Association Study of KCNJ11 and PPARG Genetic Variants as Potential Biomarkers for Improved Treatment Outcomes in Type 2 Diabetes Mellitus

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Abstract

PPARG is implicated in the risk of T2DM due to its role in adipocyte differentiation and energy homeostasis. The prevalence of T2DM has been increasing rapidly in recent decades, driven by its pathogenesis which involves insulin resistance and beta-cell dysfunction. Numerous studies suggest that the KCNJ11 polymorphism increases the risk of T2DM.This study aims to identify and validate the risk associated with two genetic variants: the PPARG (rs7656250) and the KCNJ11 (rs5219), in a subset of the Indian population. Genotyping of the genetic variants was performed using PCR, RFLP and Sanger sequencing. Significant differences in genotype and allele frequencies were observed between T2DM cases and controls.Both the dominant (KCNJ11: OR, 5.26; 95% CI, 2.93–9.45; p < 0.0001; PPARG: OR, 15; 95% CI, 6.54–34.3; p < 0.0001) and codominant models (KCNJ11: OR, 0.45; 95% CI, 0.27–0.76; p = 0.002) revealed a strong association of KCNJ11 rs5219 and PPARG rs7656250 with T2DM risk.

However, PPARG rs7656250 in the codominant model (OR, 0.62; 95% CI, 0.37–1.04; p = 0.07) did not show significant association. These findings underscore the potential of KCNJ11 and PPARG genetic variants as biomarkers for assessing T2DM risk in the Indian population.

Keywords: PPARG, rs7656250, KCNJ11, rs5219, Polymorphism, Diabetes Mellitus,

Introduction

Type 2 diabetes mellitus (T2DM) is one of the most prevalent metabolic disorders, characterized by the body's inability to effectively utilize insulin, leading to elevated blood sugar levels. Approximately 90% of all diabetes cases are T2DM. The condition often begins with insulin resistance, progressing to pancreatic beta-cell dysfunction and insufficient insulin production. Its onset is influenced by a combination of genetic predisposition and environmental factors, typically manifesting in adults over 40 years of age. However, recent studies highlight an alarming rise in T2DM among younger populations, linked to the global obesity epidemic[3,16,29].

Although the precise etiology of T2DM remains unclear, two hallmark features are insulin resistance (IR) and impaired insulin secretion (IS), both of which are pivotal to its pathophysiology[21]. Several key genes have been implicated in T2DM susceptibility, with KCNJ11 and peroxisome proliferator-activated receptor gamma (PPAR-γ) being particularly significant[5,18-20]. The PPARG gene, located on chromosome 3, plays a critical role in adipocyte differentiation, insulin sensitivity and energy homeostasis. It is expressed predominantly in adipose tissue but also in the liver and heart. PPARG exists in three isoforms: PPAR-α, PPAR-β/δ and PPAR-γ, each of which has physiological relevance to diabetes[6,8,13]. Synthetic PPAR-γ agonists such as pioglitazone and rosiglitazone, are known to improve insulin sensitivity, to increase HDL cholesterol levels and to reduce triglycerides[9,17,24].

The PPARG gene produces proteins of 477 or 505 amino acids, depending on the specific promoter activated. PPAR-γ2, found in adipose tissue, is encoded by an additional exon (exon B), which gives it a unique N-terminal region[28]. In contrast, PPAR-γ1 is expressed in multiple tissues while PPAR-γ3 is localized to macrophages and the large intestine. Human genetics studies suggest that PPARG contributes to insulin responsiveness, glucose homeostasis and the regulation of blood pressure, with evidence linking it to various chronic diseases[26,28,32]. Additionally, PPARG activators have been shown to reduce cardiovascular risks while improving glycemic control[22,29].

The KCNJ11 gene, located at 11p15.1 on the human chromosome, encodes the inward rectifier potassium ion channel (Kir6.2), a key component of the ATP-sensitive potassium (KATP) channel. This channel, formed by the interaction of Kir6.2 with Sulfonylurea Receptor 1 (SUR1), regulates insulin secretion in pancreatic beta cells. In response to rising glucose levels, the KATP channel closes, leading to depolarization of the cell membrane, activation of voltage-gated calcium channels and subsequent insulin release[2,11,22,25].

