Association of TCF7L2 Gene Polymorphisms (rs7903146 and rs7901695) with Type 2 Diabetes Mellitus and Gestational Diabetes Mellitus in East Godavari, Andhra Pradesh, India

Surekha Aravalamma Dasari*, Hima Girisha Dasari, Sai Kandula, Sudhakar Godi

Research Scholar Andhra University Visakhapatnam, Medico GSL Hospital Rajahmundry,
Diabetologist Kandula Sai Diabetic Care Hospital, Registrar in Nannaya University Rajahmundry.

ABSTRACT

Introduction:

Type 2 Diabetes Mellitus (T2DM) and Gestational Diabetes Mellitus (GDM) are prevalent metabolic disorders with rising incidence in India. Variants in the TCF7L2 gene, particularly rs7903146 (C>T) and rs7901695 (T>C), have been linked to T2DM risk, but their role in GDM remains unclear in specific regional populations.

Objectives:

This study investigated the association of TCF7L2 polymorphisms with T2DM and GDM in individuals from East Godavari, Andhra Pradesh.

Materials and Methods:

A case-control study was conducted with 150 T2DM patients, 150 GDM patients, and 300 healthy controls (150 per group). Genomic DNA was isolated from peripheral blood, and SNP genotyping was performed using PCR. Statistical analysis included allele and genotype frequency comparisons, odds ratios (OR), 95% confidence intervals (CI), chi-square tests, and assessment of the Hardy-Weinberg Equilibrium (HWE).

Results:

Significant associations were observed between both SNPs and type 2 diabetes mellitus (T2DM). The T allele of rs7903146 and the C allele of rs7901695 were significantly lower in cases than controls (T: 33.33% vs. 77.67%, OR = 0.0075; C: 35% vs. 78%, OR = 0.0091; p = 0.0001). Both SNPs deviated from HWE in T2DM cases. No significant association was found for GDM, with similar allele frequencies between cases and controls, and no significant deviation from HWE for rs7901695.

Conclusion:

TCF7L2 polymorphisms are significantly associated with T2DM but not with GDM in this population, indicating a genetic predisposition to T2DM in East Godavari.

Keywords:

Type 2 Diabetes Mellitus (T2DM), Gestational Diabetes Mellitus (GDM), TCF7L2 gene, rs7903146, rs7901695, Single Nucleotide Polymorphisms (SNPs), Genetic association

Introduction

Diabetes mellitus is a multifactorial metabolic disorder characterized by chronic hyperglycemia due to impaired insulin secretion, insulin action, or both. Among its variants, Type 2 Diabetes Mellitus (T2DM) is the most prevalent, with increasing incidence globally and a particularly steep rise in India due to rapid urbanization, sedentary lifestyles, and genetic predisposition (International Diabetes Federation [IDF], 2021). Gestational Diabetes Mellitus (GDM), defined as glucose intolerance first recognized during pregnancy, is also a growing public health concern, with implications for both maternal and fetal health (American Diabetes Association [ADA], 2022).

Genetic factors are known to significantly influence the pathogenesis of both T2DM and GDM. Among the many loci identified, the Transcription Factor 7-Like 2 (TCF7L2) gene on chromosome 10q25.2 has emerged as one of the strongest genetic risk factors for T2DM (Grant et al., 2006). Polymorphisms such as rs7903146 (C>T) and rs7901695 (T>C) within this gene have been extensively studied for their role in insulin secretion and β-cell function. These single-nucleotide polymorphisms (SNPs) are believed to modulate Wnt signaling pathways, which are critical in pancreatic islet cell development and glucose homeostasis (Zhou et al., 2014).

While several studies have confirmed the association of TCF7L2 polymorphisms with T2DM across various ethnicities (Lyssenko et al., 2007; Saxena et al., 2006), the evidence linking these variants to GDM is inconsistent and likely population-specific (Zhang et al., 2013). In South Asian populations, including those in India, limited data exist regarding the role of TCF7L2 polymorphisms in GDM, particularly in underrepresented rural regions such as East Godavari in Andhra Pradesh.

Objective:

The present study aims to investigate the association of TCF7L2 gene polymorphisms rs7903146 and rs7901695 with T2DM and GDM among individuals from East Godavari, Andhra Pradesh. Understanding these genetic links could provide valuable insight into the molecular basis of diabetes in this regional population and inform early risk prediction and targeted intervention strategies.

METHODOLOGY:

In this study, the genomic DNA extraction from whole blood was done using NucleoSpin Blood starting by lysis of samples with buffer which contains chaotropic ions and Proteinase K, with later DNA binding on silica membrane in NucleoSpin Blood Columns, washing to remove the contaminants and elution with a small amount of slightly basic buffer for obtaining of PCR-grade genomic DNA. The blood samples were taken using heparinized EDTA tubes, and any clotted samples were first centrifuged. The Buffy coat was pipetted into microcentrifuge tubes containing Proteinase K and B3 buffer, allowed to stand, and treated with ethanol to facilitate DNA binding. After the centrifugation, the silica membranes were washed with BW and B5 buffers to remove excess ethanol from the membrane, and the DNA was eluted with BE buffer. The DNA solution obtained was then placed on ice, aliquoted, and stored at – 20°C for further use.

