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Polymorphism of Toll-like Receptor-9 (TLR9) rs187084 (−1486T>C) in Iraqi Patients with Type 2 Diabetes Mellitus

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Annotation: This study investigated the association between the Toll-like receptor 9 (TLR9) rs187084 (−1486T>C) polymorphism and the risk of type 2 diabetes mellitus (T2DM), as well as its impact on serum TLR9 levels, in a cohort of 45 T2DM patients and 35 healthy controls. Genotyping revealed significant differences in the distribution of CC, CT, and TT genotypes between groups. The TT genotype was significantly more prevalent in T2DM patients (P = 0.004), indicating increased disease susceptibility, while CC and CT genotypes were underrepresented, suggesting protective effects. Allele frequency analysis supported this association: the T allele was more frequent in patients (46.67%) than controls (22.86%), while the C allele predominated in controls (77.14%) (P = 0.018). Genotypic risk analysis indicated that TT genotype carriers had a sevenfold increased risk of T2DM (OR = 7.111, 95% CI: 1.889–26.76, P = 0.007). Gender-specific analysis revealed a stronger association in females (OR = 10.00, P = 0.040) and a protective role of the CC genotype in males (OR = 0.153, P = 0.03). The recessive

inheritance model showed a significant protective effect (OR = 0.1406, P = 0.003), while dominant and over dominant models showed non-significant trends. These findings suggest that TLR9 rs187084 polymorphism contributes to T2DM susceptibility via immune-inflammatory pathways and may serve as a potential biomarker for risk stratification and targeted therapies.

Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder marked by hyperglycemia due to insulin resistance and/or impaired insulin secretion. With a global prevalence exceeding 500 million people, T2DM has emerged as a significant public health challenge. While environmental and lifestyle factors play critical roles, genetic and immune-mediated mechanisms are increasingly recognized in T2DM pathogenesis.

Toll-like receptors (TLRs), particularly TLR9, play a pivotal role in innate immunity by recognizing unmethylated CpG motifs in microbial DNA. Activation of TLR9 triggers inflammatory responses implicated in insulin resistance and β-cell dysfunction. Polymorphisms in the promoter region of the TLR9 gene, such as rs187084 (−1486T>C), may alter gene expression and thereby modulate T2DM susceptibility.

This study explores the association of TLR9 rs187084 polymorphism with T2DM risk in an Iraqi population and evaluates the impact of genotype and allele frequencies, gender distribution, and genetic inheritance models.

Materials and Methods

This case-control study was conducted from July 1 to October 30, 2024, at the Department of Biology, University of Wasit. A total of 80 participants—45 T2DM patients (24 males, 21 females) and 35 healthy controls (15 males, 20 females)—were recruited using a convenience sampling method. The age range was 40–78 years in both groups.

Genomic DNA was extracted using the Quick-gDNA Blood MiniPrep kit (Zymo Research). Genotyping of TLR9 rs187084 was performed using the TaqMan SNP Genotyping Assay and real-time PCR.

Statistical analysis was conducted using SPSS v21.0. P-values < 0.05 were considered statistically significant. Odds ratios (OR) with 95% confidence intervals (CI) were calculated to estimate the strength of associations.

Results

A total of 45 T2DM patients and 35 healthy controls were included in this study. Genotyping revealed three genotypes—CC, CT, and TT—among both groups. The TT genotype was significantly more frequent in T2DM patients (40%) compared to controls (8.57%), while the CC genotype was more frequent among controls (62.86%) compared to patients (46.67%) (P = 0.004).

The T allele was significantly more common in T2DM patients (46.67%) than in controls (22.86%) (P = 0.018), indicating its role as a potential risk factor.

Table 1: **Genotype distribution of *TLR9* rs187084 −1486T>C among T2DM patients and controls**

Groups	Genotype no. (%)			Allele frequency .no (%)	
	CC	CT	TT	C	T
Control	22(62.858)	10(28.57)	3(8.571)	54 (%77.14)	16 (%22.86)
T2DM Patients	21(46.666)	6(13.334)	18(40)	48 (%53.33)	42 (%46.67)
Chi square χ2	10.654			9.659	
P-value	0.004			.001	
Significance	Sig.				

Sig. $P < 0.05$

Genotypic association analysis showed that individuals with the TT genotype had a sevenfold increased risk of T2DM (OR = 7.111, 95% CI: 1.889–26.76, P = 0.007). The CC and CT genotypes showed a non- significant protective trend.