The KCNJ11 gene does not contain introns and includes single nucleotide polymorphisms (SNPs) that influence T2DM risk. Among these, the rs5219 polymorphism (E23K variant) has garnered significant attention due to its association with impaired insulin secretion. This polymorphism involves a guanine-to-adenine substitution at

codon 23, resulting in a lysine-to-glutamic acid substitution. This change reduces the ATP sensitivity of the KATP channel, leading to increased channel activity and suppressed insulin secretion[17,18,20,21].

Therapeutic strategies for T2DM include lifestyle interventions and oral antidiabetic agents, with sulfonylureas such as gliclazide, glibenclamide, glimepiride and glipizide being among the most widely used treatments[4,27]. This research highlights the critical roles of PPARG and KCNJ11 genes in T2DM pathophysiology, offering insights into their potential as therapeutic and diagnostic biomarkers.

Material and Methods

Research Subject Selection Criteria: The study was conducted on 240 participants who visited the Outpatient Department (OPD) at Dr. O.P. Chaudhary Hospital and Research Center in Lucknow, Uttar Pradesh, from 2019 to 2025. All participants were fully informed about the objectives, methodology and procedures of the study before taking their consent.

Participants were divided into two groups: 120 T2DM patients and 120 healthy controls. Only T2DM patients above the age of 32 were included in the study, while individuals with severe co-morbidities were excluded. The study protocol was reviewed and approved by the Institutional Ethics Committee (IEC) of Sardar Patel Post Graduate Institute of Dental and Medical Sciences, Lucknow, Uttar Pradesh.

Selection Criteria

- **Inclusion Criteria:**
  - Participants aged over 30 years.
  - Individuals diagnosed with T2DM or in the early stages of diabetes.
- **Exclusion Criteria:**
  - Individuals with no evidence of serious comorbidities or diabetes.

Biochemical Parameters: Trained healthcare professionals recorded the height, weight and blood pressure (systolic and diastolic) of each participant. Hypertension was defined as a blood pressure reading above 145/95 mmHg or the use of antihypertensive medications. Smoking habits were assessed and habitual smokers were categorized as regular smokers. T2DM-related biochemical parameters were measured in both cases and controls using an automated clinical chemistry analyzer. Serum levels of high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG) and total cholesterol (TC) were estimated spectrophotometrically.

Blood Sample Collection: Venous blood samples (3 mL) were collected from T2DM patients and healthy controls in EDTA vials, using ethylenediaminetetraacetic acid as the anticoagulant. The samples were stored at −20 °C in a deep freezer for subsequent biochemical and genetic analysis.

Isolation of Genomic DNA: Genomic DNA was extracted from the collected blood samples using the manual phenol-chloroform extraction method.

Genotyping of Targeted SNPs: The targeted genes were amplified using polymerase chain reaction (PCR). Each PCR reaction (20 µL) contained the following components:

- 10 µL Master Mix (Gene I)
- 0.7 µL each of forward and reverse primers
- 7 µL of autoclaved molecular-grade water for volume adjustment.

The primer sequences, amplicon sizes and restriction enzymes used for the genotyping of KCNJ11 and PPARG genes are detailed in table 1.

Procedure for Genotyping PPARG Gene: The PCR program for amplifying the PPARG gene consisted of the following steps:

1. Initial denaturation at 95 °C for 5 minutes.
2. 35 cycles of:
   - Denaturation at 95 °C for 30 seconds.
   - Annealing at 60 °C for 30 seconds.
   - Extension at 72 °C for 30 seconds.
3. Final extension at 72 °C for 5 minutes.

The amplified PCR products were subjected to restriction digestion using Type II Restriction Enzyme HpaII (NEB) at 37 °C for 1 hour. Digestion results were analyzed on a 3% agarose gel run at 100 V for 1 hour.

Procedure for Genotyping KCNJ11 Gene: The PCR program for amplifying the KCNJ11 (rs5219) gene included:

1. Initial denaturation at 95 °C for 5 minutes.
2. 30 cycles of:
   - Denaturation at 95 °C for 30 seconds.
   - Annealing at 66.2 °C for 30 seconds.
   - Extension at 72 °C for 30 seconds.
3. Final extension at 72 °C for 5 minutes.

