Association of *TCF7L2* (rs7903146) and *KCNQ1* (rs2237892) Polymorphisms with Type 2 Diabetes Mellitus and Plasma Glucose Levels in an Andhra Pradesh Population

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Abstract

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder influenced by both genetic and environmental factors. Variants in genes involved in glucose metabolism, including *TCF7L2* and *KCNQ1*, have been widely implicated in T2DM susceptibility. The present study aimed to evaluate the association of *TCF7L2* rs7903146 (C/T) and *KCNQ1* rs2237892 (C/T) polymorphisms with T2DM and to examine their correlation with plasma glucose levels in the Andhra Pradesh population. A case–control study was conducted on 136 T2DM patients and 136 healthy controls. Genomic DNA was isolated from peripheral blood samples, and genotyping was performed using allele-specific PCR for *TCF7L2* and tetra-ARMS PCR for *KCNQ1*. Genotype and allele frequencies were analyzed using chi-square tests, odds ratios and p-values. Plasma glucose levels were measured by the GOD–POD method, and genotype–phenotype associations were assessed using one-way ANOVA followed by Tukey's HSD post hoc test. The TT genotype and T allele of *TCF7L2* rs7903146 were significantly associated with increased T2DM risk, while the CC genotype showed a protective effect. In contrast, *KCNQ1* rs2237892 did not show a significant association with T2DM at the genotype or allele level. Genotype–phenotype analysis revealed that CT genotype carriers of *TCF7L2* rs7903146 exhibited significantly higher plasma glucose levels in both T2DM patients and healthy subjects. For *KCNQ1* rs2237892, a significant genotype-dependent variation in plasma glucose levels was observed only among T2DM patients, particularly between CT and TT genotypes, while no such association was noted in healthy controls. The findings suggest a strong role of *TCF7L2* rs7903146 in T2DM susceptibility and glucose dysregulation, whereas *KCNQ1* rs2237892 may influence plasma glucose levels primarily in diabetic individuals. These results highlight the importance of population-specific genetic markers in understanding T2DM pathophysiology.

Keywords:

Diabetes Mellitus, TCF7L2, KCNQ1, plasma glucose levels, insulin levels

1. Introduction

Type 2 Diabetes Mellitus (T2DM) is a complex illness defined by impaired insulin resistance, insulin secretion, and disturbances in lipid and protein metabolism, resulting from a combination of genetic predisposition and environmental influences (1). Globally, there has been considerable emphasis on examining the immune response and genetic factors that may elevate the risk of diabetes. Gene mutations, especially polymorphisms in the promoter region, can influence gene transcription, resulting in abnormal expression of the corresponding mRNAs and dysfunction of the synthesized proteins. Moreover, these modifications can potentially influence people' susceptibility to diabetes. Researchers have discovered that genetic variations in immune-related genes influence several diseases. Consequently, researchers are increasingly concentrating on discovering gene variants, including single nucleotide polymorphisms (SNPs), associated with an elevated risk of T2DM. Identifying these genes may aid in the recognition of risk factors and the development of targeted therapies for patients in the future (2). Nonetheless, many questions concerning gene polymorphism and its correlation with disease remain unanswered. Moreover, the results differ among various groups. Potential complicating factors may include ethnicity, age, and regional distribution. Diverse ethnic groups display differing levels of variation in SNPs. *TCF7L2* (rs7903146) and *KCNQ1* (rs2237892) are among the most significantly correlated genetic loci for T2DM across diverse populations. Examining their polymorphisms elucidates the genetic factors influencing susceptibility to and progression of T2DM.

TCF7L2 (Transcription Factor 7-Like 2) serves as the transcriptional activator in the canonical Wnt-signaling pathway, which regulates incretin hormone synthesis, pancreatic β-cell growth, vascular growth, and may participate in insulin signalling (3). The *TCF7L2* rs7903146 mutation, which is commonly linked to T2DM, has been linked with hypertension (4), diabetic retinopathy, coronary artery disease (CAD) (5), and nephropathy (6).

The *KCNQ1* (Potassium Voltage-Gated Channel Subfamily Q Member 1) gene encodes a pore-forming subunit of the voltage-gated potassium (K+) channel, referred to as $K_v7.1$, which is physiologically significant in various tissues, particularly the cardiovascular system, pancreas, and kidneys (7.Liin et al., 2015). Genetic variants in *KCNQ1* are significantly linked to T2DM, insulin production, and impaired fasting blood glucose (8). Polymorphisms in KCNJ11 have been primarily linked to T2DM (9) and later to hypertension (10), CAD (11), and diabetic retinopathy (12).

While the correlation of polymorphisms in the *TCF7L2* and *KCNQ1* genes with T2DM has been repeatedly validated across several ethnicities, its relationship with vascular problems remains inconclusive (6). The impact of individual SNPs on the likelihood of diabetic complications is often minimal; thus, the aggregate effect of numerous risk alleles as a genetic risk score may serve as a more effective instrument for assessing the risk of T2DM-related problems (13). Hence, the present study sought to examine the relationship between the polymorphisms of *TCF7L2* C/T (rs7903146) and *KCNQ1* C/T (rs2237892) withT2DM development.