Table 2: Association of TLR9 rs187084 −1486T>C with T2DM patients and healthy controls in term of genotypes

Genotypes	control	patients	OR	OR95%CI	P-value	Significance
CC	22	21	0.517	0.209 to 1.273	0.151	Ns.
CT	10	6	0.384	0.124 to 1.190	0.097	Ns.
TT	3	18	7.111	1.889 to 26.760	0.003	Sig.

OR, odds ratio; 95% CI, 95% confidence interval.

Statistically significant result (p < 0.05). Sig.

Ns.Non-significant P>0.05

When stratified by gender, the TT genotype was associated with a tenfold increased risk in females (OR = 10.00, 95% CI: 1.10–90.60, P = 0.040), whereas in males, the CC genotype showed significant protection (OR = 0.153, 95% CI: 0.028–0.833, P=0.03).

Table 3. Association of *TLR9* rs187084 −1486T>C with T2DM females patients and healthy controls in term of genotypes

Genotypes	control	patients	OR	OR95%CI	P-value	Significance
CC	11	9	0.681	0.202 to 2.301	0.537	Ns.
CT	9	5	0.416	0.110 to 1.567	0.195	Ns.
TT	1	7	10.00	1.103 to 90.596	0.040	Sig.

OR, odds ratio; 95% CI, 95% confidence interval.

Statistically significant result (p < 0.05). Sig.

Ns.Non-significant P>0.05

Genetic inheritance models further supported these findings. The recessive model (CC + CT vs. TT) indicated a significant protective effect (OR = 0.1406, 95% CI: 0.037–0.529, P = 0.003). The dominant and over dominant models did not reach statistical significance.

Table 4: Association of TLR9 rs187084 −1486T>C with T2DM males patients and healthy controls in term of genotypes

Genotypes	control	patients	OR	OR95%CI	P value
CC	12	12	0.1538	0.0284 to 0.8339	0.03
CT	0	1	1.8511	0.0706 to 48.5535	0.7118
TT	2	11	5.0769	0.9287 to 27.7555	0.0608

OR, odds ratio; 95% CI, 95% confidence interval.

Statistically significant result (p < 0.05). Sig.

Ns.Non-significant P>0.05

Discussion

In the present study, we examined the association between the TLR9 gene polymorphism rs187084 (−1486T>C) and the risk of developing type 2 diabetes mellitus (T2DM) in a case–control cohort. Our findings indicate a statistically significant relationship between the TT genotype and increased susceptibility to T2DM, particularly among female patients, while the C allele (CC and CT genotypes) appears to offer a protective effect, especially in males.

The rs187084 SNP is located in the promoter region of the TLR9 gene, indicating a potential role in modulating transcriptional activity. This aligns with prior research suggesting that promoter polymorphisms can influence gene expression and immune responses (Li *et al*., 2017). The results of the current study showed that individuals with the TT genotype had a markedly higher risk of T2DM (OR = 7.111, *P*= 0.007), while those with the CC genotype exhibited a protective trend, particularly among males (OR = 0.153, *P* = 0.03). Similar gender-specific associations have been observed in other immune-mediated diseases such as Behçet's disease, where TLR9 polymorphisms demonstrated a significant effect in males but not females (Azizi *et al*., 2024).

The gender-related differences in genotype effects observed in the present study are noteworthy. Among females, the TT genotype was associated with a tenfold increased risk of developing T2DM, whereas in males, this genotype showed a fivefold increased risk, although it did not reach statistical significance (*P* = 0.060). Conversely, the CC genotype appeared to protect males from the disease, suggesting a possible interaction between sex hormones and TLR9 gene expression. This hypothesis is supported by previous studies reporting differential TLR expression patterns between sexes, potentially influenced by estrogen and testosterone levels (Mancuso *et al*., 2021).

Comparison with previous literature reveals both consistencies and divergences. For example, Alzahrani *et al*. (2023) reported a significant association between rs187084 and T2DM in a Saudi Arabian population, consistent with the findings of the current study. They also noted correlations between TLR9 genotypes and lipid profile alterations, particularly total cholesterol and HDL-C levels, further supporting a role for TLR9 in metabolic regulation. In contrast, a study by Zhang *et al*. (2012) in a Chinese Han population did not find a statistically significant association between rs187084 and T2DM or coronary artery disease, though they observed a borderline trend towards higher CC genotype frequency in diabetic patients (*P* = 0.055). These inconsistencies may be attributed to genetic background differences, sample size variation, or environmental factors affecting gene–disease interaction.

