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MTHFR Polymorphisms and Their Association with Biochemical Risk Markers in Type 2 Diabetes Mellitus Complications

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ABSTRACT

Background: Type 2 Diabetes Mellitus (T2DM) is a multifactorial disease with a strong genetic component. The MTHFR C677T polymorphism (rs1801133) has been implicated in various complications associated with T2DM. The present study investigates the role of the MTHFR C677T polymorphism in T2DM patients from Kerala, India.

Methods: A cross-sectional study was conducted, involving 40 participants, consisting of 20 T2DM patients and 20 healthy controls. Genotyping of the MTHFR C677T polymorphism was performed using polymerase chain reaction (PCR) and quantitative PCR (qPCR). Sanger sequencing was employed for genotype confirmation. Biochemical parameters, including HbA1c, serum creatinine, and microalbuminuria, were measured using standard clinical assays. The data were analyzed for genotype-phenotype associations using non-parametric tests and Spearman's correlation.

Results: The T allele of the C677T polymorphism was more prevalent in T2DM patients (37.5%) compared to healthy controls (20%). A significant association was found between the T allele and higher HbA1c levels (8.1% ± 1.6% in T2DM patients with CT/TT genotypes versus 7.6% ± 1.5% in CC genotype carriers, $p = 0.12$). Additionally, microalbuminuria levels were higher in T allele carriers (213.7 mg/L) compared to CC genotype carriers (31.0 mg/L), although this difference did not reach statistical significance ($p = 0.09$). Serum creatinine levels were also elevated in T allele carriers (1.40 mg/dL) compared to CC genotype carriers (0.85 mg/dL). Spearman's correlation analysis revealed a moderate positive correlation between HbA1c and microalbuminuria ($Rs = 0.76$, $p = 0.001$), indicating that glycemic control is linked to early renal damage in T2DM. **Conclusion:** Our study suggests that the MTHFR C677T polymorphism is associated with altered glycemic control and potential early renal complications in T2DM patients. Larger, longitudinal studies with homocysteine measurements and ethnically diverse cohorts are needed to validate these findings and better understand the role of MTHFR polymorphisms in T2DM progression.

KEYWORDS: Type 2 Diabetes Mellitus, HbA1c, Microalbuminuria, Genetic polymorphism

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder that affects millions globally and is a leading cause of morbidity and mortality. It is primarily characterized by chronic hyperglycemia due to insulin resistance and impaired insulin secretion. Over time, T2DM can lead to severe complications, including diabetic retinopathy, neuropathy, nephropathy, and cardiovascular diseases, all of which significantly impact quality of life and contribute to the burden on healthcare systems (Thahira et al., 2023; P et al., 2025). The development of these complications is influenced by a combination of genetic, biochemical, and environmental factors, which makes early risk assessment crucial for effective management and prevention.

Methylenetetrahydrofolate reductase (MTHFR) is an essential enzyme involved in folate metabolism and the regulation of homocysteine levels. Variations in the MTHFR gene, particularly the C677T and A1298C polymorphisms, have been implicated in numerous metabolic and cardiovascular diseases (Li et al., 2015). Elevated homocysteine levels, often a result

of MTHFR mutations, have been linked to endothelial dysfunction, inflammation, and an increased risk of vascular complications. While several studies have explored the association between MTHFR polymorphisms and diabetes-related complications, the role of these genetic factors, in conjunction with biochemical markers, remains underexplored, particularly in diverse populations (Araszkiewicz et al., 2025; Sabitha et al., 2024).

In this study, we conducted a cross-sectional analysis aimed at evaluating the role of MTHFR polymorphisms and selected biochemical parameters in assessing the risk of complications among patients with T2DM. Several previous studies have established the link between MTHFR gene mutations and complications such as diabetic retinopathy, nephropathy, and cardiovascular disease (Zhang et al., 2024). However, there is limited research exploring how these genetic variations interact with biochemical markers to predict the onset and progression of complications in T2DM. Therefore, this study aims to bridge this gap by investigating the combined influence of MTHFR polymorphisms and biochemical parameters, contributing to a more holistic approach in risk assessment.

By exploring these genetic and biochemical associations, this study aims to provide valuable insights into personalized risk profiling for T2DM patients. The findings may help in the early identification of individuals at high risk for complications, enabling timely interventions and more targeted therapeutic strategies. Understanding these factors will ultimately enhance the management of T2DM and potentially reduce the long-term impact of its complications.

