Diabetes, Metabolic Syndrome and Obesity

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ORIGINAL RESEARCH

Association of NLRP3 Inflammasome rs10754558 Polymorphism with Type 2 Diabetes Mellitus and Its Complications: Clinical and Bioinformatics Study

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Introduction: The *NLRP3* inflammasome is thought to be an important element in innate immunity; aberrant activation might be caused by many inflammatory conditions, including diabetes. The study aims to investigate the association of the *NLRP3* inflammasome rs10754558 polymorphism with susceptibility to type 2 diabetes mellitus (T2DM) and its complications using clinical and bioinformatics.

Methods: In this case control study, 250 T2DM cases and 150 matched-age and gender healthy subjects were genotyped for rs10754558. Clinical, biochemical, and inflammatory markers (*NLRP3*, IL-1β) were measured. Associations with complications assessed using logistic regression. In silico analyses were carried out to evaluate miRNA binding and pathway interactions.

Results: T2DM cases had a significantly higher frequency of the rs10754558 C allele than controls (20.8% vs 13.3%, $p = 0.007$). Nephropathy/CVD were significantly associated with the CC genotype (83.3%, $p < 0.001$). Higher levels of *NLRP3*, IL-1β, FPG, and HbA1c ($p < 0.05$) were observed in GC/CC genotype carriers. The C allele alters predicted miRNA binding in the 3′ UTR increase mRNA stability. PPI network pathway enrichment highlighted the central roles of *NLRP3* in IL-1β signaling.

Conclusion: The *NLRP3* rs10754558 C allele was associated with higher risk of T2DM and vascular complications in Saudi patients and correlated with elevated NLRP3 and IL-1β levels. These population-specific findings highlight the biological relevance of the NLRP3–IL-1β axis in metabolic inflammation and provide a foundation for future functional and clinical studies.