The PCR products were digested with type II Restriction Enzyme BanII (NEB) at 37 °C for 4 hours. The digested products were analyzed on a 3% agarose gel run at 100 V for 1 hour.

Analytical Statistics: Statistical analyses were performed using SPSS software version 20.0. Continuous variables were expressed as mean ± standard deviation (SD). The Student's *t*-test was used to calculate *p*-values. Genotype and allele frequencies were evaluated using the chi-square test, Hardy–Weinberg equilibrium and odds ratios (OR) with corresponding *p*-values. Biochemical parameters for both groups were analyzed and ANOVA was employed to determine significant correlations. The results were tabulated following biochemical testing.

Results and Discussion

Table 1 summarizes the biological and clinical characteristics of the study participants. Significant associations were observed in FBS, PPS, VLDL, HDL, SBP and DBP between cases and controls. Age was also significantly correlated between the two groups. The DNA concentration for each sample varied from 12 to 55 ng/µl, with an average purity index ranging from 1.8 to 2.0. After the PCR reaction, a 275 bp DNA fragment was successfully amplified (Fig. 1). Upon treating the PCR products with the HpaII enzyme, distinct patterns were observed: The wild-type homozygote allele yielded a single fragment of 275 bp. The mutant homozygote produced two fragments measuring 184 bp and 91 bp. Heterozygotes exhibited three fragments of 275 bp, 184 bp and 91 bp.