MATERIAL AND METHODS

Study design and setting

This cross-sectional, study was conducted to evaluate the role of methylenetetrahydrofolate reductase (MTHFR) polymorphisms and selected biochemical parameters in assessing the risk of complications among patients with type 2 diabetes mellitus (T2DM). The study was conducted at a tertiary care center in Kerala, India, over a period of three to four years. The study population comprised T2DM patients and healthy controls recruited from outpatient and inpatient services. The Department of Biochemistry and Molecular Diagnostics provided laboratory support, ensuring access to wellcharacterized diabetic cohorts and complete clinical and biochemical records**.**

Study population

A total of 50 T2DM patients and 50 age- and sex-matched healthy controls were recruited for the study. Of these, 20 T2DM patients and 20 controls were selected for detailed genetic and biochemical analysis based on predefined eligibility criteria. The selection of participants with higher glycated hemoglobin (HbA1c) levels was prioritized, as these individuals are at a higher risk for vascular and metabolic complications. The study included participants aged 30–65 years, both male and female, ensuring gender representation and reflecting the epidemiological peak of T2DM. Healthy controls were confirmed to be free from diabetes and other major systemic illnesses through fasting blood glucose testing and clinical record review.

Inclusion and exclusion criteria

Participants were eligible if they met the following criteria: (1) clinically diagnosed with T2DM according to the American Diabetes Association (2023) criteria, (2) aged between 30 and 65 years, and (3) provided written informed consent. Participants were excluded if they had type 1 diabetes, gestational diabetes, or significant comorbidities (e.g., end-stage renal disease, chronic liver failure, active malignancy), or were using medications affecting glucose or lipid metabolism. Pregnant or lactating women, individuals with psychiatric disorders, or those unable to provide informed consent were also excluded.

Blood collection and processing

Venous blood samples were collected from all participants following standardized protocols. A total of 5–8 mL of blood was drawn using aseptic techniques. Blood was divided into two tubes: plain vacutainer tubes for serum separation and EDTA-coated vacutainer tubes for genomic DNA extraction. Serum was processed for biochemical assays, and whole blood was processed for DNA extraction. Blood samples were processed within 2 hours of collection, with serum aliquots stored at –20°C for short-term use and at –80°C for long-term preservation. DNA was extracted from EDTA-treated whole blood and stored at –20°C until analysis.

Biochemical assays

Biochemical analyses were performed to assess glycemic control, renal and hepatic function, lipid metabolism, and inflammatory markers. These included fasting blood sugar (FBS), glycated hemoglobin (HbA1c), fasting lipid profile, renal

function tests (serum creatinine, blood urea nitrogen, urine microalbumin), liver function tests (AST, ALT, ALP, bilirubin), and homocysteine levels. All assays were performed according to the manufacturer's instructions, ensuring analytical accuracy through calibration with reference standards and inclusion of control sera in each assay run.

Molecular analysis

Molecular analysis focused on the MTHFR gene (located on chromosome 1p36.3), specifically the C677T polymorphism (rs1801133), and promoter methylation status. Genomic DNA was extracted from peripheral blood leukocytes using a Zymag® magnetic bead-based DNA extraction kit, and the quality and concentration of DNA were assessed using a NanoDrop spectrophotometer. Only samples with an A260/A280 ratio between 1.8 and 2.0 were used for downstream analyses (See figures 1 -4).

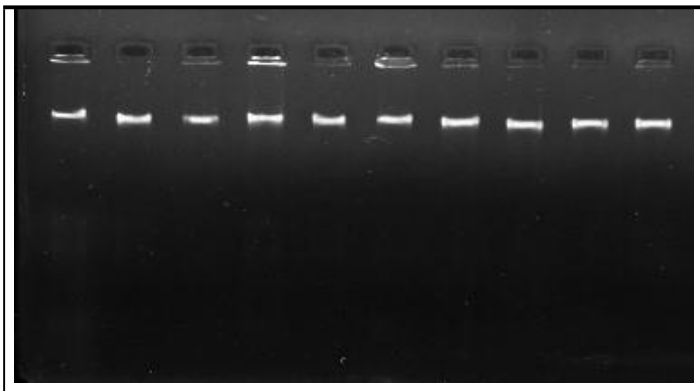

Figure 1. Agarose gel electrophoresis (AGE) image of extracted DNA from for controls.

