Clinical Case Reports International

Research Article
Published: 07 Jun, 2023

Association Study of *KCNJ11* and *TCF7L2* Genetic Variants with Type 2 Diabetes Mellitus in Southern Punjab

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Abstract

Diabetes is the most chronic disease that is caused due to genetic and environmental factors. The common genetic factor that is associated with T2D is single nucleotide polymorphism. The aim of the present study was to assess the association between rs5219 of the *KCNJ11* gene and rs11196205 of the *TCF7L2* gene with diabetes type 2. Total of 300 samples (150 T2DM patients and 150 non-diabetic controls) together with demographic factors were taken from public and private hospitals of the Southern Punjab. This was a case-control study and contained 150 T2D patients and 150 controlled samples. To analyze the polymorphisms in *KCNJ11* and *TCF7L2*, PCR-RFLP and Tetra-arm PCR were used. The findings of this study were that in *KCNJ11* gene, homozygous AA genotype was predominant (83.33%) than the GG genotype (14%) and heterozygous genotype GA was present in very a small amount (2.66%) while in case of *TCF7L2* gene, CC 28.00%, GG 27.33% and GC 44.67%. The insignificant statistical association was found between rs5219, rs11196205 and T2D as p-value of *KCNJ11* (rs5219) is 0.4125 and *TCF7L2* (rs11196205) is 0.6. The conclusion of this study is that rs5219 of the *KCNJ11* gene and rs11196205 of *TCF7L2* gene are not associated with diabetes type 2 in the Southern Punjab population.

Keywords: *KCNJ11*; *TCF7L2*; PCR-RFLP; Tetra-arm PCR; Insignificant

Introduction

Type 2 Diabetes Mellitus is a chronic multifactorial disorder described by high blood glucose level (hyperglycemia), which is due to the resistance of insulin or lower insulin secretion [1]. Both genetic and environmental factors play a significant role in the development of type 2 diabetes mellitus. Environmental factors are age, obesity, lack of physical activity, hypertension, diet, and smoking [2]. International Diabetes Federation reported that in 2017, the number of patients with diabetes was 425 million and this ratio will increase to 629 million in 2045 [3]. Pakistan is South Asia Country and diabetes and obesity is more common in South Asia. 12,000 deaths are noted every year in Pakistan due to diabetes. Different studies revealed that people in urban areas are more affected by this disease due to lack of physical activity, hypertension, and intake of high-calorie food [4]. For diabetes, Genome-Wide Association Study (GWAS) identified different susceptibility genes. Genetic factor is more involved in the pathogenesis of type 2 diabetes mellitus because of Single Nucleotide Polymorphism (SNP) [5]. Different studies found many genes that are associated with type 2 diabetes mellitus. Among these genes, the potassium voltage-gated channel subfamily J member 11 (*KCNJ11*) gene and *TCF7L2* gene are the main candidate genes for the pathogenesis of type 2 diabetes mellitus. *KCNJ11* gene is situated on the human chromosome at 11p15.1 and consists of one exon with no intronic sequences [6]. Inward rectifier potassium ion channel (Kir6.2) protein is encoded by this gene. *KCNJ11* gene plays a significant role in the secretion of insulin. Kir6.2 protein with Sulfonylurea Receptor 1 (SUR1), forms K_{ATP} channel [7]. In the K_{ATP} channel, when these two proteins are combined then it mediates the secretion of insulin [8]. When the level of glucose increases then it results in the closing of the K_{ATP} channel and activation of voltage-gated calcium channels. The Channel of K_{ATP} allows the potassium ions entry. The Intracellular level of calcium ion increases when the cell membrane becomes depolarized due to the increased amount of potassium ions and result in insulin secretion [9]. Insulin production and its secretion are controlled through glucose metabolism by the K_{ATP} channel. Many *KCNJ11* gene SNPs have been identified and among these SNPs, rs5219 has been gaining more consideration for its association with type 2 diabetes mellitus [10]. *KCNJ11* gene polymorphism rs5219 is caused when at codon 23, glutamic acid is substituted into lysine amino acid [11]. In this variation, guanine is changed into adenine. The Sensitivity of the potassium channel is reduced due to this variation and this substitution caused

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Received Date: *22 May 2023*
Accepted Date: *02 Jun 2023*
Published Date: *07 Jun 2023*

Citation:
Shaheen S, Aslam S, Shaheen M, Iqbal RK, Awan A, Jamil H. Association Study of KCNJ11 and TCF7L2 Genetic Variants with Type 2 Diabetes Mellitus in Southern Punjab. Clin Case Rep Int. 2023; 7: 1555.

insulin resistance and leads to diabetes mellitus [12].