Keywords: *NLRP3*, IL-1β, rs10754558, miRNA, inflammasome, T2DM

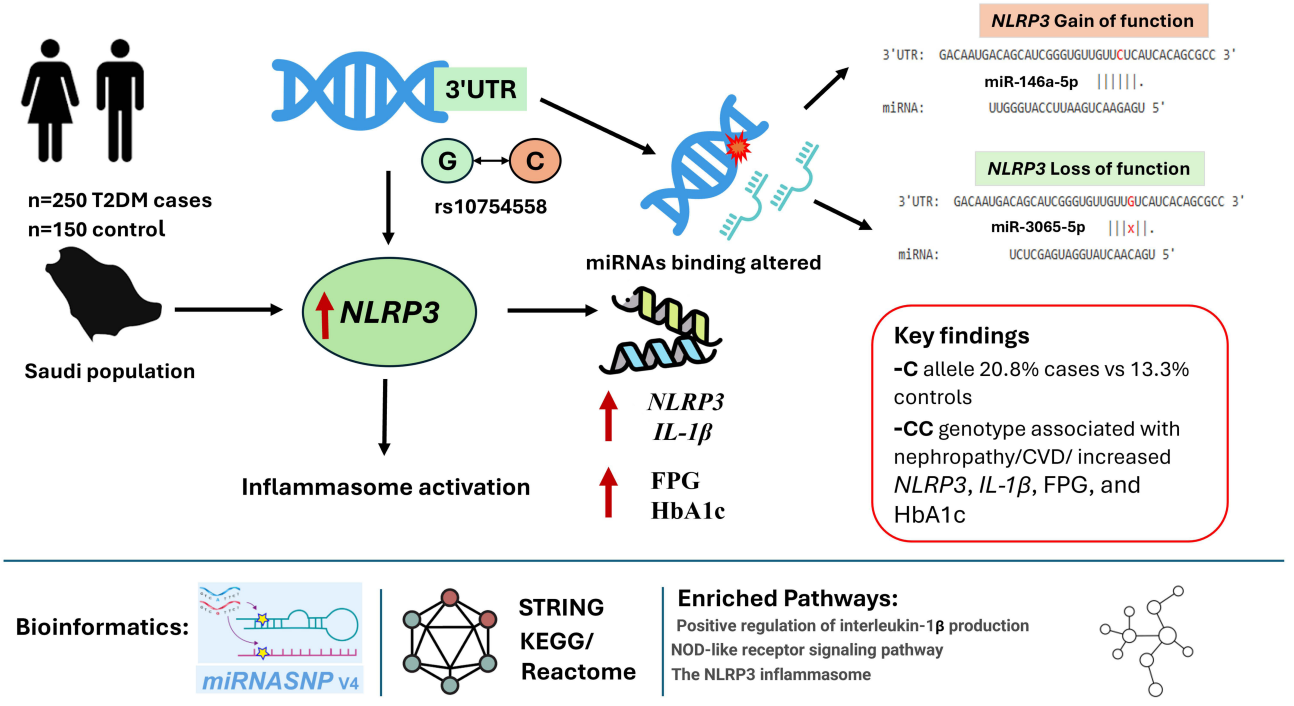
Introduction

Diabetes mellitus is considered a set of metabolic illnesses characterized by hyperglycemia, which damages several body tissues and results in dysfunction.[1] With an elevating incidence estimated at 500 million cases globally by 2030, type 2 diabetes mellitus (T2DM) is recognized as a serious public health issue.[2] Over 90% of diagnosed cases worldwide are T2DM. T2DM is linked with chronic macro- and microvascular complications.[3] According to the World Health Organization (WHO), the Kingdom of Saudi Arabia ranks as the seventh highest country globally in the prevalence of diabetes. In addition, T2DM is considered the main type and accounts for 28% as of 2023, with a raised incidence rate.[4]

Persistent low-grade inflammation is considered a hallmark of T2DM, promoting a significant role in disease progression and the development of chronic complications, including microvascular issues such as retinopathy, neuropathy, and nephropathy. Moreover, cardiovascular diseases (CVD) and peripheral arterial occlusive disease (PAOD) are

https://doi.org/10.2147/DMSO.S586787
Received: 11 December 2025
Accepted: 23 February 2026
Published: 10 March 2026

Graphical Abstract



macrovascular complications. To improve risk assessment and predict therapies, it is crucial to understand the molecular mechanisms underlying the inflammatory response.[5]

In response to infection or cellular injury, the NLRP3 inflammasome (a vital element of the innate immune system) triggers the activation of caspase-1 and the secretion of pro-inflammatory cytokines, including IL-1β and IL-18. Many inflammatory conditions, such as cryopyrin-associated periodic syndromes, diabetes, atherosclerosis, and Alzheimer's disease, have been linked to the aberrant activation of the NLRP3 inflammasome.[6] A variety of molecular and cellular events, including ionic mitochondrial malfunction, reactive oxygen species generation, and lysosomal damage, may contribute to activation. The mechanism by which NLRP3 responds to these signaling processes and initiates the NLRP3 inflammasome assembly process remains unknown.[6] *NLRP3* stimulates caspase-1 maturation and the subsequent secretion of IL-1β and IL-18 during metabolic events, such as activation by glucose toxicity, lipotoxicity, or oxidative stress. This can aggravate insulin resistance and create pancreatic β-cell dysfunction.[7] Additionally, aberrant NLRP3 signaling has been implicated in atherosclerosis, vascular inflammation, and endothelial dysfunction, thereby linking this pathway to cardiovascular risk in diabetics.[8,9]

NLRP3 gene variations may modulate an individual's susceptibility to developing diabetes and its associated consequences. The single-nucleotide polymorphism (SNP) rs10754558, located in the 3′ untranslated region (3′UTR) of the *NLRP3* gene, is well known to influence inflammasome activity, resulting in the secretion of inflammatory cytokines.[10] Previous research in Asian populations has demonstrated that risk alleles of rs10754558 are associated with increased NLRP3 expression, higher IL-1β levels, and increased susceptibility to T2DM.[11] However, despite the elevated prevalence of T2DM and its associated consequences among Middle Eastern populations, data remain inconsistent across ethnic groups.