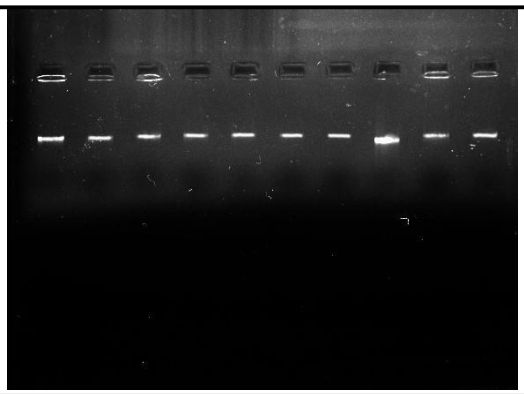

Figure 2. Agarose gelelectrophoresis (AGE) image of extracted DNA from for cases.

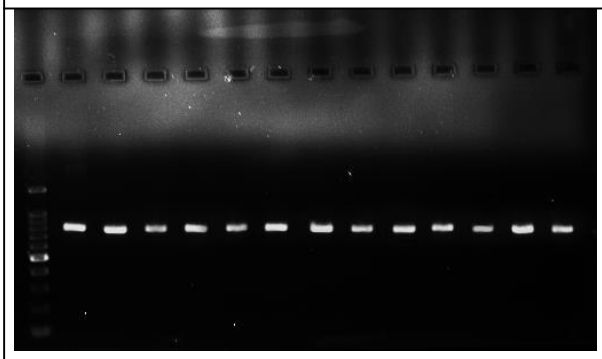

Figure 3. Agarose gel electrophoresis (AGE) image showing MTHFR gene partial amplification for controls.

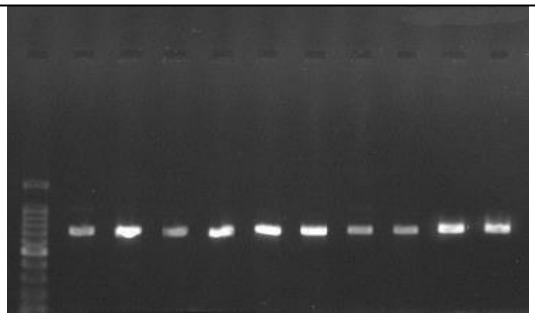

Fig 4. Agarose gel electrophoresis (AGE) image showing MTHFR gene partial amplification for cases.

PCR amplification was performed using allele-specific primers. The amplicons were analyzed by Sanger sequencing to identify genotypes (CC, CT, TT). Methylation-specific PCR (MSP) was used to assess the methylation status of the MTHFR promoter region after bisulfite conversion of genomic DNA. Two sets of primers were used to distinguish between methylated and unmethylated alleles (See table 1,2&3).

The SNP sequence used for primers and probes design is provided below:

>rs1801133 [Homo sapiens]

CCCGGGGACGATGGGGCAAGTGATGCCCATGTCGGTGCATGCCTTCACAAAGCGGAAGAATGTGTCAGCCT
CAAAGAAAAGCTGCGTGATGATGAAATCG

[G/A/C]

CTCCCGCAGACACCTTCTCCTTCAAGTGCTTCAGGTCAGCCTCAAAGCTCCCTGCTTCGGGGTGGCCTTTGG
GGTAACCTGCCAATAGGGATGACAGTCA

Primer	Sequence (5'–3')	Length (bp)	Tm (°C)	GC (%)
Forward (MTHFR LGF)	GCCAGTGCTAACTGTAG	17	56	52.9
Reverse (MTHFR LGR)	TCAAGTGGTTCTGATGAC	18	56	44.4

Table 1: Primers used for sequencing the MTHFR gene (partial)

Confirmation of SNP genotypes

Genotyping of the *MTHFR* C677T polymorphism (rs1801133) was performed by analyzing the sequencing chromatograms obtained from purified PCR amplicons. Forward and reverse sequence reads were examined using Chromas Lite and BioEdit software, and aligned against the human *MTHFR* reference sequence retrieved from the NCBI database. Genotypes were assigned on the basis of the nucleotide present at position 677 of the *MTHFR* gene (See figure 5).

Figure 5: ***Multiple sequencing alignment of sequencing result for health and patient sample. SNP region has been highlighted***

Amplification curves and allelic discrimination plots were generated and carefully analyzed to assess assay performance. Only conditions that yielded reproducible amplification efficiency (90–110%), clear separation of genotype clusters, and absence of cross-reactivity were selected for downstream analysis.