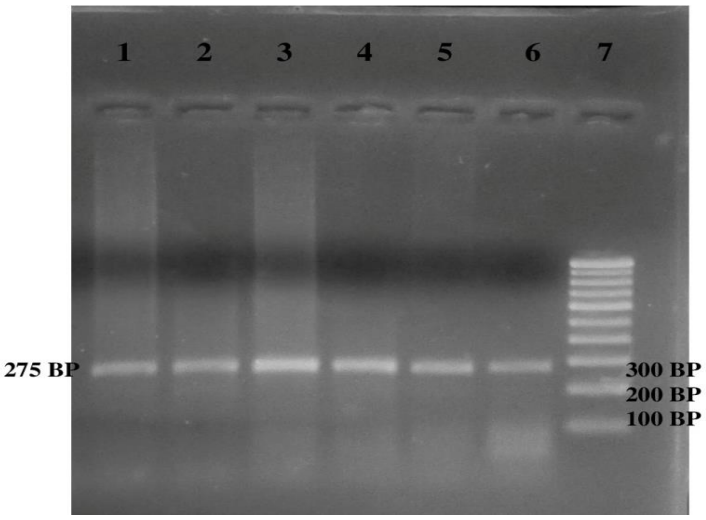

Figure 1: PCR of PPARGrs7656250

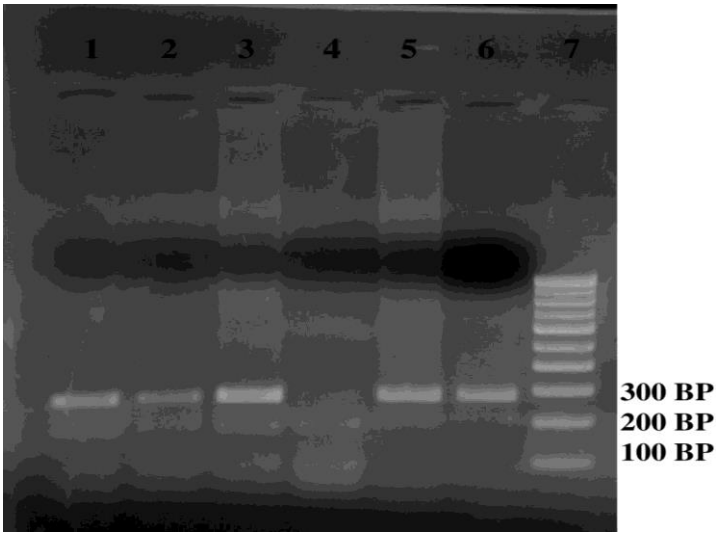

Figure 2: Restriction Digestion of PPARGrs7656250

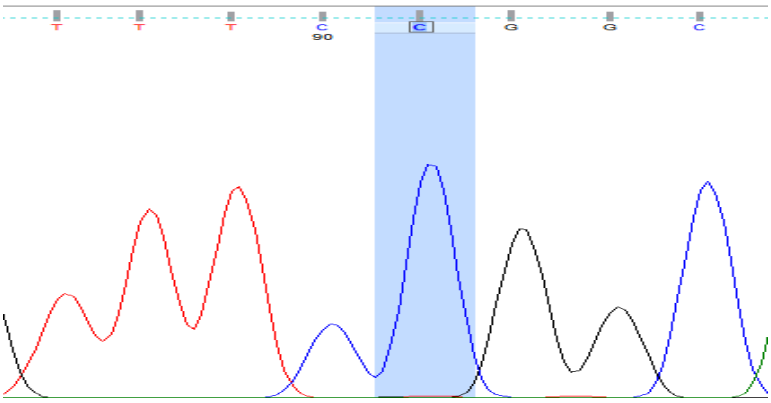

Figure 3: Wild Condition of PPARGrs7656250 verified through Sanger Sequencing

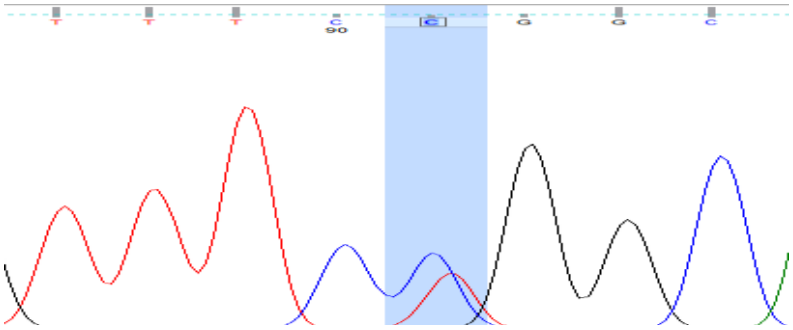

Figure 4: Heterozygous Condition of PPARGrs7656250 verified through Sanger Sequencing