Despite strong evidence linking *TCF7L2* (rs7903146) and *KCNQ1* (rs2237892) polymorphisms with T2DM globally, region-specific genetic data from South India are also scarce. The Andhra Pradesh population, characterized by distinct genetic and environmental backgrounds, remains underrepresented in existing association studies. Therefore, population-based evaluation of genotype and allele frequency distributions is essential to elucidate the role of these variants in T2DM susceptibility in this region.

To address these gaps, the present study was designed to investigate the genotype and allele frequency distribution of *TCF7L2* (rs7903146) and *KCNQ1* (rs2237892) polymorphisms in T2DM patients and healthy controls from the Andhra Pradesh population.

2. Materials and Methods

The identification of *TCF7L2* C/T (rs7903146) and *KCNQ1* C/T (rs2237892) polymorphisms from the T2DM patients, performed by the experimentation includes genomic DNA extraction, PCR amplification of *TCF7L2* and *KCNQ1* promoter regions, and agarose gel electrophoresis.

2.1 Genomic DNA Extraction

Genomic DNA was isolated from peripheral blood samples of 136 individuals (T2DM patients and healthy controls) using the salting-out method, which involves protein precipitation with a saturated sodium chloride solution followed by DNA recovery from the supernatant. This method provides high-quality DNA suitable for downstream molecular analyses (14).

2.2 Qualitative analysis of DNA

Genomic DNA quality was evaluated using 1% agarose gel electrophoresis in 1× TAE buffer. DNA samples were electrophoresed at 100 V, visualized under a UV transilluminator, and documented to assess DNA integrity.

2.3 Amplification of *TCF7L2* and *KCNQ1* polymorphisms by ARMS-PCR

For the designing of primers,the genomic sequences surrounding the target SNPs, *TCF7L2* C/T (rs7903146) and *KCNQ1* C/T (rs2237892), were obtained from the National Center for Biotechnology Information (NCBI) database. The two special set of forward and reverse primers were designed for each SNP identification by using the primer1 program(http://primer1.soton.ac.uk/public_html/primer1.html) (15). The details of the primers are summarized in Table 1.

Table 1.List of Forward and Reverse Primers (Inner and Outer) Applied for Detection of SNPs

SNP	Forward & Reverse primer (Outer & Inner)	Primer sequence
TCF7L2 C/T (rs7903146)	FP - *TCF7L2*-C	GAACAATTAGAGAGCTAAGCACTTTTTAGAAAC
	FP - *TCF7L2*-T	GAACAATTAGAGAGCTAAGCACTTTTTAGAGAT
	Common RP – TCF7/L2R	AGATGAAATGTAGCAGTGAAGTGC

KCNQ 1 C/T (rs2237 892)	FO-P – *KCNQ1*	CTGTGGGTACACAGCTTCCCT
	RO-P – *KCNQ1*	CCTGGGTCATCAGACTAGGGTAG
	F-IP – *KCNQ1* (C)	GTCACAGGACTTTGCCAACC
	RI-P – *KCNQ1* (T)	TTTCTAGGCCCCTCACCACA

The DNAamplification was carried out using ALLELE SPECIFIC for *TCF7L2* and TETRA-ARMS PCR for *KCNQ1***.**

For *TCF7L2*, allele specific PCR amplification (Table 2) was used taking 5 µL of PCR taq mixture which contain $MgCl_2$, and dNTPs, 1 µL each of reverse primer, 1µL forward primer (C allele) and 1 µL of forward primer (T allele), in seperate tubes for each allele.

For *KCNQ1* amplifications (Table 3), the PCR reaction mixture was prepared by taking 1 µL of template DNA, 1µL forward outer primer, 1 µL reverse outer primer, 2 µL forward inner primer, 2 µL reverse inner primer (all the primers at 10 pmol/mL concentration), 5 µL of PCR taq mixture.

Table 2.PCR Thermal Profile for TCF7L2 (rs7903146)

***TCF7L2*(rs7 903146)**	**STEP**	**TEMP.**	**TIME**	**NUMBER OF CYCLES**
STEP 1	Initial denaturation	94°C	3 min	1
STEP 2	Denaturation Annealing Extension	94°C 50°C 72°C	1 min 1 min 5 min	32
STEP 3	Final extension	72°C	5 min	1

Table 3.PCR Thermal Profile for KCNQ1 (rs2237892)

***KCNQ1*(rs22 37892)**	**STEP**	**TEMP.**	**TIME**	**NUMBER OF CYCLES**
STEP 1	Initial denaturation	95°C	5 min	1
STEP 2	Denaturation Annealing Extension	94°C 59°C 72°C	45s 45s 45s	40
STEP 3	Final extension	72°C	5 min	1