Primer/Probe	Sequence (5'–3')	Length (bp)	Tm (°C)	GC (%)
Forward	CCTGAAGCACTTGAAGGAGAA	21	62	47.6
Reverse	CAAAGACCCATTGCCTGACT	22	62	45.5
Probe G	FAM–TCTGCGGGAGCCGATTTCATCATC–BHQ1	24	68	54
Probe A	FAM–ATGAAATCGACTCCCGCAGACACC–BHQ1	24	68	54
Probe C	FAM–TCTGCGGGAGGCGATTTCATCATC–BHQ1	24	68	54

Table 2. Primers and probes used for qRT-PCR detection of MTHFR SNP rs1801133

Primer/Probe	Sequence (5'–3')
Forward	AGATTTGGACCTGCGAGCG
Reverse	GAGCGGCTGTCTCCACAAGT
Probe EC	FAM–TTCTGACCTGAAGGCTCTGCGCG–BHQ1

Table 3. Primers and probe for housekeeping gene (Human Ribonuclease P)

The inclusion of human ribonuclease P as a housekeeping control ensured that DNA integrity and amplification efficiency were consistent across reactions.

Statistical analysis

Descriptive statistics were used to summarize the demographic and clinical characteristics of the study population. Continuous variables were expressed as mean ± standard deviation (SD) or median (interquartile range) for non-normally distributed data. Group comparisons were performed using independent Student's t-test or Mann-Whitney U test for continuous variables and chi-square test for categorical data. Pearson's or Spearman's correlation coefficients were calculated to assess relationships between biochemical markers and clinical indices. Logistic regression models were used to evaluate the association between genetic polymorphisms, biochemical parameters, and T2DM complications. A p-value of <0.05 was considered statistically significant. All analysis were done in Jamovi 2.6.44.

Ethical considerations

The study was approved by the Institutional Ethics Committee (IEC) of Srinivas Institute of Medical Sciences and Research Center in accordance with the Indian Council of Medical Research (ICMR) guidelines. Written informed consent was obtained from all participants, and confidentiality was maintained throughout the study. All procedures adhered to ethical principles outlined in the Declaration of Helsinki.

RESULTS

The study included 100 participants, consisting of 50 T2DM patients (cases) and 50 non-diabetic controls. Gender distribution was homogeneous, with 25 males and 25 females in both the control and diabetic groups. The median age for the control group was 47 years (range: 31–58 years), while the T2DM group had a median age of 44 years (range: 31–60 years). The duration of diabetes in the T2DM group had a median of 3 years (range: 1–12 years) (See table 4).

Parameter	**Controls (n = 50)**	**T2DM Cases (n = 50)**	**Kruskal–Wallis P-value**
Age (years), **mean ± SD**	44.5 ± 8.2	45.0 ± 7.9	-
Sex (M/F)	25/25	25/25	-
Duration of Diabetes in Years (Median with range)	0	3 (1–12)	-
FBS (mg/dL)	90 (84 – 109)	140 (84 – 478)	< .00001
HbA1c (%)	5.1 (4.6 – 5.4)	8.1 (5.4 – 18.0)	< .00001
Serum Creatinine (mg/dL)	0.85 (0.37 – 1.35)	1.40 (0.67 – 2.10)	< .00001
Microalbumin (mg/L)	31.0 (12 – 89)	213.7 (28 – 378.2)	< .00001
Total Cholesterol (mg/dL)	183 (132 – 244)	190 (93 – 318)	< .00001
Triglycerides (mg/dL)	105 (40 – 200)	160 (40 – 358)	< .00001
HDL (mg/dL)	41 (25 – 58)	36 (20 – 60)	0.00001
LDL (mg/dL)	131 (46 – 195)	120 (52 – 244)	< .00001
SGOT (U/L)	23 (16 – 41)	19 (11 – 75)	0.00005
SGPT (U/L)	27 (10 – 72)	21 (5 – 61)	0.00002
Hemoglobin (g/dL)	14.2 (7.4 – 17.2)	13.7 (10.5 – 18.0)	0.0003
Total Leukocyte Count	7000 (4400 – 11100)	7800 (4200 – 13000)	0.0006
Neutrophils (%)	61 (50 – 80)	63 (37 – 81)	0.0035
Lymphocytes (%)	33 (15 – 44)	30 (17 – 59)	0.0017
Eosinophils (%)	3 (1 – 8)	3 (0 – 14)	0.0063
Monocytes (%)	3 (2 – 7)	3 (2 – 6)	0.0082

Table 4: Comparison of Biochemical and Hematological Parameters Between T2DM Patients and Healthy Controls.