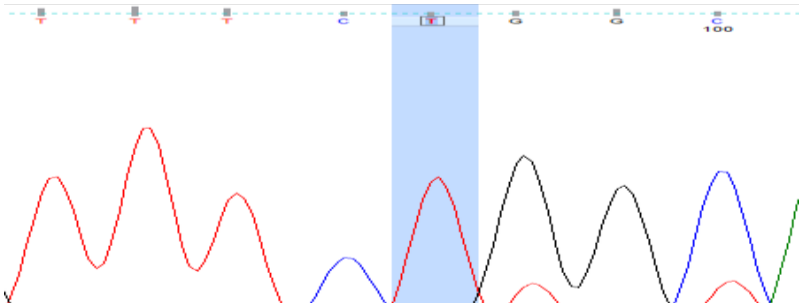

Figure 5: Mutant Condition of PPARGrs7656250 verified through Sanger Sequencing

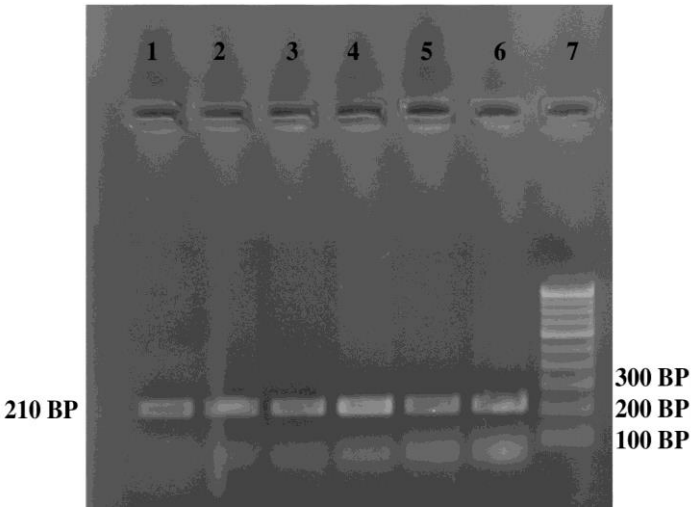

Figure 6: PCR of KCNJ11rs5219

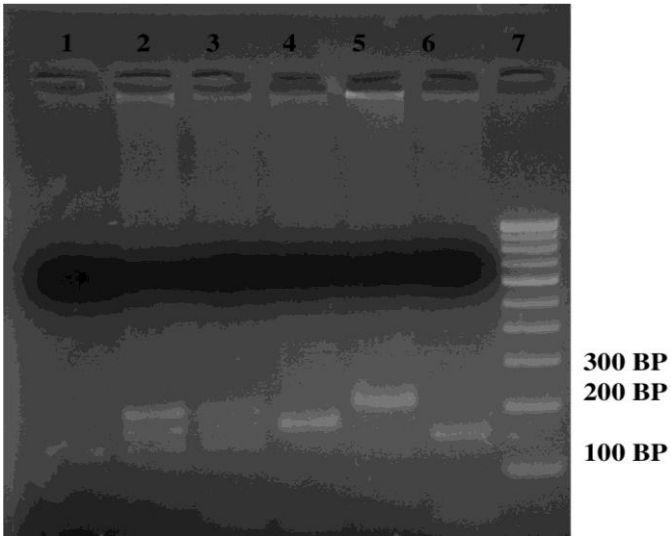

Figure 7: Restriction Digestion of KCNJ11rs5219

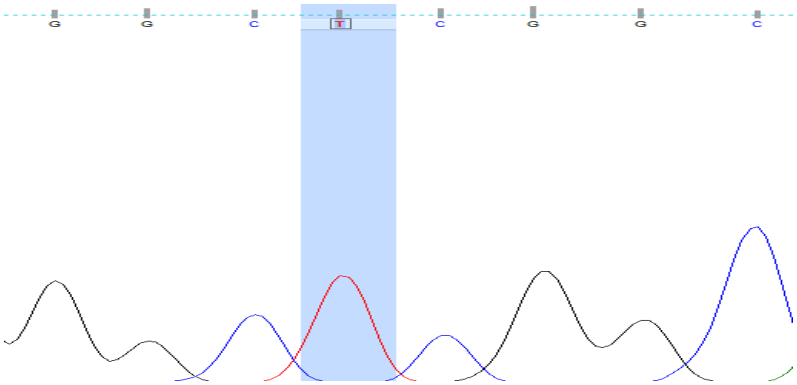

Figure 8: Wild Condition KCNJ11rs5219 verified through Sanger Sequencing

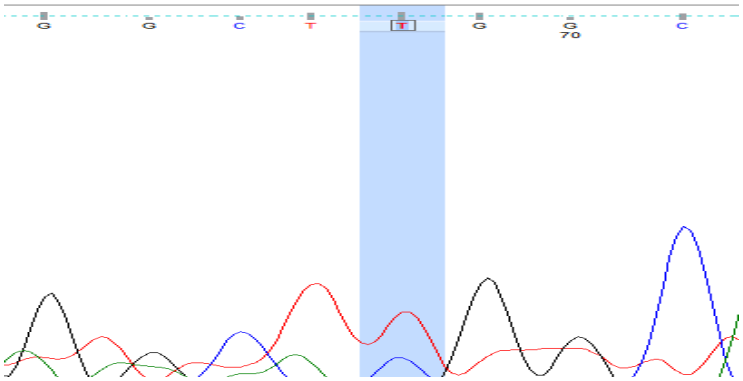

Figure 9: Heterozygous Condition of KCNJ11rs5219 verified through Sanger Sequencing