2.4 Estimation of plasma glucose

The serum plasma glucose levels in CC, CT, and TT genotypes of *TCF7L2* and *KCNQ1* in both case subjects and control individuals were quantified using the GOD-POD method, which relies on the concept that glucose is oxidised by GOD, resulting in the production of gluconic acid and hydrogen peroxide. Subsequently, the hydrogen peroxide undergoes a process of decomposition, facilitated by the enzyme peroxidase, resulting in the formation of water and nascent oxygen. Following that, the newly formed oxygen reacts with the chromogenic oxygen acceptor, 4-aminophenazone, in the presence of phenol, resulting in the production of a pigmented compound called quinoneimine. This compound may be quantified using a spectrophotometer. The colour intensity generated id directly proportional to the glucose content in the sample. To determine the glucose concentration, 10 µL of serum from each blood sample was taken into separate test tubes, and to this, 1 mL of a glucose reagent containing GOD-POD was added. Simultaneously, blank and standard were maintained by taking 10 µL of distilled water and 100 mg/dL concentrated glucose solutions. The contents were mixed thoroughly and allowed to incubate for 10-15 minutes at a temperature of 37ºC. The colour intensity was quantified at a wavelength of 505 nm using spectrophotometry. The glucose concentration in the test sample was determined using the following formula:

$$Glucose(mg/dL) = \frac{Abof test - Abof blank}{Abof standard} X100$$

2.5 Statistical Analysis

The number and frequency of genotypes are obtained by counting and percent. The distribution of the genotype frequencies of *TCF7L2* (rs7903146) and *KCNQ1* (rs2237892)

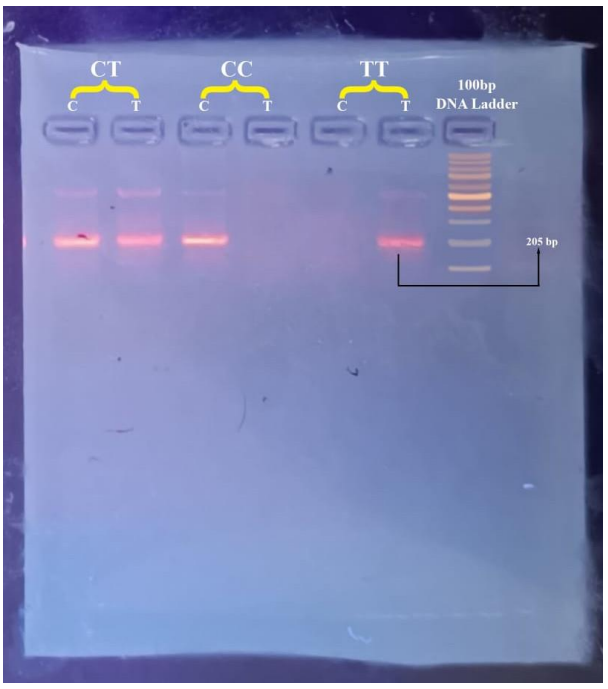
polymorphisms and Hardy-Weinberg Equilibrium among patients and control subjects were calculated using the chi-squared test. The odd ratio (OR) and its corresponding 95% confidence interval (CI) were also measured to assess the vulnerability of the disease. The OR and 95% CI were calculated for the homozygous, heterozygous, and recessive models, and allele models. P value < 0.05 was considered as statistically significant. A Turkey's HSD test was performed to find the association between the plasma glucose quantities with the respective polymorphic genotypes of *TCF7L2* (rs7903146) and *KCNQ1* (rs2237892)in T2DM patients as well as healthy subjects.

3. Results

3.1 Molecular Analysis of *TCF7L2* C/T (rs7903146) polymorphism

Genotyping of the *TCF7L2* rs7903146 (C/T) polymorphism was successfully performed using a single-tube allele-specific PCR approach employing three primers (one common reverse primer and two allele-specific forward primers). The amplification yielded a distinct fragment of 205 bp, which was used for genotype determination. Individuals showing amplification (Figure 1) with only the C-allele–specific primer were classified as CC genotype, those with amplification only for the T-allele–specific primer were classified as TT genotype, while samples exhibiting amplification for both allele-specific reactions were identified as CT heterozygotes. The clear and reproducible 205 bp bands enabled unambiguous discrimination of all three genotypes.

Figure 1. Amplified TCF7L2 C/T (rs7903146) gene on 2% agarose gel



The genotype distribution (Table 4) of *TCF7L2* rs7903146 differed significantly between T2DM patients and healthy controls. The TT genotype was significantly more frequent in T2DM subjects compared to controls (p = 0.007; OR = 2.05), while the CC genotype showed a protective association (p = 0.014; OR = 0.41). The CT genotype did not show a significant association with T2DM.

Table 4.