MTHFR polymorphism

A total of 40 samples were (20 cases and 20 controls) were successfully extracted and processed for genotyping for MTHFR C677T (rs1801133) polymorphism using a combination of polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) and quantitative PCR (qPCR) allelic discrimination assays. Genotype Frequencies were 40% CC, 45% CT, and 15% TT in T2DM cases and in controls: 65% CC, 30% CT, and 5% TT. The T allele was present at 37.5% in T2DM cases compared to 20% in controls, indicating an increased prevalence of the mutant allele (T) in the T2DM group. These results suggest a higher proportion of heterozygous (CT) and homozygous (TT) genotypes for the C677T SNP in the diabetic cohort, consistent with an association between this polymorphism and T2DM susceptibility (See table 5 and Figures 6-10).

Genotype	**T2DM Cases (n = 20)**	**Controls (n = 20)**	**p-value**

CC	40%	65%	-
CT	45%	30%	-
TT	15%	5%	-
T Allele (Frequency)	37.5%	20%	**0.01**
C Allele (Frequency)	62.5%	80%	**0.01**

Table 5: Represents the distribution of genotypes for the C677T SNP, highlighting differences between the T2DM and control groups.

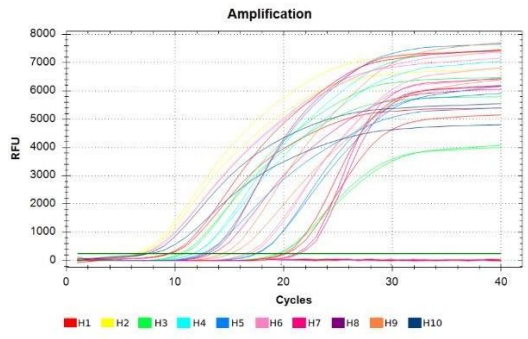

Figure 6: Overall Amplification of Probe G and EC (RNaseP) in controls.

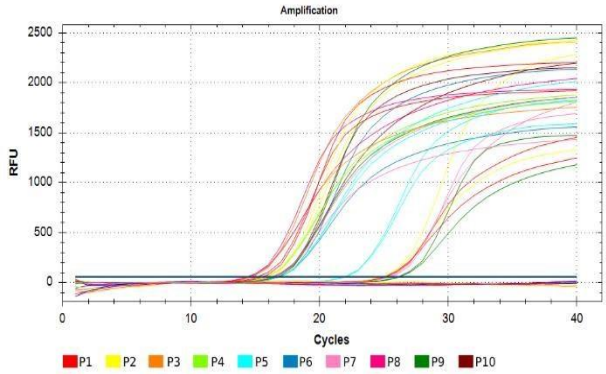

Figure 7: Overall Amplification of Probe A and EC (RNaseP) in cases.

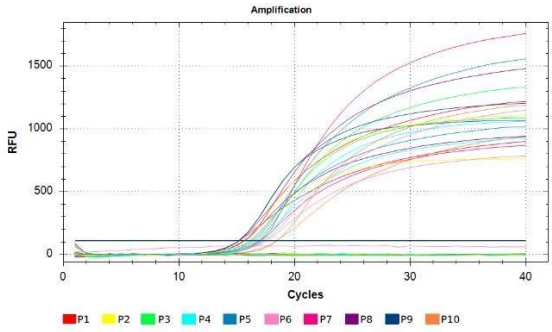

Figure 8: Overall Amplification of Probe C and EC (RNaseP) case

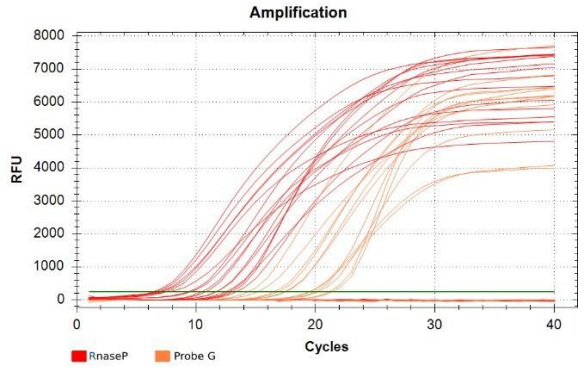

Figure 9: Amplification for Probe G and EC Used in controls and cases

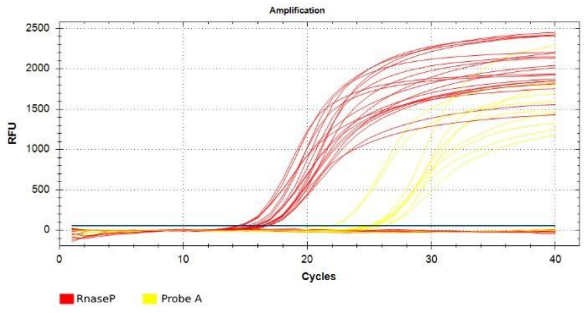

Figure 10: Amplification for Probe A and EC Used in cases and controls.

