Association of KCNJ11 genetic variations with risk of Type 2 diabetes mellitus (T2DM) in North Indian population

Vasiuddin Khan[a*], Deepti Bhatt[a*], Shahbaz Khan[a*], Amit Kumar Verma[a], Rameez Hasan[a], Sahar Rafat[a], Yamini Goyal[a], Prahlad Singh Bharti[b], M.Y. Shareef[c], Mohammed A. Alsahli[d], Arshad Husain Rahmani[d], Kapil Dev[a#]

a-Department of Biotechnology, Jamia Millia Islamia, New Delhi

b-Department of Biophysics, All Indian Institute of Medical Sciences, New Delhi

c-Faculty of Dentistry, Jamia Millia Islamia, New Delhi

d-Department of Medical Laboratories, College of Applied Medical Sciences, Qassim University, Buraydah, Saudi Arabia

* - equal contribution

- corresponding author

ABSTRACT

Type 2 diabetes mellitus (T2DM) is a polygenic metabolic disease described by hyperglycemia, which is caused by insulin resistance or reduced insulin secretion. Interaction between various genetic variants and environmental factors triggers T2DM. The main aim of this study was to find the risk associated with genetic variant (rs5210) of KCNJ11gene in the development of T2D in Indian Population**.** A total number of 300 cases of T2D and 100 control samples were studied to find the polymorphism in KCNJ11 through PCR-RFLP. The genotype and allele frequencies in T2DM cases were significantly different from the control population**.** We found significant association of KCNJ11 (rs5210) gene polymorphism with T2DM in North Indian patients indicating role of this variant in developing risk for T2DM.

Keywords: Type 2 Diabetes; KCNJ11; RFLP; SNP

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1. Introduction

Type 2 Diabetes is a chronic metabolic disorder described by reduced insulin secretion, insulin action and elevated blood glucose. Combination of both environmental and genetic factors play prominent role in the pathogenesis of T2DM. Environmental factors include obesity, diet, physical inactivity, age, hypertension, abnormal cholesterol levels and smoking [1].

As per International Diabetes Federation's (IDF) Diabetes Atlas, 8th edition, it has been estimated that number of diabetes patients are expected to increase from 425 million in 2017 to 629 million by 2045. India is home to 74 million people with diabetes mellitus with approximately 8.7% prevalence among adult population and considered as the "diabetes capital" of the world (IDF, 8th edition).

Genome–wide association studies (GWAS) found various susceptibility genes for diabetes [2-4]. However, the mechanisms these genes follow for the development of diabetes is still not clear. Many gene polymorphism findings demonstrated the association of various SNPs in the pathogenesis of T2DM and in different population, each SNPs may exhibit dissimilar association with T2DM. Therefore, there is a necessity to discover various genetic risk markers/ factor of T2DM for its prevention and treatment. It has been stated that Indians may have different genetic predisposition to diabetes than Europeans [5].

In human, significance of KCNJ11 in insulin secretion was suggested by its function in permanent neonatal diabetes [6] and familial persistent hyperinsulinemic hypoglycaemia of infancy [7]. Insulin resistance and reduced insulin secretion together leads to T2DM development. Due to the position and function of KCNJ11 gene in regulating glucose-stimulated insulin secretion, KCNJ11 has gained significant attention as a potential candidate for T2D susceptibility. KCNJ11 gene located at 11p15.1 on human chromosome and lacks

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intronic sequences, this gene is a member of the potassium channel gene family [8]. KCNJ11 gene encodes Kir6.2, an inward-rectifier potassium ion channel, which is 390 amino acid protein with intracellular N- and C-terminals and two transmembrane domains M1 and M2. Kir6.2 protein forms KATP channel with sulfonylurea receptor 1 (SUR1).This KATP channel, through glucose metabolism controls insulin secretion and production [9]. In this study, we evaluated the association of KCNJ11 gene variant (rs5210) with T2DM risk in North Indian patients.

2. Results

2.1 Distribution of genotypes in cases and control with KCNJ11 (A>G) genotypes.

We determined differences in distribution of genotype between cases and controls. From the total 300 cases, homozygous wild type (AA) was 120(40%); heterozygous type (AG) was 136(45.3%) and homozygous mutant type (GG) was 44(14.6%), whereas from the total 100 control samples, 58(58%) were wild (AA); 32(32%) were heterozygous and 10(10%) were mutant as presented in Table 1. We observed significant difference in the dominant genotype for cases vs control (AG+GG vs AA Odd ratio=2.07 [95% CI 1.30-3.27] p value-0.0002). These findings suggests an association between the rs5210 variant and T2DM risk in the overall analysis (AG vs. AA: OR = 2.05 [95% CI: 1.25–3.37], p = 0.0006).