The genes which are elaborate in the cell multiplication and distinctness are regulated by the Transcription Factor 7-like 2 genes (*TCF7L2*) [13]. This gene contains the High Mobility Group (HMG) box and also elaborates in the Wnt signaling channel that plays the principal role in galactose stability and this gene is the master regulator of the proinsulin a nuclear receptor for B-catenin is encoded by this gene that involved in lipid metabolism. Variants of *TCF7L2* gene increased the risk of T2DM with an impaired insulin secretion showed a strong association [14]. Wnt wave channel is essential during normal functioning and this function is done by the same receptors in which Frizzled (Fz) and LDL receptor-related proteins are included [15]. β-catenin is also important for the activation of many genes when it binds to the TCF receptor. In which Glucagon-Like-Peptide-1 (GLP-1) is also included in this way the beta-catenin level is increased and also increased the growth of beta cells. When the mutation in the *TCF7L2* gene impaired the secretion glucagon-like peptide-1 due to which production of insulin is low and beta-catenin level also reduced, also disturb the beta-cell proliferation [16].

Objectives of this study was to determine the SNP association with body mass index, fasting blood glucose, random blood glucose in controlled and cases subjects. The aim of this study was to determine the correlation between rs5219 of *KCNJ11* gene and rs11196205 of *TCF7L2* with type 2 diabetes mellitus in the population of Southern Punjab, if any.

Material and Method

Study participants

A total of 300 were selected for the analysis including 150 cases and 150 controlled subjects from government and private hospitals of the Southern Punjab.

Data collection

To study the epidemiological factors such as age, gender, body mass index, smoking, and personal medical history, questionnaire was conducted for this purpose.

Anthropometric and biochemical evaluation

By using a standard protocol, anthropometric measurements such as height, weight, and waist were acquired. The calculation of BMI was done by dividing the body weight in kilograms by square of height in meters. A routine investigation of patients such as fasting and random blood glucose level, HbA1c was measured.

DNA extraction

In EDTA tubes, Sample of blood (3ml to 5 ml) from each participant was preserved and kept at -4°C for storage. Inorganic DNA extraction method was used for DNA extraction and DNA was collected from the collected samples of blood and stored at -20°C.

Genotyping *KCNJ11* E23K Variant

For amplification of *KCNJ11* E23K polymorphic region, PCR-RFLP method was used for this purpose, Oligonucleotide primers sequences 5'GACTCTGCAGTGAGGCCCTA 3' and 5'ACGTTGCAGTTGCCTTTCTT 3' were used. Master Mix of 25 ul was prepared that consist of 3 µl DNA, 2 µl of 25 mM $MgCl_2$, 2 µl dNTPs, 12 pmol of each primer, 1 µl Taq polymerase and 5 µl Buffer. The amplification started with a preliminary denaturing step at 94°C for 5 min along with 28 cycles of 94°C for 30 s, annealing at 59°C for 30 s, and ultimate extension at 72°C for 30 s with a final elongation step at 72°C for 5 min. 209 bp PCR product was analyzed on 1.8% agarose gel electrophoresis. PCR product was restricted by *Ban* II enzyme. Restriction was carried out in volume of 20 ul. 8 ul PCR product, 1 ul *Ban* II enzyme, 5 ul tango buffer and 6 ul distilled water. Keep it at 37 for °C sixteen hours. By electrophoresis on 3% agarose gel, restriction products were separated and presence of bands was shown under the fluorescence of UV light. 100 bp ladder was used to know the exact position of particular band size which differentiated the genotypes of *KCNJ11* i.e., for 150, 59 bp (GG), 150, 59, 209 bp (GA) and 209 bp (AA).

Genotyping of *TCF7L2* rs11196205 variants

The genotyping of rs11196205 (C/G) was done by the Tetra-ARMS-PCR (Tetra Primer Amplification Polymerase Chain Reactions) for the detection of single nucleotide polymorphism in *TCF7L2* gene. The primer that was designed and their product size for rs11196205 in *TCF7L2* gene is following and mention in this table (Table 1).