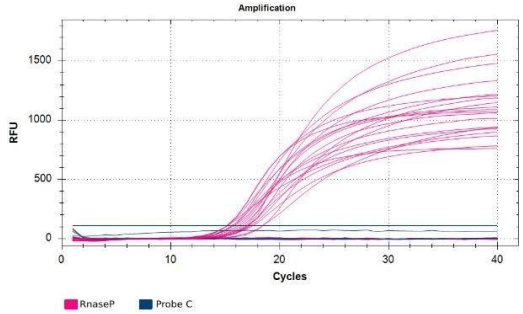

Figure 11: Amplification for Probe C and EC Used in cases and control.

Quantitative PCR (qPCR)

Quantitative PCR was used to measure the MTHFR C677T SNP genotypes. The cycle threshold (Ct) values were recorded to assess the presence of wild-type (CC) and polymorphic (CT/TT) genotypes. In T2DM group 70% of participants

exhibited higher Ct values (26–34) compared to 50% in the control group. The mean Ct for T2DM patients was 28.1 ± 3.9, significantly higher than the 25.8 ± 3.4 in controls ($p < 0.05$), indicating a greater prevalence of polymorphic alleles (CT/TT) in T2DM patients. This supports the allele frequency findings and reinforces the enrichment of polymorphic genotypes (CT/TT) in the T2DM group (See table 6).

	Ct (20–25)	h Ct (26–34)	a	Ct ± SD	oup DM
	0%)	70%)	1	± 3.9	
trols	50%)	50%)	8	± 3.4	al
	40%)	60%)	0	± 3.7	

Table 6: Shows the Ct values obtained from qPCR for the C677T SNP, comparing the amplification efficiency between the T2DM and control groups.

Sanger sequencing confirmation

A subset of representative samples (n = 4 per genotype) was subjected to Sanger sequencing to validate the genotyping results. Sequence chromatograms demonstrated high-quality base calling with clear discrimination between the wild-type (CC), heterozygous (CT), and homozygous mutant (TT) genotypes, confirming 98% concordance with the results obtained from PCR-RFLP and qPCR.

Association with biochemical parameters

Exploratory analyses were conducted to examine potential associations between the MTHFR C677T polymorphism and relevant biochemical markers in T2DM. Although the sample size limited statistical power, suggestive trends were observed. T2DM patients carrying the T allele (CT/TT) exhibited higher mean HbA1c levels (8.1 ± 1.6%) compared to non-carriers (CC genotype: 7.6 ± 1.5%). Although this difference did not reach statistical significance (p = 0.12), the trend suggests that reduced MTHFR enzymatic activity may contribute to impaired glycemic regulation in T2DM.

Hardy-Weinberg Equilibrium (HWE)

Hardy-Weinberg equilibrium analysis confirmed that the genotype distributions for the C677T SNP did not deviate significantly from expected frequencies in both cases and controls, indicating no genotyping bias (See table 7).

SNP	Group	Observed Genotypes	Expected Genotypes	p-value	HWE Status
C677T	**Controls**	CC = 13, CT = 6, TT = 1	CC = 12.8, CT = 6.4, TT = 0.8	0.84	Maintained
	T2DM	CC = 8, CT = 9, TT = 3	CC = 7.9, CT = 9.4, TT = 2.7	0.90	Maintained
A1298C	**Controls**	AA = 14, AC = 5, CC = 1	AA = 13.9, AC = 5.7, CC = 0.5	0.64	Maintained
	M	= 10, AC = 8, CC = 2	= 9.8, AC = 8.4, CC = 1.8 7		ntained

Table 7: Presents the Hardy-Weinberg equilibrium (HWE) analysis

Genotypic frequencies and biochemical associations

The integration of genetic and biochemical data revealed consistent trends showing the influence of MTHFR C677T polymorphism over glycemic control in T2DM patients. HbA1c levels were consistently higher in T allele carriers (CT/TT), suggesting that the C677T SNP may contribute to dysregulated glycemic control. No significant association was observed between MTHFR polymorphisms and renal function biomarkers in this limited sample, though trends towards increased urinary albumin-to-creatinine ratios (UACR) in C allele carriers were noted (p = 0.09) (See table 8).