Table 1: Distribution of KCNJ11 genotype among cases and control

Genotype	Number of Cases (n-300)	Number of Control (n-100)	Odd Ratio (95%CI)	P value
AA	120 (40%)	58 (58%)	Ref	Ref
AG	136 (45.3%)	32 (32%)	2.05(1.25-3.37)	0.0006*

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GG	44 (14.6%)	10 (10%)	2.12(0.99-4.5)	0.016*
Carrier(AG+GG)	180 (60%)	42 (42%)	2.07(1.30-3.27)	0.0002*

*significant at p<0.05

2.2 Genotype distribution and Allele frequencies of KCNJ11 gene among T2D cases and controls

Allele frequencies were found to be 0.63 and 0.74 for allele A in cases and control respectively and for allele G, it was 0.37 and 0.26 for cases and control respectively. The genotype and allele frequencies were significantly different from the control population .Chi square p-value obtained was 0.007 at p<0.05 significant; Odd Ratio was 2.12 with 95% CI in the range of 0.99-4.52 as depicted in table 2.

Table 2: KCNJ11 polymorphism in T2D cases and controls

Population	**No of subjects**	**No of individuals (%) Genotypic frequency**			**Allelic frequency**		**Chi square test P- value**
		(AA)	(AG)	(GG)	A	G	
Type 2 Diabetes cases	300	120 (40%) 0.40	136 (45.3%) 0.45	44 (14.6%) 0.15	0.63	0.37	0.007*
Controls	100	58 (58%) 0.58	32 (32%) 0.32	10 (10%) 0.10	0.74	0.26	
OR	2.12						
95%CI	0.99-4.52						

*significant at p<0.05

2.3 Comparative analysis of clinicopathological characteristics in T2D cases and control

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Various clinicopathological characteristics were compared between cases and control by using student t -test. Significant difference was found in the mean values of Age, BMI, PPG, FPG, HbA1c, T-Cholesterol, Systolic BP, Diastolic BP and Triglycerides between cases and control, whereas no significant difference was found in HDL as depicted in table 3.

Table 3: Comparative analysis of clinicopathological characteristics in Type 2 Diabetes cases and control

Characteristic	T2DM (n=300)	Control (n=100)	t-test	
			t	Sig. (2-tailed)
				P Value
Age(Years)	40.33±9.76	35.29±7.96	4.67	< .0001*
BMI(kg/m^2)	28.8±5.22	24.83±2.33	7.36	< .0001*
PPG(mg/dl)	208.5±48.49	135±13.02	14.96	< .0001*
FPG(mg/dl)	140±35.64	90.22±7.11	13.86	< .0001*
FPI	9.6±1.35	8.66±0.71	6.65	<.0001*
HbA1c	7.12±1.03	5.75±0.54	12.72	< .0001*
T-Cholesterol(mg/dl)	245.58±15.14	152.63±18.82	49.89	< .0001*
Systolic BP(mmHg)	146.79±17.05	106.07±10.39	22.51	< .0001*
Diastolic BP (mmHg)	102.87±16.19	75.85±10.91	15.55	< .0001*
Triglycerides(mg/dl)	356.32±100.48	140.98±5.52	21.4	< .0001*

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HDL-C (mg/dl)	45.89±11.47	46.21±8.7	0.25	0.80053
LDL-C (mg/dl)	194.60±27.38	106.42±19.92	29.68	< .0001*

Data presented as mean±standard deviation,*significant at p<0.05

2.4 Observed and expected genotype of KCNJ11 polymorphism in control.

Observed and expected genotype frequencies of KCNJ11 gene polymorphism in controls showed no deviation from Hardy-Weinberg equilibrium expectations. Chi square test demonstrated that there was no significant deviation Hardy-Weinberg equilibrium for KCNJ11 SNP genotypes (P>0.05) as shown in table 4.

T**able 4:** Observed and expected genotype of KCNJ11 polymorphism in control.

Genotype	**Observed Genotype**	**Expected Genotype**	**P-Value**	**Chi Square(X^2)**
AA	58	54.76	0.24222	2.836
AG	32	38.48		
GG	10	6.76		

2.5 Clinicopathological characteristic of Type 2 Diabetes patients among wild and carrier alleles of KCNJ11 gene

We analysed the Clinicopathological data with carrier (AG+GG) and found out that there is no statistically significant association between the carrier allele with age (p-value-0.138), BMI (p-value-0.771), PPG (p-value-0.051), HB1Ac (p-value-0.622), Cholesterol (p-value-0.081), Systolic BP (p-value-0.215), Diastolic BP (p-value-0.170) , HDL (p-value-0.525) and

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Triglyceride (p-value-0.422). Positive correlation was observed between FPG (p-value-0.002), FPI (p-value-0.012) and LDL (p-value-0.034) with Carrier allele (AG+GG) as shown in table 5 (the result is significant at P<0.05).