Master Mix of 25 ul was prepared that consist of 3 µl DNA, 2 µl of 25 mM $MgCl_2$, 2 µl dNTPs, 12 pmol of each primer, 1 µl Taq polymerase and 5 µl Buffer. The amplification started with a denaturation followed by 30 cycles in which initial denaturation at 95°C for 6 min, final denaturation at 95°C, annealing at 64°C for 45s, and elongation at 72°C for 45s and a final elongation step at 72°C for 6 min. PCR product was analyzed on 2% agarose gel electrophoresis and presence of bands was shown under the fluorescence of UV light. 100 bp ladder was used to know the exact position of particular band size which differentiated the genotypes of *TCF7L2* i.e., for 235 (GG), 253 (CC) and 434 (GC).

Statistical analysis

Minitab (version 18) software was used for analyzing the results. The significance level was considered at $P<0.05$. The correlation between DM and all demographical variables was studied by using binary logistic regression followed by logistic regression analysis which was carried out to find the association between DM and selected biochemical factors. A Chi-square test was performed for determining the risk factors frequency between control and diabetic patients. Also, the frequency of genotypes was determined by direct counting whereas, the frequency of alleles was found out with the help of Hardy Weinberg equilibrium calculator and to find the correlation between diabetes and rs5219 (SNP) of the *KCNJ11* Chi-square test was applied.

Table 1: Primers and the product size of *TCF7L2* genotypes.

S.NO	SNP	Primer Type	Sequence	Amplification Size	Product Size
1	rs11196205	Inner Forward	CTGAAAGTTCTCAACATTTATAACTGCC	434bp	**C:**253bp **G:**235bp
		Inner Reverse	CAACCATAACTCTCTTACATACTGGTC		
		Outer Forward	TAGATTGTCTCCTTTTGTTTCTGCTAC		
		Outer Reverse	TAAACATCTGACCTTGAAGCCTACC		

Results

Genotyping of *KCNJ11* (rs5219)

KCNJ11 PCR amplification was carried out and produced a band size of 209 bp as shown in Figure 1.

The 209 bp band was digested with ban 2 enzyme and gave different genotypes pattern such as GG: 59 bp and 150 bp, AA: 209 bp, GA: 59 bp, 150 bp and 209 bp (Figure 2).

Tetra-ARM-PCR for *TCF7L2* analysis

The Tetra-ARM-PCR for*TCF7L2* (rs11196205 C/G) that give the amplification of following products size in which 434 bp band as a control, 253 bp C wild type allele and 235 for the G mutant allele (Figure 3).

Association of various demographic and biochemical factors with diabetes

Through binary logistic regression, different demographic factors were contrasted between cases who were suffering from diabetes and healthy individual. It was noted that family history that has p-value =0.000 and Planned diet that has p-value =0.001 were more significant between patients and control while smoking showed the significant relation between normal healthy individual and patients and has a p-value 0.005. The statistical relation was insignificant between control and patients about age, exercise, treatment regularity, gender, and marital status because they have a p-value greater than 0.05.

Through logistic regression testing, various biochemical parameters were contrasted between T2D patients and healthy individuals. It was noted that all biochemical parameters were not associated with the incidence of developing T2D because all biochemical parameters have a p-value greater than 0.05 that denotes a non-significant association.

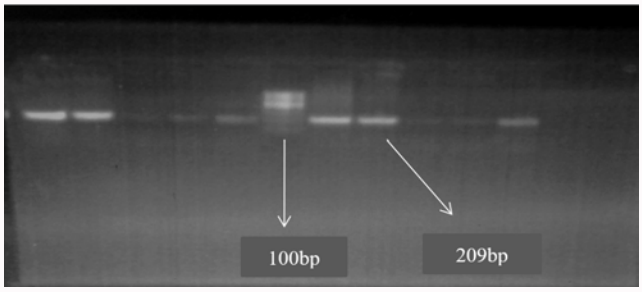

Figure 1: The figure indicating the PCR product gel of KCNJ11 before restriction. Lane 1-5 and 7-11 show the PCR product size of 209bp and lane 6 indicating the 100bp Ladder.