Genotype Distribution and Association Analysis of TCF7L2 (rs7903146) C/T Polymorphism in T2DM Cases and Healthy Controls

Statistical parameter		***TCF7L2* C/T (rs7903146)**		
Genotypes		**CC**	**CT**	**TT**
T2DM subjects (N = 136)	No.	26	69	41
	%	19.11	50.74	30.15
	Frequency	0.19	0.51	0.3
Healthy subjects (N = 136)	No.	64	52	20
	%	47.06	38.24	14.71
	Frequency	0.47	0.38	0.15
χ^2value		6.041	2.388	7.229
Fisher 's exact test		0.406	1.327	2.05
p value		0.014	0.122	0.007
df		1	1	1

OR (95% CI)	0.4063 (0.243-0.679)	1.3269 (0.8620-2.0425)	2.050 (1.1420-3.6799)

Allelic analysis (Table 5) revealed a significantly higher frequency of the T allele among T2DM patients than controls (p = 0.048; OR = 2.20), whereas the C allele showed no significant association. These findings indicate that the T allele and TT genotype of *TCF7L2* rs7903146 are associated with increased susceptibility to T2DM in the Andhra Pradesh population.

Table 5.

Allele Frequency Distribution and Association Analysis of TCF7L2 (rs7903146) C/T Polymorphism in T2DM Cases and Healthy Controls

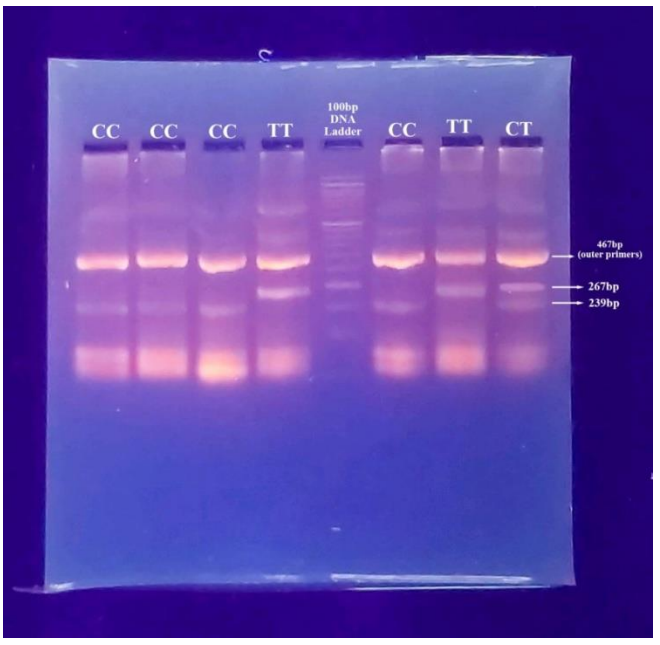
Statistical parameter		***TCF7L2* C/T (rs7903146)**	
Alleles		**C**	**T**
T2DM subjects (N = 136)	No.	114	22
	%	83.5	16.5
	Frequency	0.835	0.165
Healthy subjects (N = 136)	No.	126	10
	%	92.5	7.5
	Frequency	0.925	0.075
χ^2value		0.600	4.50
Fisher ‘s exact test		0.9048	2.2
p value		0.4386	0.0488
df		1	1
OR (95% CI)		0.9048 (0.6392-1.1281)	2.20 (1.0041 - 4.8204)

3.2 Molecular Analysis of *KCNQ1* C/T (rs2237892) polymorphism

Genotyping of the *KCNQ1* C/T (rs2237892) polymorphism was successfully carried out using the tetra-ARMS PCR technique. Agarose gel electrophoresis (Figure 2) showed a consistent outer control fragment of 467 bp in all samples, confirming successful amplification.

Figure 2.

Amplified KCNQ1 C/T(rs2237892) on 3% agarose gel



Allele-specific inner fragments were observed at 267 bp for the T allele and 239 bp for the C allele, allowing clear discrimination of genotypes. Samples displaying the 239 bp fragment along with the control band were classified as CC homozygotes, whereas samples showing the 267 bp fragment were identified as TT homozygotes. Individuals exhibiting both 239 bp and 267 bp fragments in the presence of the 467 bp control band were designated as CT heterozygotes.

The genotype distribution of the *KCNQ1* rs2237892 (C/T) polymorphism in T2DM patients and healthy controls is presented in Table 6. The CC genotype was predominant in both groups, with no significant difference between T2DM subjects and controls (p = 0.662). The CT genotype showed a higher frequency in T2DM patients compared to controls; however, this association did not reach statistical significance (p = 0.052; OR = 3.33). The TT genotype was rare in both groups and showed no significant association with T2DM.

Table 6.