Estimation of DNA was done by determining its concentration with the help of a spectrophotometer kept at 260 nm. Choosing the absorbance range of 0.15 to 1. Actually, at this wavelength, the A260 value was important for checking DNA purity, where the A260/A280 ratio was used. A ratio close to 1 was used for this technique. It is content and 8 typically relate to pure DNA, while any variations can be interpreted to mean contamination with protein or phenol, among others. The DNA concentration (µg/ml) was calculated using the formula.

To check the quality of genomic DNA, a 1% agarose gel was prepared, and the sample was run through it. The low percentage was used to prepare the gel, which required 0.8 g agarose in 100 mL 1X TEB containing ethidium bromide. Incorporating the gel and casting it to form a solid base, DNA samples were pipetted into these wells, and the Samples were subjected to electrophoresis, which ranged from 50–100 volts. The movement of DNA through the gel under an electric current facilitated the use of a UV transilluminator, which aided in the determination of the presence and quality of the DNA sample.

The protocol followed for genotyping the rs7901695 and rs7903146 polymorphisms was the Real-Time PCR technique that used Allele Refractory Mutation System (ARMS) PCR. Optimal conditions for this SNP genotyping involved the use of specific primers as well as PCR cycling conditions. Next, genomic DNA was isolated employing the NucleoSpin Blood Kit; likewise, normal and mutant PCR master mixes were created. The ABI 7500 Real-Time PCR System was set with thermal parameters such as the initial step and the amplification steps. The samples were then put in a 96-well PCR plate, properly sealed, and spun in the PCR before initiating it. When it came to data analysis, we included analysis of fluorescence data and identification of genotypes; moreover, we ensured the reliability of the assay with quality control and validation steps.

Primer sequences			
Primer	**Sequence**	**Annealing temperature**	**Amplicon length (bp)**
7903146F	GCTAAGCACTTTTTAGATAC	60^{0C}	247
7903146R	ACTATGTATTGTTGCCAGTC		
7901695F	AATGGTATCATAAAATCTAC	64^{0C}	207
7901695R	TGTGCAAAATGTTCATAGTA		

Data analysis was done on Simple frequency count, and quantitative characteristics include: mean ± SD using SPSS version 16. These measures were important for evaluating the epidemiological and molecular variables starting from the patients and up to the control subjects. Genetic variation of the population based on genotype studies was determined using the allelic frequency, which involves the frequency of homozygous and heterozygous genotypes from the total sample size. Significances of descriptive and comparative tests of allele and genotype frequencies of the groups were tested using the chi-squared (χ^2) test, which tests the association of categorical data.

Further, the t-test was used to analyze the means, evaluating potential differences of numeric patient and control values concerning molecular or epidemiological indices. To measure the association between two categorical variables, odds ratios were computed, which give the odds of an event in a given group compared to another group. They allowed the examination of relationships and comparisons of health conditions between groups, and offered a sound basis for the evaluation of results.

Results

A total of 600 individuals were included in this case-control study: 150 patients with Type 2 Diabetes Mellitus (T2DM), 150 patients with Gestational Diabetes Mellitus (GDM), and 300 healthy control subjects (150 for each case group). Genotyping for the TCF7L2 polymorphisms rs7903146 (C>T) and rs7901695 (T>C) was successfully performed using ARMS-PCR followed by gel electrophoresis and Real-Time PCR analysis.

Genotype and Allele Frequencies in T2DM vs. Controls

For the rs7903146 SNP, the T allele frequency was significantly lower in the T2DM group (33.33%) compared to controls (77.67%). The odds ratio (OR) for the T allele was 0.0075 (95% CI: 0.0010–0.0548; $p = 0.0001$), indicating a strong association between the presence of the T allele and T2DM susceptibility. Similarly, genotype frequencies showed significant differences between cases and controls, with a higher prevalence of the CC genotype in controls.

For the rs7901695 SNP, the C allele frequency was 35% in the T2DM group compared to 78% in controls. The OR was 0.0091 (95% CI: 0.0012–0.0666; $p = 0.0001$), suggesting a strong inverse association. A significant difference in genotype distribution was also observed ($p < 0.001$), with the TT genotype more frequent in T2DM patients, while the CC genotype was more common among controls.

Both SNPs (rs7903146 and rs7901695) deviated significantly from Hardy-Weinberg Equilibrium (HWE) in the T2DM patient group ($p < 0.01$), indicating possible selection bias or genetic predisposition in this population. In contrast, genotype distributions in the control group were consistent with HWE ($p > 0.05$).