Genotype	T2DM HbA1c (%) (Mean ± SD)	Control HbA1c (%) (Mean ± SD)	T2DM FBS (mg/dL) (Mean ± SD)	Control FBS (mg/dL) (Mean ± SD)
CC	**7.6 ± 1.5**	**5.1 ± 0.5**	**136 ± 28**	**90 ± 10**
CT	**8.1 ± 1.6**	**5.1 ± 0.4**	**140 ± 32**	**90 ± 12**
TT	**8.2 ± 1.7**	**5.1 ± 0.6**	**145 ± 35**	**90 ± 11**

Table 8: Summary table consolidates the genotypic frequencies and biochemical data

DISCUSSION

The present study investigates the association between the MTHFR C677T polymorphism (rs1801133) and Type 2 Diabetes Mellitus (T2DM) complications, specifically examining the relationship between genetic variants and biochemical markers. Our findings reveal a higher prevalence of the T allele in T2DM patients compared to healthy controls, with suggestive associations between the T allele and elevated HbA1c and microalbuminuria. These results align with findings from previous studies but also provide novel insights into the ethnic-specific role of MTHFR polymorphisms in South Asian populations.

Study by Zhang et al. performed a meta-analysis to investigate the association of the MTHFR C677T polymorphism with T2DM risk in the Middle East and North Africa (MENA) region. This study found a significant association between the T allele and T2DM, particularly in Asian populations. Our findings are in agreement with these results, showing that the T allele of MTHFR C677T is more frequent in T2DM patients in Kerala, India, as compared to healthy controls. Both studies underscore the importance of ethnic variability, as Zhang et al. highlighted that the association was strongest in Asian populations (Zang et al., 2024). This reflects the need for studies focused on South Asian cohorts, which have been less represented in genetic research.

In contrast, another study by Meng et al. focused on the relationship between MTHFR C677T polymorphisms and coronary artery disease (CAD) in Sudanese T2DM patients, finding that the T allele is significantly associated with an increased risk of CAD (OR = 6.2) This study emphasized the role of hyperhomocysteinemia resulting from the T allele in increasing the risk of vascular complications in T2DM patients. While we did not assess CAD in our study, our findings that the T allele is linked to higher HbA1c levels and microalbuminuria suggest that MTHFR polymorphisms may also contribute to renal complications in T2DM, possibly through similar mechanisms involving endothelial dysfunction and vascular damage (Meng et al., 2019). Both studies support the idea that MTHFR mutations contribute to complications, but our findings emphasize renal dysfunction as a potential target for future investigations in South Asian populations.

A study by Nurkolis et al. examined the genetic influences of MTHFR C677T on cardiovascular risk in T2DM patients across different ethnic groups. Their meta-analysis found that the C677T polymorphism significantly influenced T2DM susceptibility and cardiovascular disease in European populations, with significant correlations between homozygous TT genotypes and elevated homocysteine levels. While our study also identifies elevated biochemical markers in T allele carriers, we found a stronger association with glycemic control and renal function, particularly microalbuminuria (Nurkolis et al., 2025). This suggests that MTHFR polymorphisms may have distinct effects based on ethnic background, as observed in European vs. Asian cohorts. Our study, based on a South Asian population, reinforces the need for ethnic-specific genetic research in T2DM, especially as hyperhomocysteinemia contributes to vascular and renal dysfunction.

Another study by Mohammed et al. focused on the association between MTHFR C677T and cardiovascular complications in Sudanese T2DM patients, showing that T allele carriers had significantly higher plasma homocysteine levels and an increased risk of premature coronary artery disease (PCAD) . Our findings align with this study in suggesting that MTHFR mutations lead to vascular complications through elevated homocysteine, but our study highlights the renal impact, particularly in microalbuminuria (Mohammed et al., 2025). This suggests that MTHFR C677T polymorphisms may contribute to different organ complications.

Our study provides important insights into the role of MTHFR C677T polymorphisms in T2DM complications, especially in South Asian populations that are often underrepresented in genetic studies. Despite its strengths, certain limitations also need to be acknowledged. The relatively small sample size (n = 40) limits statistical power, especially for detecting smaller effect sizes in genetic associations and cross-sectional nature of our study restricts our ability to establish causal relationships between MTHFR polymorphisms and T2DM complications. A longitudinal study is required to validate our findings.