Statistics of genotypes from KCNQ1 C/T gene polymorphism in T2DM cases and healthy individuals

Statistical parameter		***KCNQ1* C/T(rs2237892)**		
Genotypes		**CC**	**CT**	**TT**
T2DM subjects (N = 136)	No.	125	10	1
	%	91.9	7.35	0.74
	Frequency	0.919	0.073	0.0074
Healthy subjects (N = 136)	No.	132	3	1
	%	97.05	2.21	0.74
	Frequency	0.975	0.022	0.0074
χ^2value		0.1907	3.7692	-
Fisher ‘s exact test		0.947	3.33	-
p value		0.6624	0.0522	0.502
df		1	1	-
OR (95% CI)		0.9470 (0.6733-1.3319)	3.333 (0.8977-12.377)	3.0 (0.0121-74.23)

Allelic distribution analysis (Table 7) revealed comparable frequencies of the C and T alleles in both T2DM patients and healthy individuals, with no significant association observed (p > 0.05). These results indicate that the *KCNQ1* rs2237892 polymorphism was not significantly associated with T2DM in the studied Andhra Pradesh population.

Table 7.

Allele frequencies and statistics of KCNQ1 C/Tgene polymorphism in T2DMand healthy individuals

Statistical parameter		***KCNQ1* C/T(rs2237892)**	
Alleles		**C**	**T**
T2DM subjects (N = 136)	No.	135	1
	%	99.63	0.37
	Frequency	0.9963	0.0037
Healthy subjects (N = 136)	No.	135	1
	%	99.63	0.37
	Frequency	0.9963	0.0037
χ^2value		0.0	0.0
Fisher ‘s exact test		0.947	0.0
p value		0.6624	0.502
df		1	1
OR (95% CI)		1.0 (0.7141-1.4004)	1.0 (0.0619 - 16.1518)

3.3 Genotype–Phenotype Correlation Analysis of *TCF7L2* and *KCNQ1* Polymorphisms with Plasma Glucose Levels

3.3.1 Correlation of Plasma Glucose Levels with *TCF7L2* rs7903146 Genotypes

The descriptive statistics of plasma glucose levels stratified by *TCF7L2* rs7903146 (C/T) genotypes in T2DM patients and healthy subjects are presented in Table 8. Among T2DM patients, individuals carrying the CT genotype exhibited the highest mean plasma glucose levels (155 mg/dL), followed by TT (142 mg/dL) and CC genotypes (137.7 mg/dL). In healthy subjects, plasma glucose levels were comparatively higher in CT genotype carriers (98.5 mg/dL) than in CC (86.1 mg/dL) and TT (90.6 mg/dL) genotypes.

One-way ANOVA analysis demonstrated a statistically significant difference in plasma glucose levels among *TCF7L2* genotypes in T2DM patients (F = 6.21, Table 9). Post hoc Tukey's HSD analysis (Table 10) revealed a significant increase in plasma glucose levels in CT genotype carriers compared to CC ($p = 0.006$) and TT ($p = 0.043$) genotypes, whereas the difference between CC and TT genotypes was not statistically significant ($p > 0.05$).

Similarly, in healthy subjects, one-way ANOVA showed a significant genotype-dependent variation in plasma glucose levels (F = 8.39, Table 11). Tukey's HSD post hoc analysis (Table 12) indicated that individuals with the CT genotype had significantly higher plasma glucose levels compared to CC ($p = 0.001$) and TT ($p = 0.040$) genotypes, while no significant difference was observed between CC and TT genotypes.

Overall, the analysis demonstrates a significant association between the CT genotype of *TCF7L2* rs7903146 and elevated plasma glucose levels in both T2DM patients and healthy individuals, indicating a genotype-dependent influence of this polymorphism on glucose regulation

Table 8.

Descriptive statistics of plasma glucose levels in T2DM patients and healthy subjects with CC, CT, and TT genotypes of TCF7L2 C/T (rs7903146) polymorphism

S.No	Statistical parameter	T2DM patients			Healthy subjects		
		CC	CT	TT	CC	CT	TT
1	Mean	137.7	155	142	86.1	98.5	90.6
2	Standard deviation	8.88	13.61	11.30	6.87	5.04	8.26
3	Standard error	2.81	4.30	3.57	2.17	1.59	2.61
4	Variance	78.9	185.11	127.78	47.21	25.38	68.26
5	Kurtosis	-0.854	0.209	-0.541	-0.975	2.442	0.053
6	Skewness	0.294	0.296	0.527	-0.524	-1.241	0.380
7	Range	26	47	35	19	18	27
8	Minimum	127	132	128	75	87	78
9	Maximum	153	179	163	94	105	105
10	Confidence level (95%)	6.354	9.733	8.086	4.915	3.604	5.911

Table 9.

One way ANOVA analysis of plasma glucose levels in T2DM subjects with CC, CT, and TT genotypes of TCF7L2 C/T (rs7903146) polymorphism

Source	SS	df	Ms	F
Treatment (between groups)	1622.6	2	811.3	6.21
Within groups	3526.1	27	130.5963	
Total	5148.7	29		

Table 10.

Tukey's HSD analysis of plasma glucose levels in T2DM subjects with CC, CT, and TT genotypes of TCF7L2 C/T (rs7903146) polymorphism

Pairwise comparisons	Tukey's HSD	Q value	P value
CC : CT	17.30	4.79	0.00601
CC : TT	4.30	1.19	0.68102
CT : TT	13.0	3.60	0.0435

Table 11.