Genotype and Allele Frequencies in GDM vs. Controls

In the GDM group, the T allele frequency at rs7903146 was 27.33%, compared to 20.67% in the control group. This difference was not statistically significant (OR = 1.2967; 95% CI: 0.8090–2.0784; $p = 0.2804$). Similarly, genotype distributions did not differ significantly between GDM cases and controls.

For rs7901695, the C allele frequency was 34% in the GDM group and 28% in the control group. The OR was 1.1126 (95% CI: 0.7074–1.7499; $p = 0.6442$), suggesting no meaningful association between this SNP and GDM in the studied population. Genotype distributions were also not significantly different, and both case and control groups for rs7901695 conformed to HWE ($p > 0.05$). However, the rs7903146 genotype distribution in the GDM group showed a deviation from HWE ($p = 0.0053$).

Summary of Findings

A significant association exists between TCF7L2 polymorphisms (rs7903146 and rs7901695) and Type 2 Diabetes Mellitus in the East Godavari population.

No statistically significant association was found between these polymorphisms and Gestational Diabetes Mellitus.

Deviation from HWE in T2DM and GDM cases suggests underlying genetic selection pressures or potential population stratification.

Genotype and Allele Frequencies of TCF7L2 Polymorphisms in T2DM and GDM Patients Compared to Controls

SNP	Group	Genotype Frequency (%)	Allele Frequency (%)	OR (95% CI)	p-value	HWE p-value
rs7903146	T2DM	CC: 18 CT: 65 TT: 17	C: 66.67 T: 33.33	T allele: 0.0075 (0.0010–0.0548)	0.0001	<0.01
	Control	CC: 10 CT: 25 TT: 65	C: 22.33 T: 77.67			>0.05
	GDM	CC: 15 CT: 45 TT: 40	C: 72.67 T: 27.33	T allele: 1.2967 (0.8090–2.0784)	0.2804	0.0053
	Control	CC: 20 CT: 47 TT: 33	C: 79.33 T: 20.67			>0.05
rs7901695	T2DM	TT: 22 TC: 56 CC: 22	T: 65 C: 35	C allele: 0.0091 (0.0012–0.0666)	0.0001	<0.01
	Control	TT: 8 TC: 28 CC: 64	T: 22 C: 78			>0.05
	GDM	TT: 24 TC: 42 CC: 34	T: 66 C: 34	C allele: 1.1126 (0.7074–1.7499)	0.6442	>0.05
	Control	TT: 27 TC: 45 CC: 28	T: 72 C: 28			>0.05

Discussion

This case-control study examined the association of TCF7L2 gene polymorphisms—rs7903146 (C>T) and rs7901695 (T>C)—with Type 2 Diabetes Mellitus (T2DM) and Gestational Diabetes Mellitus (GDM) in a population from East Godavari, Andhra Pradesh. The findings revealed a strong and statistically significant association of both polymorphisms with T2DM but not with GDM.

Our results showed that the T allele of rs7903146 and the C allele of rs7901695 were significantly underrepresented in the T2DM group compared to healthy controls. The OR values (0.0075 and 0.0091, respectively) indicate a protective effect of these alleles in this population. These findings are in agreement with several studies reporting that the T allele of rs7903146 is one of the strongest genetic risk factors for T2DM globally, including in South Asian populations (Grant et al., 2006; Lyssenko et al., 2007; Chandak et al., 2007).

The deviation from Hardy-Weinberg Equilibrium (HWE) observed in the T2DM group further supports a genetic predisposition or selection pressure related to these loci. This could be indicative of population-specific genetic influences or stratification, a common observation in complex traits like T2DM (Helgason et al., 2007).

In contrast, no statistically significant association was found between either SNP and GDM. Allele and genotype frequencies between GDM patients and controls did not differ substantially, and OR values were not significant. These findings suggest that the genetic etiology of GDM may differ from that of T2DM in this population, despite both conditions sharing pathophysiological features such as insulin resistance and β-cell dysfunction. This observation aligns with reports from other studies where the association of TCF7L2 variants with GDM showed inconsistencies across ethnicities and geographic regions (Zhang et al., 2013; Pervjakova et al., 2011).

The deviation from HWE observed for rs7903146 in the GDM group, despite a non-significant association, may indicate subtle population structure or small sample artifacts. However, further investigation with larger sample sizes and haplotype analysis would be necessary to elucidate the precise role of these polymorphisms in GDM susceptibility.

Overall, the strong association of TCF7L2 variants with T2DM but not GDM in this study underscores the importance of genetic context and population specificity in the interpretation of association studies. It also emphasizes the need for localized genetic epidemiology to develop effective predictive and preventive strategies in diverse regions such as East Godavari.

Conclusion

The study concludes that TCF7L2 polymorphisms rs7903146 and rs7901695 are significantly associated with T2DM in the East Godavari population, suggesting a genetic predisposition to the disease. However, no significant association was found between these polymorphisms and GDM in the same population. Further studies with larger sample sizes and diverse populations are needed to validate these findings and explore the underlying mechanisms.

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