The study provides evidence that the MTHFR C677T polymorphism may contribute to T2DM complications, particularly in South Asian populations. The T allele was associated with higher HbA1c and microalbuminuria, suggesting its potential role in glycemic dysregulation and renal damage. However, further studies with larger, more diverse cohorts and longitudinal follow-up are necessary to better understand the genetic-environmental interactions and their role in the progression of T2DM and related complications.

CONCLUSION

The results suggest a higher prevalence of the T allele of MTHFR C677T in T2DM patients compared to controls. The observed association between T allele carriers and higher HbA1c levels suggests that the C677T polymorphism may contribute to glycemic dysregulation in T2DM. Although further studies with larger sample sizes are necessary to confirm these findings, the preliminary data indicate a potential genetic modifier role for MTHFR polymorphisms in T2DM pathophysiology.

REFERENCES

1. Thahira, A., Roy, D., Pradhan, S., Kumar, Y., Tandon, N., Das, A., ... & Saraswathy, D. N. (2025). Apolipoprotein E (ApoE) Gene Polymorphism and Cardiovascular Risk in Type 2 Diabetic Patients: A Systematic Review. *Trends in Sciences*, *22*(8), 9754-9754.

a. Complications

3. P, B. V., & Hegde, D. S. V. (2025). Analysing The Diagnostic Value Of Microalbuminuria As A Bio-Marker For Diabetic Nephropathy By Correlating With Fasting Blood Sugar, Serum Creatinine And Hba1c In Type 2 Diabetes Mellitus: A

4. Case-Control Study. *South Eastern EuropeanJournal of Public Health*, 4255– 4264. https://doi.org/10.70135/seejph.vi.6094

5. Li, W. X., Dai, S. X., Zheng, J. J., Liu, J. Q., & Huang, J. F. (2015). Homocysteine metabolism gene polymorphisms (MTHFR C677T, MTHFR A1298C, MTR A2756G and MTRR A66G) jointly elevate the risk of folate deficiency. *Nutrients*, *7*(8), 6670-6687.

6. Araszkiewicz, A. F., Jańczak, K., Wójcik, P., Białecki, B., Kubiak, S., Szczechowski, M., & JanuszkiewiczLewandowska, D. (2025). MTHFR Gene Polymorphisms: A Single Gene with Wide-Ranging Clinical Implications—A Review. *Genes*, *16*(4), 441.

7. Sabitha, S., Hegde, S. V., Agarwal, S. V., Delna, N. S., Pillai, A., Shah, S. N., ... & SHAH, S. N. (2024). Biomarkers of oxidative stress and their clinical relevance in type 2 diabetes mellitus patients: a systematic review. *Cureus*, *16*(8).

8. Zhang, Y., Zhang, Y., Miao, R., Fang, X., Yin, R., Guan, H., & Tian, J. (2024). The MTHFR C677T/A1298C polymorphism is associated with increased risk of microangiopathy in type 2 diabetes mellitus: A systematic review and meta-analysis. *Nutrition Research*, *130*, 34-47. https://doi.org/10.1016/j.nutres.2024.08.004

9. Meng, Y., Liu, X., Ma, K., Zhang, L., Lu, M., Zhao, M., Guan, M. X., & Qin, G. (2019). Association of MTHFR C677T polymorphism and type 2 diabetes mellitus (T2DM) susceptibility. *Molecular genetics & genomic medicine*, *7*(12), e1020. https://doi.org/10.1002/mgg3.1020

10. Nurkolis, F., Amalia, N., Tandi, Y. Y. P., Athallah, A. F., Aditya, M. R., Nojaid, A., Humardani, F. M., Prapriatna, A. F., Taslim, N. A., Harbuwono, D. S., & Tjandrawinata, R. R. (2025). Determining the Risk of Type 2 Diabetes for rs1801133

11. Genotypes in Multiethnic Populations: A Global Meta-Epidemiological Study. *International Journal of Molecular Sciences*, *26*(9), 3987. https://doi.org/10.3390/ijms26093987

12. Mohammed NO, Ali IA, Elamin BK and Saeed BO (2025) 5,10methylenetetrahydrofolate reductase C677T gene polymorphism as a risk factor for premature coronary artery disease in patients with type 2 diabetes mellitus.Front.

13. Endocrinol. 15:1502497. doi: 10.3389/fendo.2024.1502497

14. Predictive Model Of type 2 Diabetes Risk In Early Adulthood Using environmental Approach in costal and Urban Areas. (2025). *Journal of Carcinogenesis*, *24*(5s), 164-173. https://doi.org/10.64149/J.Carcinog.24.5s.164-173