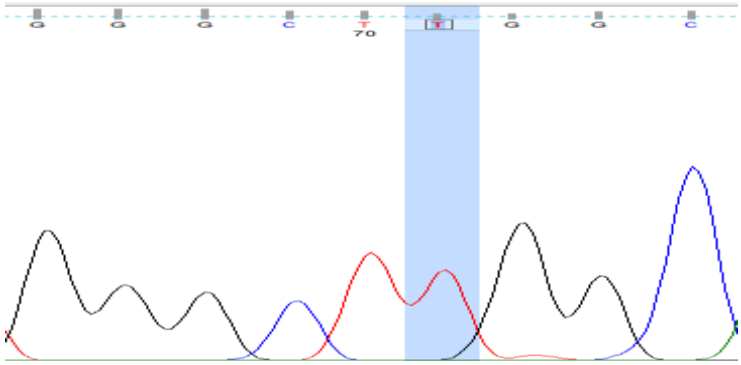

Figure 10: Mutant Condition of KCNJ11rs5219 verified through Sanger Sequencing

Table 1
Shows the Primer Sequences of Target Genes along with Amplicon size

Gene Name	Primer Sequence	Amplicon Size	Restriction Enzyme
KCNJ11	**F Primer:** 5′-GACTCTGCAGTGAGGCCCTA-3′	210 bp	BanII
rs5219	**R Primer:** 5′-ACGTTGCAGTTGCCTTTCTT-3′		
PPARG	**F Primer:** 5′-TCACCATCTTCCTCCATCA-3′	275 bp	HpaII
rs7656250	**R Primer:** 5′-GGGTAAGTCTTGCCTCCTT-3′		

PPARG Genotypic Analysis: Table 2 illustrates the genotypic variation associated with the PPARG gene in both groups. In the control group, the frequency of genotypes (CC, CT, TT) was 33.3%, 55% and 11.6% respectively while in the T2DM group, it was 50%, 43.3% and 6%. The observed p-value indicated a statistically significant difference between the two groups. Genotype frequencies for all participants adhered to Hardy-Weinberg equilibrium. The allele frequencies in the patient group were 116.6% for the C allele and 78.3% for the T allele. In contrast, the

control group exhibited allele frequencies of 143.3% (C) and 56.6% (T). However, the results suggested no statistically significant association of this SNP with T2DM in this population (P = 0.06).

The impact of the PPARG rs7656250 polymorphism on T2DM was evaluated using five distinct genetic models: Homozygous model (CC vs TT): OR = 2.62 (95% CI: 1.00–6.83, P = 0.04) Heterozygous model (CT vs TT): OR = 1.90 (95% CI: 1.10–3.26, P = 0.01) Dominant model (CC vs TT+CT): OR = 15 (95% CI: 6.54–34.3, P < 0.0001) Recessive model (CC+CT vs TT): OR = 1.84 (95% CI: 0.74–4.58, P = 0.18) Codominant model (CT vs TT+CC): OR = 0.62 (95% CI: 0.37–1.04, P = 0.07).

KCNJ11 Genotypic Analysis: Table 3 highlights the significant association of biochemical traits between the two study groups genotyped for the KCNJ11 rs5219 polymorphism. The target gene was amplified using PCR and genotyping was performed via RFLP followed by Sanger sequencing. After PCR amplification, a 210 bp fragment was obtained. Restriction digestion using the HpaII enzyme at 37°C for 4 hours yielded the following patterns: The wild-type homozygote allele yielded a single fragment of 150 bp. The mutant homozygote produced a single fragment of 178 bp. Heterozygotes displayed three fragments: 210 bp, 178 bp and 150 bp (Fig. 4).

Table 4 presents the genotypic variation for the KCNJ11 rs5219 gene. The frequency of genotypes (TT, TC, CC) was 54.1%, 37.5% and 8.33% respectively in the control group compared to 18.3%, 56.6% and 25% in the T2DM group. A highly statistically significant difference was observed between the two groups (P < 0.0001). The allele frequencies in the patient group were 93.3% for the T allele and 106.6% for the C allele, while in the control group, they were 145.8% (T) and 54.1% (C). The results indicated a strong and statistically significant association of KCNJ11 rs5219 with T2DM in this population (P < 0.0001). The effect of the KCNJ11 rs5219 polymorphism on T2DM was assessed using the following genetic models: Homozygous model (TT vs CC): OR = 8.86 (95% CI: 3.73–21.0, P = 0.0001) Heterozygous model (TC vs TT): OR = 4.46 (95% CI: 2.41–8.24, P = 0.0001) Dominant model (TT vs CC+TC): OR = 5.26 (95% CI: 2.93–9.45, P < 0.0001) Recessive model (TT+TC vs CC): OR = 3.66 (95% CI: 1.70–7.90, P = 0.0009) Codominant model (TC vs CC+TT): OR = 0.45 (95% CI: 0.27–0.76, P = 0.002).