Therefore, this study aimed to evaluate the association of *NLRP3* rs10754558 (29940G>C) with T2DM susceptibility and to examine its association with glycemic profiles, inflammatory markers, and micro- and macrovascular

complications of T2DM in a Saudi population. To strengthen mechanistic interpretation, we combined clinical-genotyping data with bioinformatics analyses, including variant annotation, miRNA binding prediction, and pathway/network enrichment.

Materials and Methods

Study Population

In this case-control study, 250 patients with T2DM (case group) and 150 healthy people (control group) participated. Participants were recruited from several hospitals in Madinah, Kingdom of Saudi Arabia. T2DM diagnosis, as defined by the World Health Organization (WHO), includes diagnostic criteria that include an HbA1c Level of ≥6.5% and a fasting blood glucose (FBG) level of ≥7.0 mmol/L. Exclusion criteria include patients who suffer from severe illnesses, cancer, liver diseases, known autoimmune disorders, and end-stage kidney diseases requiring dialysis. In addition, women who are pregnant, individuals with types of diabetes other than T2DM, and children were excluded. Diabetes complications diagnosis was set as previously described.[12] Diagnosis of nephropathy was defined by a glomerular filtration rate (GFR) lower than 60 mL/min and a urine albumin-to-creatinine ratio (UACR) ≥30 mg/g,[13] with the exclusion of borderline or type 1 diabetes. Additionally, diagnosis of retinopathy is based on fundus findings (hemorrhages, microaneurysms, and neovascularization) using retinal imaging.[14] Moreover, neuropathy, as defined by the American Academy of Neurology, involves a nerve conduction study and clinical signs.[15] Furthermore, diagnosis of cardiovascular diseases (CVD) is made according to ADA guidelines using clinical and laboratory markers.[16] Furthermore, PAOD was diagnosed based on the previously mentioned criteria.[17]

Permission for this research was obtained from the "Research Ethics Committee" at Taibah University (Approval No. CLS 2020116). This research was conducted in accordance with the "1964 Helsinki Declaration and its recent amendments". Informed consent was signed by all participants in this study before participating.

Biochemical Analysis

NLRP3 level was measured using an ELISA assay developed by Elabscience (USA). IL-1β was measured with an ELISA kit from Thermo Fisher (USA). Glucose, lipids, urea, creatinine, and albumin levels were measured enzymatically with a Dimension® EXL™ 200 full biochemistry autoanalyzer (Siemens, Erlangen, Germany). UACR was estimated according to the previous description.[18] HbA1c was measured with a D-10™ Hemoglobin Analyzer manufactured by Bio-Rad (Nyocard, USA).

DNA Extraction and Genotyping

Following the manufacturer's guidelines, genomic DNA was extracted from blood leukocytes using the QIAamp DNA Mini Blood Kit (Qiagen, Hilden, Germany). A spectrophotometer (UV-1900i Plus, SHIMADZU, Kyoto, Japan) was employed to determine the concentration and purity of DNA, and only samples with an A260/A280 ratio between 1.8 and 2.0 were included. The extracted DNA was then stored at −20°C until further analysis.

Design and Validation of PCR Primer

The following primers were used to amplify the *NLRP3* rs10754558 (G>C) polymorphism, according to the NCBI reference sequence (GenBank accession no. NC_000001.11):

- Forward: 5′-ACCCAGGCTTTCTATTTGCTTT-3′
- Reverse: 5′-ATGAGGTCACCAAGAGGAACATT-3′

Amplicon size was 235 bp. NCBI Primer-BLAST confirm the primer specificity that indicate with other genomic regions no cross-reactivity.