One way ANOVA analysis of plasma glucose levels in healthy subjects with CC, CT, and TT genotypes of TCF7L2 C/T (rs7903146) polymorphism

Source	SS	df	Ms	F
Treatment (between groups)	788.0667	2	394.033	8.39

Within groups	1267.8	27	46.9553
Total	2055.8667	29	

Table 12.

Tukey's HSD analysis ofplasma glucose levels in healthy subjects with CC, CT, and TT genotypes of TCF7L2 C/T (rs7903146) polymorphism

Pairwise comparisons	Tukey's HSD	Q value	P value
CC : CT	12.40	5.72	0.0011
CC : TT	4.50	2.08	0.3215
CT : TT	7.90	3.65	0.0404

3.3.2 Correlation of Plasma Glucose Levels with *KCNQ1* rs2237892 Genotypes

The descriptive statistics of plasma glucose levels according to *KCNQ1* rs2237892 (C/T) genotypes in T2DM patients and healthy subjects are summarized in Table 13. Among T2DM patients, individuals carrying the CT genotype showed the highest mean plasma glucose levels (162.8 mg/dL), followed by CC (152.2 mg/dL) and TT genotypes (142.4 mg/dL). In contrast, plasma glucose levels in healthy subjects were comparable across CC, CT, and TT genotypes, with mean values remaining within the normal range.

One-way ANOVA revealed a significant difference in plasma glucose levels among *KCNQ1* genotypes in T2DM patients (F = 11.33, Table 14). Post hoc Tukey's HSD analysis (Table 15) indicated a highly significant difference between CT and TT genotypes (p = 0.0002), while comparisons between CC and CT (p = 0.051) and CC and TT (p = 0.075) did not reach statistical significance.

In healthy subjects, one-way ANOVA showed no significant variation in plasma glucose levels across *KCNQ1* genotypes (F = 0.50, Table 16). Consistently, Tukey's HSD post hoc analysis (Table 17) demonstrated no significant differences among any genotype pairwise comparisons (p > 0.05).

Overall, these findings suggest that the *KCNQ1* rs2237892 polymorphism is associated with genotype-dependent differences in plasma glucose levels in T2DM patients, particularly between CT and TT genotypes, whereas no such association was observed in healthy individuals.

Table 13.

Descriptive statistics of plasma glucose levels in T2DM patients and healthy subjects with CC, CT, and TT genotypes of KCNQ1 C/T (rs2237892) polymorphism

S. No.	Statistical parameter	T2DM patients			Healthy subjects		
		CC	CT	TT	CC	CT	TT
1	Mean	152.2	162.8	142.4	89.5	86.5	86.6
2	Standard deviation	10.96	10.58	6.58	8.003	8.46	6.15
3	Standard error	3.46	3.35	2.08	2.53	2.68	1.95
4	Variance	120.17	111.96	43.38	64.06	71.61	37.82
5	Kurtosis	-0.267	-0.634	-1.046	-1.355	-0.816	-0.126
6	Skewness	0.737	0.533	-0.1882	0.194	-0.479	0.031
7	Range	34	33	20	24	25	21
8	Minimum	139	149	132	78	73	76
9	Maximum	173	182	152	102	98	97
10	Confidence level (95%)	7.842	7.569	4.711	5.725	6.054	4.399

Table 14.

One way ANOVA analysis of plasma glucose levels in T2DM cases with CC, CT, and TT genotypes of KCNQ1 C/T (rs2237892) polymorphism

Source	SS	df	Ms	F

Treatment (between groups)	2081.8667	2	1040.933	11.33
Within groups	2479.6	27	91.837	
Total	4561.4667	29		

Table 15.

Tukey's HSD analysis ofplasma glucose levels in T2DM cases with CC, CT, and TT genotypes of KCNQ1 C/T (rs2237892) polymorphism

S. No.	Pairwise comparisons	Tukey's HSD	Q value	P value
1	CC : CT	10.60	3.50	0.0506
2	CC : TT	9.80	3.23	0.0749
3	CT : TT	20.40	6.73	0.0002

Table 16

One way ANOVA analysis of plasma glucose levels in healthy subjects with CC, CT, and TT genotypes of KCNQ1 C/T (rs2237892) polymorphism

Source	SS	df	Ms	F
Treatment (between groups)	58.066	2	29.033	0.5
Within groups	1561.4	27	57.8296	
Total	1619.4667	29		

Table 17.

Tukey's HSD analysis ofplasma glucose levels in healthy subjects with CC, CT, and TT genotypes of KCNQ1 C/T (rs2237892) polymorphism

S. No.	Pairwise comparisons	Tukey's HSD	Q value	P value
1	CC : CT	3.0	1.25	0.6559
2	CC : TT	2.90	1.21	0.6741
3	CT : TT	0.10	0.04	0.9995

4. Discussion

Recent GWAS have found multiple genetic loci linked to T2DM risk and glycemic control, notably variations in the *TCF7L2* and *KCNQ1* genes, which have received considerable attention (16).