Table 2
Bio-chemical parameters associated with PPARGrs7656250

Biochemical Parameter	T2DM Patients (120)	Control (120)	*p*- Value
Age (Years)	43.4 ± 13.2	36.4 ± 7.73	0.000
FBS (mg/dL)	109.7 ± 6.56	85.8 ± 8.47	0.000
PPS (mg/dL)	211.4 ± 60.35	120.1 ± 12.15	0.000
VLDL (mg/dL)	16.6 ± 9.42	15.07 ± 8.32	0.244
HDL (mg/dL)	63.3 ± 77.0	53.09 ± 10.11	0.191
SBP (mm/Hg)	177.6 ± 10.4	109.51 ± 11.9	0.000
DBP (mm/Hg)	129.7 ± 22.3	70.15 ± 6.38	0.000

Table 3
Genotypic Correlation between Case and Control associated with PPARGrs7656250

Variables	T2DM Patients (120)	Control (120)	OR (95% CI)	*p*- Value
CC	40 (33.3%)	60 (50%)	Reference	
CT	66 (55%)	52 (43.3%)	1.90(1.10– 3.26)	0.01
TT	14 (11.6%)	8 (6.6%)	2.62(1.00-6.83)	0.04
X^2 Value	-		7.29	-
Dominant model				
CC	40 (33.3%)	60 (50%)	Reference	
TT+CT	80 (66.6%)	60 (50%)	15(6.54-34.3)	<0.0001
Recessive model				
CC+CT	106 (88.3%)	112 (93.3%)	Reference	
TT	14 (11.6%)	8 (6.6%)	1.84(0.74-4.58)	0.18
Codominant model				
CT	66 (55%)	52 (43.3%)	Reference	
CC+TT	54 (45%)	68 (56.6%)	0.62(0.37-1.04)	0.07
Alleles				
C	140 (116.6%)	172 (143.3%)	Reference	
T	94 (78.3%)	68 (56.6%)	1.69(1.15-2.49)	0.006

Table 4
Biochemical parameters associated with KCNJ11rs5219

Biochemical Parameter	T2DM Patients (120)	Control (120)	*p*- Value
Age (Years)	43.6 ± 12.9	36.5 ± 8.07	0.000
FBS (mg/dL)	109.9 ± 6.7	85.0 ± 8.2	0.000
PPS (mg/dL)	216.0 ± 58.0	121.3 ± 12.5	0.000
VLDL (mg/dL)	17.5 ± 9.6	15.5 ± 8.50	0.102
HDL (mg/dL)	62.3 ± 70.4	53.1 ± 10.4	0.159
SBP (mm/Hg)	177.8 ± 11.9	108.5 ± 11.9	0.000
DBP (mm/Hg)	129.8 ± 22.5	70.0 ± 6.13	0.000

Table 5
Genotypic Correlation between Case and Control associated with KCNJ11rs5219

Variables	T2DM Patients (120)	Control (120)	OR (95% CI)	*p*- Value
TT	22 (18.3%)	65 (54.1%)	Reference	
TC	68 (56.6%)	45 (37.5%)	4.46(2.41-8.24)	<0.0001
CC	30 (25%)	10 (8.33%)	8.86(3.73-21.0)	<0.0001
X^2 Value	-		35.9	-
Dominant model				
TT	22 (18.3%)	65 (54.1%)	Reference	
CC+TC	98 (81.6%)	55 (45.8%)	5.26(2.93-9.45)	<0.0001
Recessive model				
TT+TC	90 (75%)	110 (91.6%)	Reference	
CC	30 (25%)	10 (8.3%)	3.66(1.70-7.90)	0.0009
Codominant model				
TC	68 (56.6%)	45 (37.5%)	Reference	
CC+TT	52 (43.3%)	75 (62.5%)	0.45(0.27-0.76)	0.002
Alleles				
T	112 (93.3%)	175 (145.8%)	Reference	
C	128 (106.6%)	65 (54.1%)	3.07(2.10-4.50)	<0.0001

Table 6
Genotypes of KCNJ11 and PPARG together and their impact on clinicopathological markers