PCR Analysis

Using 1 U of Taq DNA polymerase (Thermo Fisher Scientific, USA), and a thermal cycler used was a Bio-Rad T100, PCR was performed, a 25 μL reaction reagent, which included: 100 ng of genomic DNA, 1× PCR buffer with $MgCl_2$, 200 μM dNTPs, 0.2 μM of each primer. 2% agarose gel electrophoresis with ethidium bromide staining, accompanied by a 100 bp DNA ladder using to visualize the resulting products.

Genotyping

Using PCR followed by direct Sanger sequencing, the rs10754558 polymorphism was assessed. Chromas software v2.6.6 (Technelysium, Australia) was used to analyze sequencing chromatograms to confirm genotypes. To ensure the accuracy of genotyping, 10% of the samples were randomly selected and re-genotyped by an independent researcher, showing 100% concordance. Additionally, a negative control (no DNA template) was included in every batch of PCR to exclude any potential contamination. Moreover, the genotype distribution was assessed using the Hardy-Weinberg equilibrium (HWE) for both cases and controls.

Bioinformatics Analysis

In silico Variant Annotation

To investigate the functional role of rs10754558, we retrieved variant information from dbSNP. Ensembl Genome Browser (https://www.ensembl.org/), and gnomAD Global and Middle Eastern allele frequencies were compared. Genomic conservation was visualized through the NCBI Genome Data Viewer (GDV).

(https://www.ncbi.nlm.nih.gov/gdv?org=homo-sapiensandgroup=hominoidea).

Functional Predictions and miRNA Analysis

The effect of the G>C substitution on 3′UTR regulation was assessed using miRNASNPv4.0 to predict gain/loss of microRNA binding sites. RegulomeDB (https://www.regulomedb.org/) was used to annotate transcription factor binding sites, histone marks, and regulatory motifs.

Protein–Protein Interaction and Pathway Analysis

Protein interaction partners of *NLRP3* were identified using the STRING database v12.0 (https://string-db.org/). Pathway enrichment was performed. Reactome Pathway Database, and KEGG Pathway Analysis. Overrepresented pathways were considered significant at FDR < 0.05.

Statistical Analysis

The analysis was conducted using SPSS version 23 software and Python (scikit-learn and matplotlib packages). Unpaired Student's *t*-test, ANOVA, Chi-square/Fisher exact test, Pearson correlation, and regression analysis were employed as appropriate. In addition, Bonferroni correction was used for multiple group comparisons. Moreover, an A P<0.05 or less indicates a statistically significant difference.

Results

Table 1 presents the baseline characteristics of the study population, including demographic, clinical, and biochemical parameters of participants, comprising a total of 250 patients with T2DM and 150 healthy controls, matched for age and gender. BMI and blood pressure were significantly higher in cases than in controls ($p<0.05$). In addition, FPG, HbA1c, NLRP3, IL-1β, TC, LDL, HDL, UACR, and creatinine were significantly higher in cases than in controls ($p<0.05$).

Table 2 shows the distribution of rs10754558 genotypes and alleles. HWE shows no significance in both cases ($p = 0.31$) and controls ($p = 0.59$). The frequency of the C allele was significantly higher in cases than controls (20.8% to 13.3% respectively, $p = 0.007$). There was a significant difference ($p = 0.019$) between the GG, GC, and CC genotypes frequencies in patients (60.8%, 36.8%, and 2.4%, respectively) than controls (74%, 25.3%, and 0.7%, respectively). Both the genotype ($p = 0.019$) and allele ($p = 0.007$) relationships remained statistically significant after Bonferroni correction ($\alpha = 0.025$).