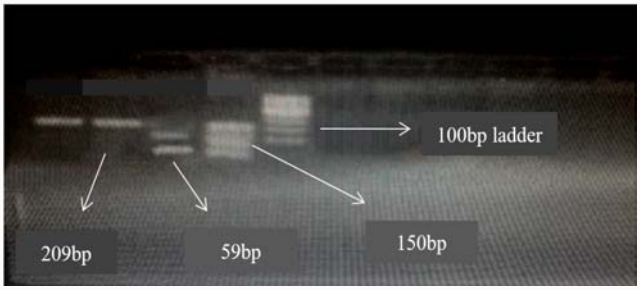

Figure 2: The figure of gel representing the *KCNJ11* gene genotypes. PCR product (209bp) of the *KCNJ11* gene (rs5219) digested with restriction enzyme Ban II. Lane 1 and 2 is mutant homozygote (AA), lane 3 is normal homozygote (GG), lane 4 is heterozygote (GA) and lane 5 is 100bp ladder.

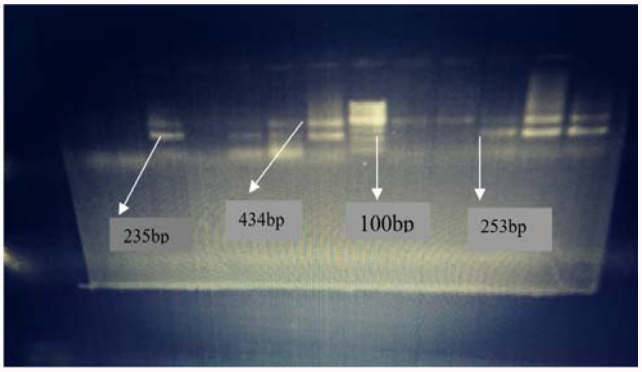

Figure 3: Product size of *TCF7L2* genotypes. The image of the Tetra-ARM-PCR for *TCF7L2* (rs11196205 C/G) represents the genotype, in this 100 bp ladder is in lane 6 and lanes 7,8 indicating the homozygous genotype and 1,3,4,5,9,10,11 indicating the heterozygous genotype.

Through binary logistic regression testing, different anthropometric measurements were contrasted between T2D patients and Normal people. It was noted that the waist and body mass index were highly significant linked with diabetes because both variables have a p-value greater 0.000 while weight was significantly associated with diabetes. The height is a non-significant parameter that was not associated with diabetes.

Association of rs5219 *KCNJ11* genotypes with the demographic, biochemical and anthropometric parameters associated with T2DM

Through the chi-square test, when different genotypes of the *KCNJ11* gene were compared with various demographic, biochemical and anthropometric parameters then it was noted that all demographic and biochemical factors showed no association between the polymorphism of *KCNJ11* and diabetes while BM1 is a highly significant parameter and was associated with *KCNJ11* polymorphism while weight, height, and waist, were non-significant factors that were not linked with genotypes of *KCNJ11*.

Association of rs11196205 *TCF7L2* genotypes with the demographic, clinical and biochemical parameters associated with T2D

From the test of chi-square when the different genotypes were contrasting with these risk factors that linked with DM. it was seen that age, genus, smoking, inheritance detail, exercise, planned diet, and marital information was the non-significant factors and are not linked with the one or more genotype (Table 2). From the test of chi-square when the different genotypes were contrasting with these clinical factors linked with DM. it was seen that BMI, waist, fasting glycogen level, diastolic plasma pressures, systolic plasma pressures, HbA1c and, random plasma glycogen level was the non-significant factors and are not linked with the one or more genotypes.

Association of genotypic and allelic frequency of rs5219 in *KCNJ11* and rs11196205 in *TCF7L2* with T2DM

The *KCNJ11* genotype of patients and control showed insignificant results as the p-value is 0.415. The allelic frequency of A was 0.886 in control and 0.846 in patients. Hardy-Weinberg equilibrium was used to determine the allelic frequency in control and cases. In TCFL2 gene, the p-value was the 0.62 that showed the non-significant linkage with the DM. The outcomes of genotypic distribution of the *KCNJ11*

Table 2: Association of demographic and biochemical parameters with type 2 diabetes.