Clinical Parameter	Combined variable of KCNJ11+ PPARG	Mean ± SD	p value
FBS	TT+TC	109 ± 1.52	0.001*
	CC+TT	110.25 ± 1.08	
	TC+CT	108.5 ± 3.02	
	CC+CT	110.18 ± 1.17	
	CC+CC	109.76 ± 1.82	
	TC+CC	111.11 ± 1.89	
PPS	TT+TC	211.7 ± 11.8	0.000*
	CC+TT	85.76 ± 2.54	
	TC+CT	209.8 ± 16.3	
	CC+CT	237 ± 17.3	
	CC+CC	221.2 ± 16.5	
	TC+CC	173.1 ± 16.9	
VLDL	TT+TC	8.6 ± 3.28	0.32
	CC+TT	15.4 ± 2.82	
	TC+CT	15.7 ± 2.51	
	CC+CT	22.16 ± 2.75	
	CC+CC	16.33 ± 2.06	
	TC+CC	17.57 ± 2.36	
HDL	TT+TC	111.7 ± 53.7	
	CC+TT	53.5 ± 5.18	

	TC+CT	56.72 ± 2.78	0.55
	CC+CT	52.2 ± 3.06	
	CC+CC	48.5 ± 3.12	
	TC+CC	57.8 ± 2.83	
SBP	TT+TC	175.5 ± 3.21	0.50
	CC+TT	174.7 ± 3.96	
	TC+CT	175.4 ± 2.05	
	CC+CT	179.6 ± 3.75	
	CC+CC	180.5 ± 3.20	
	TC+CC	182.2 ± 2.73	
DBP	TT+TC	148 ± 4.9	0.01
	CC+TT	125.0 ± 6.09	
	TC+CT	142.1 ± 5.09	
	CC+CT	110.2 ± 4.88	
	CC+CC	121.75 ± 5.27	
	TC+CC	141.7 ± 4.23	

Table 7
Cases of dependent variables and controls in regression analysis

Clinical Variables	R^2	p Value
Age	0.01	0.261
FBS	0.002	0.59
PPS	0.008	0.31
VLDL	0.01	0.13
HDL	0.000	0.75
SBP	0.02	0.09
DBP	0.03	0.05

This study targeted the PPARG rs7656250 and KCNJ11 rs5219 SNPs to explore their correlation with T2DM in an Indian population[24]. The PPARG rs7656250 SNP, located in intron 2, was specifically included due to its unique position and potential to enhance the density of SNP analyses. PPARG serves as the primary pharmacological target for thiazolidinediones, a class of antidiabetic agents that increase insulin sensitivity. While previous studies did not find significant associations between PPARG rs1801282 and rs3856806 polymorphisms and T2DM, our study suggests partial significance in the Indian population. Similarly, the KCNJ11 rs5219 polymorphism, previously studied in Kurdish, Chinese and European populations, has shown significant associations with T2DM[7,10,30,31].

Study from Gaza demonstrates the highly significant role that environmental factors and the E23K SNP play in the development of type 2 diabetes, with a significance of (P=0.000). Our study also demonstrated that age plays a substantial role in the onset of T2DM[1]. Ren et al[23] confirmed this genetic variant and its significant role in the rise of type 2 diabetes in the targeted population in the populations of Europe and America, but this SNP was first investigated in the population of Uttar Pradesh, India.

Our results corroborate these findings and confirm the strong association of this SNP with T2DM in the Indian State of Uttar Pradesh. Additionally, this study reports a novel nucleotide substitution (T>C) for KCNJ11 rs5219. Environmental factors and stress also played a significant role in T2DM onset.

Conclusion

This study identifies a significant correlation between type 2 diabetes mellitus (T2DM) and the KCNJ11 gene's rs5219 polymorphism, as well as the PPARG rs7656250 polymorphism, in an Indian population sample. The findings highlight the critical role of the KCNJ11 rs5219 polymorphism in the etiology of T2DM, offering valuable insights into its genetic underpinnings. By identifying these genetic variations, clinicians can better predict the risk of developing T2DM and to understand its pathophysiology, paving the way for personalized treatment strategies.

Although this study provides compelling evidence for these associations, inconsistent results in other genetic investigations suggest the need for further research across diverse population groups. Expanding such studies can address variations in genetic markers, phenotypic expressions and environmental interactions that contribute to T2DM susceptibility.

The substantial association observed between PPARG rs7656250 and KCNJ11 rs5219 polymorphisms with key biomarkers of T2DM underscores the potential clinical utility of genotyping these SNPs. By integrating genetic profiling into clinical practice, it may be possible to optimize blood glucose and lipid management on an individual basis,

ultimately enhancing treatment outcomes and improving the quality of care for T2DM patients.

Acknowledgement

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