Table 1 Demographic, Clinical Characteristics, and Biochemical Parameters of Participants

Parameters	Cases (n=250)	Controls (n=150)	*P*-value
Gender (males, females)	152, 98	90, 60	0.87
Age (years)	56.2±7.3	54.9±6.7	0.07
BMI (kg/m^2)	25.3 ±3.9	23.6±2.2	<0.001*
Blood pressure			
SBP (mm/Hg)	122.2±16.1	118.8±12.2	0.02*
DBP (mm/Hg)	81±5.1	79.8±3.5	0.009*
Hypertensive patients	73 (29.2%)	-	
Smoking	72 (28.8%)	35 (23.3%)	0.23
FPG (mmol/l)	7.5±0.93	5.1±0.42	<0.001*
HbA1c %	7.2±0.5	5±0.4	<0.001*
NLRP3 (ng/mL)	5.3±1.1	1.2±0.2	<0.001*
IL-1β (pg/mL)	11.6±5.2	6.9±2.1	<0.001*
TC (mmol/l)	3.98±0.4	3.39±0.2	<0.001*
TG (mmol/l)	1.59±0.4	1.52±0.29	0.06
LDL (mmol/l)	2.59±0.3	2.51±0.21	0.004*
HDL (mmol/l)	1.21±0.2	1.25±0.1	0.02*
UACR (g/mol)	27.5 ± 5.2	1.9 ± 0.9	<0.001*
Creatinine (mol/L)	82.6±13.6	72.5±14.1	<0.001*
Nephropathy	29 (11.6%)	-	-
Retinopathy	13 (6.8%)	-	-
Neuropathy	9 (4.7%)	-	-
CVD	32 (12.8%)	-	-
PAOD	20 (8%)	-	-

Notes: * significant at p<0.05. P-values were adjusted for multiple comparisons using the Bonferroni correction.

Abbreviations: BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; NLRP3, NOD-like receptor family, pyrin domain containing 3; IL-1β, interleukin 1 beta; TC, total cholesterol; TG, triglyceride; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; UACR, U-albumin/creatinine ratio; CVD, cardiovascular diseases; PAOD, peripheral arterial occlusive disease.

Table 2 Distribution of Studied Groups According to the Frequencies of *NLRP3* Inflammasome (rs10754558) Genotypes and Alleles

	Cases (n=250)	Controls (n=150)	OR (95% CI)	*P*-value	HWE (*P*-value)	
					Cases (250)	Controls (150)
Genotype					0.31	0.59
GG (wild type)	152 (60.8%)	111 (74%)				
GC	92 (36.8%)	38 (25.3%)				
CC	6 (2.4%)	1 (0.7%)	1.91 (1.17–2.69)	0.019*		
GC+CC	97 (39.2%)	39 (26%)				
Allele percent (%)						
G	396 (79.2%)	260 (86.7%)				
C	104 (20.8%)	40 (13.3%)	2.03 (1.2–3.1)	0.007**		

Notes: * significant at p<0.05. ** significant at p<0.01.

Abbreviations: CI, confidence interval; HWE, Hardy–Weinberg equilibrium.

Table 3 presents a further examination of the NLRP3 rs10754558 polymorphism in relation to T2DM complications and other factors, revealing a significant association between genotypes and nephropathy, with the prevalence being highest in CC carriers (83.3%), followed by GC (9.8%), and GG (9.9%) ($p < 0.001$). In addition, CVD exhibits

Table 3 The Association of *NLRP3* Inflammasome (rs10754558) with the Risk of Different Clinical, Demographic, and Biochemical Characteristics in Patients with T2DM