The *TCF7L2* rs7903146 has become one of the most important genetic loci linked to vulnerability to T2DM. The rs7903146 C/T polymorphism, situated in an intronic area, is associated with modified gene function and heightened susceptibility to T2DM in many groups (16) (17)(16) indicated that the rs7903146 polymorphism in *TCF7L2* is consistently associated with diminished insulin production and an elevated risk of T2DM. Multiple studies, including (17and (18), have established a significant correlation between the T allele of rs7903146 and an elevated risk of T2DM, presumably attributable to its influence on insulin production and β-cell functionality (19). The T allele enhances *TCF7L2* expression, affecting the Wnt signaling pathway and consequently disrupting glucose metabolism. Identifying this variation in the promoter region is thus crucial for comprehending the genetic foundations of T2DM.

The *KCNQ1* gene is essential for the controlling of pancreatic β-cell functionality and insulin release. The rs2237892 C/T polymorphism, in the promoter region of *KCNQ1*, has been extensively researched for its correlation with T2DM, especially in Asian populations (20). The C/T polymorphism at rs2237892 in *KCNQ1* is associated with vulnerability to T2DM, especially in Asian populations, where the T allele is connected to reduced insulin production and inadequate glycemic control (21). Comprehending the impact of these genetic variations on glycemic regulation may improve risk assessment and inform tailored management approaches for patients with T2DM (22). Therefore, the present study sought to examine the relationship between the polymorphisms of *TCF7L2* C/T (rs7903146) and *KCNQ1* C/T (rs2237892) and plasma glucose concentrations in patients with T2DM.

The current investigation revealed the frequencies of the CC, CT, and TT genotypes of *TCF7L2* rs7903146 in T2DM participants to be 0.19, 0.51, and 0.30, respectively. The frequencies in

healthy controls were 0.47 (CC), 0.38 (CT), and 0.15 (TT). Statistical analysis indicated a strong correlation between the CC and TT genotypes and T2DM ($p = 0.014$ for CC and $p = 0.007$ for TT). The odds ratio (OR) for the CC genotype was 0.4063 (95% CI: 0.243–0.679), signifying a protective effect against T2DM, whereas the TT genotype exhibited an OR of 2.050 (95% CI: 1.142–3.679), indicating a greater than twofold elevated risk for T2DM in persons possessing the TT genotype. The CT genotype exhibited no significant connection ($p = 0.122$, OR = 1.3269, 95% CI: 0.8620–2.0425). The findings align with earlier research by (16) and (18) which indicated that the T allele of the *TCF7L2* rs7903146 SNP is associated with an elevated risk for T2DM, but the C allele (CC genotype) appears to have a protective effect.

In a recent study from India, (23) found that people with the T allele of the rs7903146 (C/T) SNP had a 1.96-fold higher risk of T2DM, and that the risk was two-fold for those with the C allele. (24) however, found a *TCF7L2* rs7903146 T allele frequency similar to that of Caucasians in research done in Ghana, albeit with a little weaker correlation to T2DM. (25) also reported that the *TCF7L2* risk allele is linked with a 50% increased risk of T2DM, with a population-attributable risk varying from 10% to 25%, contingent upon allele frequency. A study by (19) emphasized the effect of the T allele of rs7903146 in *TCF7L2* on the enteroinsular axis and its role in the interaction between the incretin hormone gastric inhibitory polypeptide and its target hormones, glucagon and insulin. (26) have shown that *TCF7L2*-mediated regulation of postprandial lipid metabolism may influence diabetes risk.(27) identified a strong correlation between serotonin levels and the rs7903146 genotype in an investigation that controlled for T2DM and other prevalent metabolites.

In the present study, the highest mean plasma glucose was observed in the CT genotype (155 mg/dL), followed by the TT (142.6 mg/dL) and CC (137.7 mg/dL) genotypes. The standard deviation was greatest in the CT group (43.60), indicating more variability in glucose levels among these individuals. The presence of the T allele has been postulated to reduce insulin secretion, leading to poor glycemic control (19). The higher plasma glucose levels observed in T allele carriers (CT and TT genotypes) in both patient and control groups support this mechanism. Several studies have corroborated that the T allele of *TCF7L2* rs7903146 is a strong genetic determinant of both T2DM risk and elevated plasma glucose (18; 19). The present data are consistent with these findings, emphasizing the predictive value of this polymorphism for hyperglycemia and diabetes susceptibility. Several studies, such as (19) and (17) have shown that *TCF7L2* variants can predict diabetes risk in individuals with impaired glucose tolerance, consistent with the significantly elevated glucose levels in the present study in individuals carrying these genotypes. (28) have demonstrated that overexpression of *TCF7L2* in mice displays reciprocal phenotypes, including increased plasma insulin levels and glucose intolerance due to peripheral insulin resistance, indicating that overexpression of *TCF7L2*leads to a phenotype of T2DM. (29) reported that the plasma glucose levels in the genotypes of rs7903146 in study participants, the mean plasma concentration of glucose, were significantly higher in subjects with the CT genotype than in subjects with the CC genotype (7.52±3.48 mmol/L vs. 7.05±3.39 mmol/L, $P = 0.009$).

