in view of the genetic diversity of Indians (Indian Genome Variation Consortium, 2008) [63], the result needs to be replicated in other groups.

In our study, weak association was observed between KCNJ11 E23K (rs5219) polymorphism and type 2 diabetes (OR 1.086; 95% CI: 0.832-1.416; $P = 0.544$). Interestingly, our data for the SNP show small effect size than those reported in European and East Asian populations (OR- 1.14; OR- 1.13, respectively) [17-19] and North-Western Indian populations (OR- 1.39) [62]. However, in South Indian population KCNJ11 (rs5219) gene polymorphism is not associated to T2D. Out of four KCNJ11 variants studied (rs5219, rs5215, rs41282930,

rs1800467) only rs5215 variant showed statistically significant association with T2D susceptibility (OR=1.33, 95% CI=1.0–1.6, $P=0.005$) in South Indian population [47].

In the present study, no significant association was observed between the KCNJ11 codon E23K marker and glucose intolerance (Table 3). Only under recessive model, there is significant difference in 2 hr PPPG between homozygous risk genotype KK vs. homozygous non-risk genotype EE + heterozygous EK genotype in normal healthy control group. Also significant difference was observed with respect to WC and BMI in the normal healthy control group under recessive model (Table 5).

We also observed prevalence of the E/K genotype in both the normal healthy controls and type-2 diabetic patients (figure 1), which is in concordance with maximum studies done so far with respect to E23K (rs5219) polymorphism of KCNJ11 gene with T2D. The probable evolutionary significance of prevalence of heterozygous E/K genotype in both normal and diabetic groups has been very well discussed by Reidel et al. [64] as “The evolutionary benefits of each homozygous genotype - the K/K *thrifty-utility* vs. the E/E *thrifty storage* gene - may be balanced in heterozygotes, contributing to the high prevalence of the E/K genotype in both the general and type-2 diabetic population [9, 11, 13] and, in present times, conferring only a slight increase in risk for the development of type-2 diabetes [12, 16]”.

Conclusion

Our data for the SNP variant E23K (rs5219) of KCNJ11 show smaller effect size (OR-1.08) than those reported in European and East Asian populations (OR- 1.14; OR- 1.13, respectively) and North-Western Indian populations (OR-1.39). Further studies in larger study population are required to confirm the association as the present study did not attain statistical significance. The prevalence of more of the heterozygous genotype E/K in both type 2 diabetic patients as well as in the normal healthy controls in our population is in concordance with all other studies and points towards the evolutionary significance of balanced polymorphism in the population as discussed by Reidel et al. [64]. To our knowledge this is the first report in this population and provides valuable information for comparison with other ethnic groups as well as in determining disease susceptibility in this population.

Table 5: Association of different genetic model with Clinical characteristics in T2D patient and Control group according to E23K KCNJ11 genotype

Mean±S.D

Model	Characteristics	Cases			Controls		
		KK+EK (n=155)	EE (n=85)	P-value	KK+EK (n=142)	EE (n=87)	P-value
Dominant	BMI (kg/m²)	21.98±3.81	24.10±10.60	0.348	23.73±5.36	24.77±5.04	0.438
	WC (cm)	81.17±12.81	85.00±10.31	0.209	85.95±12.91	86.47±10.97	0.862
	FPG (mg/dl)	143.88±52.46	136.33±38.54	0.556	86.86±11.75	88.47±12.68	0.614
	PPPG (mg/dl)	218.94±86.13	195.68±54.78	0.254	125.16±20.54	133.13±20.22	0.136
Recessive		EK+EE (n=215)	KK (n=25)	P-value	EK+EE (n=208)	KK (n=21)	P-value
	BMI (kg/m²)	23.09±7.84	21.32±3.88	0.350	24.57±5.24	20.99±4.29	0.043
	WC (cm)	83.25±12.26	79.71±8.45	0.357	87.37±11.90	78.11±11.60	0.048
	FPG (mg/dl)	141.71±49.18	133.85±27.31	0.426	88.10±11.87	83.00±12.71	0.234
	PPPG ((mg/dl)	212.32±78.01	192.14±54.88	0.245	129.37±21.29	117.51±10.99	0.024

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