Item	GG (152) n, %	GC (92) n, %	CC (6) n, %	P value	
Diabetes complications					
Patients with nephropathy	15 (9.9%)	9 (9.8%)	5 (83.3%)	<0.001**	
Patients without nephropathy	137 (90.1%)	83 (90.2%)	1 (16.7%)		
Patients with Retinopathy	7 (4.6%)	5 (5.4%)	1 (16.7%)	0.42	
Patients without Retinopathy	145 (95.4%)	87 (94.6%)	5 (83.3%)		
Patients with Neuropathy	6 (3.9%)	3 (3.3%)	0	0.35	
Patients without Neuropathy	146 (96.1%)	89 (96.7%)	6 (100%)		
Patients with CVD	17 (11.2%)	10 (10.9%)	5 (83.3%)	<0.001**	
Patients without CVD	135 (88.8%)	82 (89.1%)	3 (16.7%)		
Patients with PAOD	11 (7.2%)	8 (8.7%)	1 (16.7%)	0.76	
Patients without PAOD	141 (92.8%)	84 (91.3%)	5 (83.3%)		
Other characteristics					
Smokers	38 (25%)	33 (35.9%)	1 (16.7%)	0.15	
None-smokers	114 (75%)	59 (64.1%)	5 (83.3%)		
Male	89 (58.6%)	59 (64.1%)	4 (66.7%)	0.65	
Female	63 (41.4%)	33 (35.9%)	2 (33.3%)		
NW	66 (43.4%)	37 (40.2%)	0		
OW	71 (46.7%)	45 (48.9%)	3 (50%)	0.089	
OB	15 (9.9%)	10 (10.9%)	3 (50%)		
Patients with hypertension	42 (27.6%)	28 (30.4%)	3 (50%)	0.47	
Patients without hypertension	110 (72.4%)	64 (69.6%)	3 (50%)		
Biochemical profiles	**GG (133) Mean ± SD**	**GC (112) Mean ± SD**	**CC (5) Mean ± SD**	**OR (95% CI)**	***P*-value**
NLRP3 (ng/mL)	5.9±0.8	6.1±0.7	6.9±1	3.1 (2.70–7.90)	0.004**
IL-1β (pg/mL)	11.2±2.9	11.9±2	13.9±2.3	2.6 (1.12–5.30)	0.01*
FPG (mmol/l)	7.2±1.2	7.6±1.1	7.9±1.1	2.3 (0.35–4.22)	0.017*
HbA1c (%)	7±1	7.3±0.9	7.7±0.8	3.1 (1.10–5.00)	0.02*
TC (mmol/l)	4.5±0.3	4.6±0.6	4.8±0.71	0.66 (0.14–1.19)	0.12
TG (mmol/l)	1.7±0.2	1.72±0.2	1.9±0.6	0.6 (0.09–1.13)	0.11
LDL (mmol/l)	2.66±0.3	2.67±0.3	2.92±0.6	0.86 (0.35–1.38)	0.18
HDL (mmol/l)	1.25±0.1	1.24±0.1	1.17±0.1	0.72 (0.23–1.25)	0.18
Allele comparison		**OR**	**(95% CI)**		***P*-value**
Dominant model: GC+CC vs.GG		1.81	1.15–2.78		0.01*
Recessive model: GC+GG vs CC		3.42	1.02–9.85		0.04*
Per C allele		1.62	1.15–2.22		0.006**

Notes: * significant at p<0.05. ** significant at p<0.01.
Abbreviations: CVD, cardiovascular diseases; PAOD, peripheral arterial occlusive disease; SD, standard deviation; NLRP3, NOD-like receptor family, pyrin domain containing 3; NW, normal weight; OW, overweight; OB, obese; OR, odd ratio; CI, confidence intervals.

a significant association; CC carriers are similar to nephropathy (83.3%), GC (10.9%), and GG (11.2%) ($p < 0.001$). Biochemical profiles exhibited a genotype-dependent trend. NLRP3 and IL-1β levels were elevated in allele C carriers (GC and CC) compared to GG carriers ($p = 0.004$ and $p = 0.01$, respectively). Additionally, the CC and GC genotypes had higher levels of FPG and HbA1c compared to the GG genotype ($p = 0.017$ and $p = 0.02$, respectively), with similar findings.