Table 5: Clinicopathological characteristic of Type 2 Diabetes patients

Characteristic		**Total**	**AA**	**Carrier**	**P Value**
Age	≤45	181	76	105	0.138
	>45	119	44	75	
BMI	≤25	86	34	52	0.771
	>25	214	86	128	
FPG	≤140	177	83	94	0.002*
	>140	123	37	86	
PPG	≤208	170	76	94	0.051
	>208	130	44	86	
HBA 1C	≤7	190	74	116	0.622
	>7	110	46	64	
Systolic BP	≤147	128	46	82	0.215
	>147	172	74	98	
Diastolic BP	≤103	206	77	129	0.170
	>103	94	43	51	
Cholesterol	≤245	190	83	107	0.081
	>245	110	37	73	

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FPI	≤9.6	176	60	116	0.012*
	>9.6	124	60	64	
Triglyceride	≤355	154	65	89	0.422
	>355	146	55	91	
HDL-C	≤46	189	73	116	0.525
	>46	111	47	64	
LDL-C	≤195	93	43	50	0.034*
	>195	207	65	130	

*significant association at $p<0.05$

3. Discussion

In the present case-control study, we investigated association of SNP (rs5210) within the KCNJ11 gene with the susceptibility to T2D in the North Indian origin patients. KCNJ11 polymorphism association with T2D risk has been extensively studied in European population however; their relationship in Indian subcontinent is yet to be validated.

In the present study, we observed a significant difference in the distribution of KCNJ11 genotypes among T2DM cases and healthy controls, higher mutant allele distribution was observed among cases as compared to healthy controls. Our findings indicated an association between KCNJ11 (rs5210) gene polymorphism with T2DM in North Indian patients. We also compared means of clinicopathological characteristics between cases and control and observed significant difference in all the parameters except HDL. In our study Clinicopathological data with carrier allele (AG+GG) was also analysed and found out that there is no statistically significant association between the carrier allele with age, BMI , PPG , HB1Ac , HDL, Systolic BP, Diastolic BP, Cholesterol and Triglyceride, whereas positive correlation was observed between FPG, LDL, FPI with Carrier allele.

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Our findings was found to be similar with other studies which confirmed the association of KCNJ11 variant 3p +215(rs5210) with T2D [10, 11]. Another meta-analysis study also demonstrated significant association of variants of KCNJ11 such as rs5219, rs5210, and rs5215 with T2D [12]. Similarly, meta- analysis in East Asian population, genotypic and allelic contrast also suggested significant association of KCNJ11 and T2D for rs5210 [13].Furthermore, rs5210 variant of KCNJ11 gene was indicated to be related with T2DM risk in meta-analysis of 5 studies and it was significantly heterogeneous (p = 0.02)[12]. Similar results were also observed in Mexican, Finnish and Korean population [14-16]. A study conducted in South Indian population of Hyderabad suggested an association between KCNJ11 polymorphism in T2DM susceptibility [17], which was consistent with our findings.

Our results were found to be inconsistent with other studies in which no association was found between KCNJ11 E23K polymorphism and T2D in Iranian population [18] , Czech population [19] and Moroccan population [20]. Similarly, another study also suggested that genetic variant of KCNJ11 did not show any significant association with T2DM risk in Mongolian population (OR=1.07; P=0.645) [21].

Genetic determinates will enable us to find the risk prediction of T2DM development and its pathogenesis and application of individualised treatment for therapy.

Materials and Methods

4.1 Ethical approval and study subjects

Collection of clinical data in pretext Performa and sample collection of the diabetic subjects/patients and control/non- diabetic of North Indian origin from Ansari Health Center Jamia Millia Islamia New Delhi was done with due consent and after getting Ethical clearance from the Institute Ethical Committee, Jamia Millia Islamia, New Delhi (Proposal No. 17/9/14/JMI/IEC/2015 dated 14/01/2016). The present cohort study was conducted in

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Department of Biotechnology, Jamia Millia Islamia, New Delhi, a total of 400 samples (300 cases and 100 controls) were collected, which fulfill all the relevant selection criteria.

4.2 Single Nucleotide Polymorphism (SNP) Genotyping by PCR-RFLP

Genomic DNA was extracted from fresh blood using phenol chloroform method. SNP (single nucleotide polymorphism) was detected with the use of primers i.e. forward 5'-ATCCAGGGTGTTACAAGGCA3' and reverse 5'-TTTCAGGGACCAAGTAGAGCTG-3'. These primers were used to exponentially amplify the316 bp of DNA fragment. PCR was performed and the conditions were as follows: Initial denaturation of 95^0C for 5 minutes, followed by 35 cycles of 95^0C for 30sec, 60^0C for 30sec, 72^0C for 30sec and final extension of 72^0C for 5 min in PeqSTAR 96 universal gradient thermocycler (Peqlab,VWR).Amplified products were then electrophoresed on 2% agarose gel and images were then captured by gel documentation system (Biorad) as shown in Fig.1