Demographic Parameters	Category	Cases	Control	Odd Ratio (95% CI)	P-value
Age	31-40	23	30	1.2779 (0.9333, 1.7497)	0.123^{ns}
	41-50	47	50		
	1-60	43	35		
	61-70	35	34		
	Above 70	2	1		
Gender	Male=1	71	83	0.6907 (0.3250, 1.4676)	0.334^{ns}
	Female=0	79	6		
Smoking	Single=0	34	16	3.8539 (1.4878, 9.9833)	0.005**
	Married=1	116	134		
Family History	Yes=1	88	16	9.6218 (4.5386, 20.3981)	0.000***
	No=0	62	134		
Exercise	Yes=1	84	109	0.8963 (0.4329, 1.8561)	0.769^{ns}
	No=0	66	41		
Treatment Regularity	Regular=1	117	94	1.8590 (0.9115, 3.7913)	0.084^{ns}
	Irregular=0	3	56		
Biochemical Parameters	**Category**	**Cases**	**Control**	**Odd Ratio (95% CI)**	**P-value**
Systolic Blood Pressure (mmHg)	120<=Normal	60	75	1.0062 (0.9819, 1.0311)	0.623^{ns}
	121 > = High	90	75		
	90 < = Low	0	0		
Diastolic Blood Pressure (mmHg)	80<=Normal	54	62	0.9614 (0.9252, 0.9991)	0.096^{ns}
	81 > = High	88	87		
	60 < = Low	8	1		
BMI (kg/m^2)	30>=Obese	60	8	4.5155 (2.2692, 8.9855)	0.000***
	25-30= Overweight	45	38		
	18.5-25 = Normal	43	03		
	18.5<= Underweight	2	1		
FBG (mg/dL)	80-100 = Normal	0	150	0.9919 (0.9345, 1.0529)	0.791^{ns}
	126 > = Diabetic	150	0		
RBG (mg/dL)	170-200= Normal	0	150	1.0332 (0.9940, 1.0739)	0.074^{ns}
	220 > = Diabetic	150	0		
HbA1c	4-5.6% = Normal	0	150	1.0944 (0.3716, 3.2228)	0.877^{ns}
	5.7> = Diabetes	150	0		

(***) = Highly Significant when P value is <0.001
(ns) = non-significant when P value is >0.05

gene and *TCF7L2* gene polymorphism and allelic frequency in control and T2D patients were discussed in Table 5 (Tables 2-5).

Discussion

T2DM is a Multifactorial disorder that is caused by both genetic and environmental factors whereby heritability level from family studies is predicted as 22% to 73% [17]. Missense mutation may change the ATP binding region charge and reduce the ATP channel sensitivity. The rs5219 polymorphism is because of the conversion of G to A and in many studies; this polymorphism has been shown a positive association with T2D [18]. This variation results in resistance to insulin. However, various studies did not show any association between rs5219 polymorphism and T2D [19]. The results of East Asian population exposed that mutant A allele was more frequent in control as compared to cases [20]. This polymorphism was associated with T2D in Tunisia population although in Ghanaian population, the mutant A allele was 99.9% predominant so this rs5219 variant did not exhibit any association with T2D [21]. In this current study, there was majority of AA genotype and mutant allele A in both non-diabetic and diabetic patients thus, rs5219 (E23K) polymorphism was insignificantly associated with T2D. The difference between *KCNJ11* genotypes in two groups was found statistically insignificant as p value is 0.415. Our results of *KCNJ11* gene were similar with other studies such as Iranian, Ghanian and Nigerian population which confirmed the non-significant association of *KCNJ11* polymorphism with T2DM. On the other hand, these findings were found to be inconsistent with many studies like Korean and Mexican population. This difference may be due to the diversity of ethnicity, sample size and geographical location.

Based on the results of this study, the high significance of BMI was shown with the polymorphism of the *KCNJ11* gene which conforms with the findings of the Euro-Brazilian population [22]. The Turkish

Table 3: Association of *KCNJ11* (rs5219) genotypes with type 2 diabetes mellitus.