The current study also aligns with functional observations from Alzheimer's disease research, where the GG genotype (analogous to CC in our context) of rs187084 was linked to increased TLR9 expression and reduced disease risk in Han Chinese individuals (Li *et al*., 2017). These findings support the concept that the C allele may enhance TLR9 transcription, thereby improving immunological surveillance and reducing chronic inflammation. In T2DM, chronic low-grade inflammation is a central pathophysiological mechanism, and TLR9 plays a known role in modulating innate immune responses to both bacterial and mitochondrial DNA (Brown *et al*., 2020).

The genetic models applied in the current study further substantiate these findings. The recessive model (CC + CT vs. TT) indicated a statistically significant protective effect (OR = 0.1406, *P* = 0.003), highlighting the potential adverse impact of the homozygous TT genotype. Although the dominant and over dominant models did not yield significant results, the over dominant model (CC + TT vs. CT) approached significance (OR = 2.60, *P* = 0.097), implying a more complex interplay of allele effects. These interpretations are important for understanding the inheritance

pattern and functional significance of the rs187084 polymorphism in T2DM susceptibility (Guerra *et al*., 2022).

In previous studies that have investigated the association of polymorphisms of several factors and other biomarkers among patients with type2 DM from Wasit province, (Yousif and Ghali,2021), revealed that IL-10 is a major contributor to the onset of type 2 diabetes mellitus and there may be a correlation between low levels of interleukin-10 and type two diabetes(Al-Sarray and Ahmed ,2021) found that may be a correlation between high levels of TNF-α and type 2 diabetes mellitus.(Shamkhi and Ahmed ,2021), displayed that levels of SIRT1 may be not associated with type2 diabetes mellitus. Furthermore, the cell free mitochondrial DNA increases significantly in patients with type2 diabetes mellitus (Hussein and Ghali,2022). COX-1 is a major contributor to the onset of type 2 diabetes and there may be an association between low levels of cyclooxygenase-1and type 2 diabetes (Jebil and Ghali,2021). The association analysis of IL-17AG197A gene polymorphism with T2DM displayed that heterozygous AG genotype of IL17AG197A showed a risk association among T2DM with OR=1.24 CI95% (0.31 - 5.01) p-value =1.00 and the G allele was associated with an increased risk ofT2DM (Khidhum and Ahmed,2022). (Mahmood and Ghali,2022), revealed that there was an association between the polymorphism of Osteoprotegerin (OPG) polymorphism and susceptibility to type2 diabetes mellitus. (Mahmood and Ghali,2022b), found also that there may be a correlation between high levels of OPG and T2DM. There is a strong evidence that the **Cys/Cys (mt/mt) genotype of the OGG1 gene is significantly associated with an increased risk of type 2 diabetes mellitus (T2DM)** in the Iraqi population from Wasit Province, with particularly high risk observed in both **male and female carriers**. Conversely, the **Ser/Ser (wt/wt) genotype** shows a **protective effect** against T2DM. The significant association of the **mt allele** with T2DM underscores its potential role as a **genetic risk factor**, likely due to impaired oxidative DNA damage repair in the presence of chronic hyperglycemia. These findings highlight **OGG1 polymorphisms as promising biomarkers** for T2DM susceptibility and suggest a critical role of oxidative stress-induced DNA damage in the disease pathogenesis Allawi and Ghali ,2024).

Despite the important insights provided, several limitations must be acknowledged. The relatively small sample size (45 patients, 35 controls) may reduce the generalizability of the findings and limit the power of stratified analyses. Additionally, deviations from Hardy–Weinberg equilibrium may reflect sampling biases or unaccounted population structure. The lack of functional assays, such as promoter activity or cytokine profiling, also limits the mechanistic interpretation of our findings. Future studies should aim to replicate these results in larger and more ethnically diverse populations and incorporate functional experiments to validate the regulatory impact of rs187084.

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Despite valuable insights, this study is limited by a small sample size and lack of functional validation. Further research with larger, more diverse populations and mechanistic studies is needed to confirm these findings.

Conclusions

1. The TLR9 rs187084 (−1486T>C) polymorphism is significantly associated with T2DM. TLR9 may serve as a potential genetic biomarker for T2DM susceptibility.

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