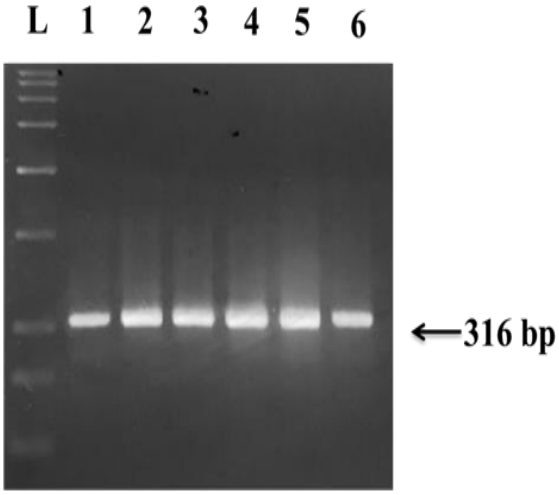

Figure 1: 2% agarose gel electrophoresis for KCNJ11 PCR product.

L-100bp DNA ladder
1-6: PCR Product of 316 bp

KCNJ11 polymorphism(6956A>G) was identified by using 6 ul PCR product with 2.5units of restriction enzyme Hpy188III (NEB) incubated for overnight at 37^0C. Hpy188III recognises

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the sequence –TCG; digesting the PCR product to yield 218bp and 98bp DNA fragments. The wild type allele (A/A) yielded 1 band of 316 bp; Heterozygous (A/G) yielded 3 bands of 316bp, 218bp and 98bp and mutant allele (G/G) yielded 2 bands of 218bp and 98bp. Digested PCR products subjected to electrophoresis in a 3% agarose gel as shown in Fig.2.

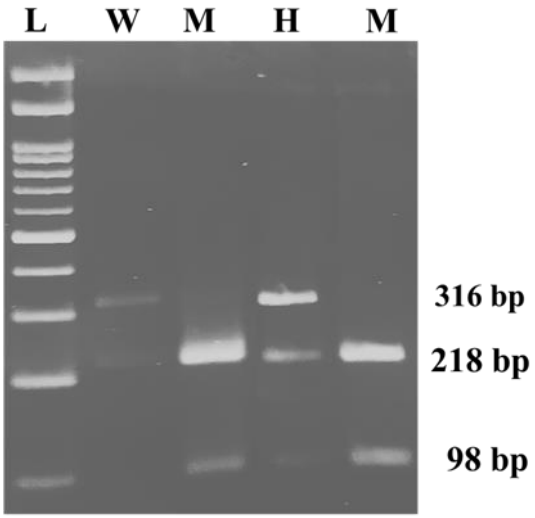

Figure 2: 3% Agarose gel electrophoresis for digested PCR product.

L-100bp DNA ladder
M- Homo Mutant (G/G)
H- Hetero (A/G)
W-Homo Wild (A/A)

4.3 Statistical Analysis

We used the SPSS Software (21.0 version, IBM, United States). Chi square test was applied for comparing genotype and allele frequencies for statistical significance between diabetic patients and controls. The Hardy–Weinberg equilibrium (HWE) was measured using the χ^2 test for goodness of fit. Comparative analysis of clinicopathologic characteristics between cases and control was performed by student t-test for equality of means. Level of significance was set at 95% (i.e. $p < 0.05$).

5. Conclusion

We identified significant association of KCNJ11 (rs5210) gene polymorphism with T2DM risk in North Indian patients suggesting the role of this variant in increased risk of developing T2D in North Indian population. However, a large cohort study is required to validate the expression of these genes in diverse populated country like India.

Author Contributions Conceptualization, MYS and KD; Data curation, VK, DB and SK; Formal analysis, VK, AKV, SR and YG; Methodology, VK and SK; Software, DB; Supervision, KD; Validation, RH; Visualization, MYS; Writing – original draft, VK, DB and SK; Writing – review & editing, PSB, MAA, AHR and KD.

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Conflict of Interest: The authors declare no conflict of interest

Ethical approval & consent to participate

Institutional Ethics Committee of Jamia Millia Islamia (Proposal No. 17/9/14/JMI/IEC/2015 dated 14/01/2016) approved this study. A written informed consent was obtained before inclusion in the study.

Abbreviations

IDF: International Diabetes Federation
KCNJ11: Potassium inwardly-rectifying channel, subfamily J, member 11
GWAS : Genome–wide association studies
SUR1: Sulfonylurea receptor 1
KATP : ATP- sensitive K channel
OR: Odds ratio
CI: Confidence interval

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HWE - Hardy-Weinberg equilibrium

SNP: Single nucleotide polymorphism

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