Demographic Parameters	Category	GG	GA	AA	P-value
Age	31-40	5	0	18	0.946^{ns}
	41-50	6	2	39	
	1-60	5	1	37	
	61-70	5	1	29	
	Above 70	0	0	2	
Gender	Male=1	12	2	57	0.615^{ns}
	Female=0	9	2	68	
Smoking	Single=0	4	2	27	0.381^{ns}
	Married=1	17	2	97	
Family History	Yes=1	13	3	72	0.745^{ns}
	No=0	8	1	53	
Exercise	Yes=1	14	1	69	0.278^{ns}
	No=0	7	3	56	
Treatment Regularity	Regular=1	13	2	102	0.051^{ns}
	Irregular=0	8	2	23	
Biochemical Parameters	**Category**	**GG**	**GA**	**AA**	**P-value**
Systolic Blood Pressure (mmHg)	120<=Normal	7	1	50	0.999^{ns}
	121 > = High	14	3	74	
	90 < = Low	0	0	1	
Diastolic Blood Pressure (mmHg)	80<=Normal	8	0	50	1.00^{ns}
	81 > = High	13	3	74	
	60 < = Low	0	1	1	
BMI (kg/m^2)	30>=Obese	1	0	1	0.000***
	25-30= Overweight	5	1	37	
	18.5-25 = Normal	7	0	38	
	18.5<= Underweight	8	3	49	
FBG (mg/dL)	80-100 = Normal	0	0	0	0.231^{ns}
	126 > = Diabetic	21	4	125	
RBG (mg/dL)	170-200= Normal	0	0	0	0.218^{ns}
	220 > = Diabetic	21	4	125	
HbA1c	4-5.6% = Normal	0	0	0	0.835^{ns}
	5.7> = Diabetes	21	4	125	

(***) = Highly Significant when P value is <0.001
(ns) = non-significant when P value is >0.05

population did not show any significant association between *KCNJ11* polymorphism and BM1 so; our results are inconsistent with the research conducted in Turkey [23].

According to the results of the present study, the *KCNJ11* association with random and fasting blood sugar was observed to be non-significant which is contrary to the results of the Turkish Population [23]. In many populations, it has been reported that E23K polymorphism of *KCNJ11* is associated with blood pressure which is contrary to this study. According to our results, SBP and DBP are not associated with diabetes that is supported by findings of the Chinese population [24]. Our study investigated the insignificant association of *KCNJ11* with gender and age which is consistent with the findings of the Syria population [25]. But *KCNJ11*was significantly associated with age and gender in the population of Euro-Brazilian which is contrary to our findings [22]. Age and gender are non-significantly associated with diabetes which following the findings of research conducted on the Iranian population [6].

The genes that are involved in the beta cell production is also known as the transcription factor 7 -like 2 gene [26]. This gene carries the High Mobility Group (HMG) box and also involved in the Wnt the signaling route that plays the capital role in glucose maintenances and that gene is the prime manager of the proinsulin [27]. A nuclear chemoreceptor for B-catenin is concealing from this gene that involved in lipid metabolism [28]. Variation in *TCF7L2* gene becomes greater the probability of Type 2 diabetes with a reduced insulin excretion showed a powerful association [14]. From the old studies, also find out that within the intronic site of Transcription factor 7 like 2 nearby some SNPs which manifest a vigorous relationship with Type 2 Diabetes [29]. Our present study on Single nucleotide polymorphism (rs11196205) in *TCF7L2* is not associated with the T2DM in Southern

Table 4: Association of *TCF7L2* (rs11196205) genotypes with type 2 diabetes mellitus.

Demographic Parameters	Category	CC	GG	GC	P-value
Age	31-40	5	10	8	0.553^{ns}
	41-50	11	13	23	
	1-60	15	15	13	
	61-70	8	10	17	
	Above 70	1	0	1	
Gender	Male=1	20	26	25	0.327^{ns}
	Female=0	20	22	37	
Smoking	Single=0	7	13	13	0.548^{ns}
	Married=1	33	35	49	
Family History	Yes=1	22	27	39	0.672^{ns}
	No=0	18	21	23	
Exercise	Yes=1	21	28	35	0.856^{ns}
	No=0	19	20	27	
Treatment Regularity	Regular=1	28	38	51	0.335^{ns}
	Irregular=0	12	10	11	
Biochemical Parameters	**Category**	**CC**	**GC**	**CC**	**P-value**
Systolic Blood Pressure (mmHg)	120<=Normal	16	17	27	0.689^{ns}
	121 > = High	24	31	35	
	90 < = Low	0	0	0	
Diastolic Blood Pressure (mmHg)	80<=Normal	13	2	3	0.707^{ns}
	81 > = High	24	31	26	
	60 < = Low	3	15	33	
BMI (kg/m^2)	30>=Obese	0	1	1	0.907^{ns}
	25-30= Overweight	11	16	16	
	18.5-25 = Normal	12	12	21	
	18.5<= Underweight	17	19	24	
FBG (mg/dL)	80-100 = Normal	2	0	1	0.2388^{ns}
	126 > = Diabetic	38	48	61	
RBG (mg/dL)	170-200= Normal	3	1	1	0.228^{ns}
	220 > = Diabetic	37	47	61	
HbA1c	4-5.6% = Normal	0	3	2	0.618^{ns}
	5.7> = Diabetes	21	54	60	

(ns) = non-significant when P value is >0.05

Punjab population and according to our awareness is the first analysis within this population.