The three main pathways offer possible explanations for the association between T2DM and the rs7903146 risk genotypes for both CT and TT. First, it has been shown that the T allele of *TCF7L2* rs7903146 makes beta cells less active, which lowers the disposition index and proinsulin secretory efficiency. Hyperglycemia can be worsened because it reduces insulin's capacity to control glucose synthesis in the liver (30). Beta cell dysfunction, a major factor in the development of T2DM, results from mutations in the *TCF7L2* gene, which disrupts the transcriptional control of target genes. Secondly, adipocyte *TCF7L2* expression and rs7903146 risk alleles have been linked to postprandial triglyceride dysmetabolism. Visceral adipose tissue *TCF7L2* expression was shown to be increased and associated with postprandial triglycerides and glycemia in a study of Asian Indians with glucose intolerance. According to (26), the modification of postprandial lipid metabolism by *TCF7L2* could increase the risk of diabetes. The *TCF7L2* gene encodes a transcription factor with an HMG box associated with blood glucose regulation. Its role is thought to pertain to the regulation of proglucagon gene expression in enteroendocrine cells via the Wnt signaling pathway (16).

Polymorphisms in *KCNQ1*, specifically rs2237892 (C/T), have been associated with disrupted glucose homeostasis and heightened risk of T2DM across several groups (21). The CT genotype group demonstrates the highest mean plasma glucose level (162.8 mg/dL), succeeded by CC (152.2 mg/dL) and TT (142.4 mg/dL). The standard deviations for CC and CT are comparable; however, they are significantly lower for TT, indicating less variability in plasma glucose among TT carriers. The mean glucose levels in CT and TT genotypes are somewhat lower than in CC, although the differences are less dramatic than those observed in T2DM patients.

The data indicate that in T2DM patients, the CT genotype of *KCNQ1* rs2237892 correlates with the highest mean plasma glucose, whereas the TT genotype is related to lower glucose levels. This indicates a potential risk correlation for the CT genotype, as elevated mean glucose levels signify inferior glycemic control and heightened diabetes risk. In healthy individuals, the influence of *KCNQ1* genotypes on plasma glucose is minimal, with only slight variations among genotypes. These results may suggest that the influence of the *KCNQ1* polymorphism is amplified by the existence of additional risk factors or underlying diabetic disease.

Prior research indicates that the T allele of rs2237892 in *KCNQ1* correlates with heightened T2DM risk and compromised glucose metabolism, particularly in Asian cohorts (31). Nevertheless, data suggests that the risk relationship may be contingent upon context and shaped by gene-environment interactions. (32) discovered a correlation between fasting plasma glucose levels and the C risk allele of rs2237892, indicating that basal insulin secretion was compromised in CC homozygotes of rs2237892. (33) discovered that the *KCNQ1* gene may influence T2DM through mechanisms unrelated to weight gain. (34) The study revealed for the first time that the *KCNQ1* rs2237892 polymorphism is associated with gestational diabetes mellitus and glucose levels in Chinese women. The research offers systematic evidence for the correlation between this polymorphism and gestational diabetes mellitus in Asian populations.

5. Conclusion

The present study demonstrated a significant association between the *TCF7L2* rs7903146 polymorphism and T2DM susceptibility in the Andhra Pradesh population, with the TT genotype and T allele conferring increased risk, while the CC genotype exhibited a protective effect. In contrast, the *KCNQ1* rs2237892 polymorphism did not show a significant molecular association with T2DM risk, indicating population-specific genetic heterogeneity. Genotype–phenotype analysis revealed that *TCF7L2* rs7903146 CT genotype carriers exhibited elevated plasma glucose levels in both T2DM patients and healthy individuals. Additionally, *KCNQ1* rs2237892 showed a genotype-dependent influence on plasma glucose levels exclusively among T2DM patients, suggesting a modulatory role in glucose regulation rather than direct disease susceptibility. Overall, these findings highlighted *TCF7L2* rs7903146 as a key genetic determinant of T2DM risk, while emphasizing the importance of integrating genetic and biochemical parameters to improve the understanding of T2DM pathophysiology.

6. Declarations

Ethics Approval and Consent to Participate

The study was conducted in accordance with the ethical standards of the institutional research committee and informed written consent was obtained from all participants prior to sample collection.

Competing Interests

The authors declare that they have no competing interests.

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7. Abbreviations

CI - Confidence interval

OR – Odds ratio

SNP - Single nucleotide polymorphism

T2DM - Type 2 Diabetes Mellitus

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