TCF7L2 (rs11196205) showed the frequency of CC wild allele 28%, GG 27.33% mutant homozygous, and GC 44.67% heterozygous in cases samples. While in control samples the frequency of CC wild allele 26.66%, GG mutant 32.00% homozygous and GC 41.33% heterozygous. According to our research, the allelic ratio of the genotype *TCF7L2* gene is not going to be significant while our results are correspondence by a study that was carried out in the South Western of Iran [30]. Our finding results also interconnected with the Arab population of Khuzestan province which is also not related toT2DM. Meta-analysis was also done on the population of East Asian in which polymorphism of (rs11196205) was examined for five-time in *TCF7L2* was strongly linked with T2DM and variation (rs11196205) in *TCF7L2* was also linked to the T2DM in the population of China and Japan's [31]. The Different study determined the strong built linkage of *TCF7L2* with T2DM at first was showed in the population of Iceland that has been copied later in Danish and America population. The causes of this unpredictability in results in several studies would be the dissimilar genetic report [27]. Our research work manifests the non-significant relationship of *TCF7L2* along with fasting and random plasma sugar, Systolic blood pressure and Diastolic blood pressure that was correspondence along to the population of Japanese, Korean and German population.

Previous findings describe that the polymorphism in the *TCF7L2* gene was linked between males, not to a female. Correspondence to our research work, the ratio of DM in females is (37%) is more as to males (25%) in diabetic individuals. In existing research work, demographic and biochemical variables were also contrast within genotypes of *TCF7L2* (rs11196205) and it is noticed that patients of diabetes manifest the no significant relationship with whole genotype.

Table 5: Distribution of genotypic and allelic frequencies of rs5219 in *KCN11* and rs11196205 in *TCF7L2* gene among cases and controls and their possible association with diabetes mellitus type 2.

Gene and SNP	Parameters	Genotype Percentage			Allelic Frequency		P-value
KCNJ11 (rs5219)	Genotype	GG	GA	AA	G	A	0.415^{ns}
	Controls	16 (10.66%)	2 (1.3%)	132 (88%)	0.1133	0.886	
	Patients	21 (14%)	4 (2.66%)	125 (83.33%)	0.1533	0.846	
TCF7L2 (rs11196205)	Genotype	CC	GG	GC	C	G	0.62^{ns}
	Controls	40 (26.66%)	48 (32.00%)	62 (41.33%)	0.426	0.573	
	Patients	42 (28.00%)	41 (27.33%)	67 (44.67%)	0.416	0.583	

(ns) = non-significant when P value is >0.05

Conclusion

The findings of this result study show that the polymorphisms rs5219 of *KCNJ11* and rs11196205 of *TCF7L2* are not associated with diabetes as in case of *KCNJ11* gene there was dominance of mutant allele A and genotype AA in non-diabetic and diabetic patients. Allele consisting of lysine in *KCNJ11* gene is not responsible for affecting the K_{ATP} channel activity and thus not related to diabetes. Our research work manifests that transcription factor 7 like 2 rs11196205 polymorphisms are not linked along with a risk of type 2 diabetes mellitus. The contrast and diversification in results of past and present findings can be effect by multiple factors like genetic differentiation, territory factors and, Metagenetic effects, sample proportions, diagnostic procedure.

Acknowledgements

We are grateful to all the patients and healthy people who were a part of this research. We thank to the Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University, Multan-60800, Pakistan for providing all the forms of support.

Authors' Contribution

Sabahat Shaheen and Sidra Aslam collected the blood sample, epidemiological and clinical data and performed the lab experiments. Mehak Shaheen reviewed the manuscript. Adeela Awan and Hira Jamil helped in statistical analysis. Rana Khalid Iqbal supervised the study, monitored and helped in carrying out the experiments on a regular basis.

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