Table 4 indicates significant correlations between the NLRP3 gene variant rs10754558 and type 2 diabetes mellitus (T2DM) susceptibility (OR = 1.72, 95% CI: 1.20–2.56, $p = 0.013$) and related complications, including nephropathy (OR = 3.10, 95% CI: 1.35–7.12, $p = 0.006$) and cardiovascular disease (CVD) (OR = 2.98, 95% CI: 1.24–6.71, $p = 0.008$).

Table 4 Shows Multivariate Logistic Regression Analysis Adjusted for C Allele Against T2DM Risk and Complications, and Pearson Correlation Between NLRP3 and IL-1β Against Biochemical Variables in Cases Group

Regression Analysis		
Item	**OR (95% CI)**	***P*-value**
T2DM risk	1.72 (1.2–2.56)	0.013*
Nephropathy	3.1 (1.35–7.12)	0.006**
CVD	2.98 (1.24–6.71)	0.008**
Pearson correlation		
NLRP3	**r^2**	***P*-value**
IL-1β	0.62	<0.001**
FPG	0.59	<0.001**
HbA1c	0.54	<0.001**
UACR	0.48	<0.001**
IL-1β		
FPG	0.46	<0.001**
HbA1c	0.41	<0.002**
UACR	0.39	0.02*

Notes: * significant at p<0.05. ** significant at p<0.01.
Abbreviations: CVD, cardiovascular diseases; *NLRP3*, NOD-like receptor family, pyrin domain containing 3; IL-1β, interleukin 1 beta.

Notable relationships were also observed between NLRP3 levels and urinary albumin-to-creatinine ratio (UACR) (p<0.001), fasting plasma glucose (FPG) (p<0.001), HbA1c (p<0.001), and interleukin-1β (IL-1β) (p<0.001). Additionally, IL-1β demonstrated strong correlations with UACR (p=0.02), HbA1c (p=0.002), and FPG (p<0.001).

The diagnostic accuracy of circulating inflammatory markers was assessed using ROC curve analysis. Both markers showed exceptional discriminatory power for identifying patients with T2DM. NLRP3 had an area under the curve (AUC) of 0.988 (95% CI: 0.980–0.995), with 85.3% sensitivity and 90.1% specificity. Similarly, IL-1β showed a strong diagnostic profile with an AUC of 0.918 (95% CI: 0.889–0.942), achieving 76.3% sensitivity and 80.2% specificity (Figure 1). Furthermore, elevated levels of these biomarkers were significantly correlated with key clinical indicators of metabolic distress and vascular injury, including fasting plasma glucose (FPG), HbA1c, and the UACR ($p < 0.001$).

Bioinformatics Analysis

Figure 2 depicts the rs10754558 (G>C) variant in the 3′UTR of the NLRP3 gene, showing the C allele as more prevalent than the G allele globally. European populations have the highest prevalence (46%), while African populations show considerable variation, with the G allele ranging from 19% to 31%. This polymorphism may affect susceptibility to NLRP3-related inflammatory conditions like T2DM. The rs10754558 SNP alters miRNA–mRNA interactions, with seven miRNAs gaining binding affinity and three losing it. Regulome DB assigned this variant a functional rank of 1f, indicating its likely regulatory role related to transcription factor binding and potential effects on post-transcriptional regulation. This figure supported with Supplementary materials (Tables S1, S2 and Figures S1, S2).

Figure 3 shows Protein–Protein Interaction and Pathway Analysis, NLRP3 is a main hub in a huge interconnected network of inflammasome-related proteins, that also includes CASP1, PYCARD, NEK7, TXNIP, AIM2, and NLRC4, according to protein–protein interaction (PPI) research using STRING. Its important function in caspase-mediated signaling cascades and inflammasome formation is emphasizing by its strong connectivity.

Functional enrichment of these interacting partners revealed strong associations with inflammatory and immune pathways. With highly significant FDR values (p<0.01), KEGG pathway analysis showed substantial enrichment of the *NLRP3*, pyroptosis, and apoptotic processes, indicating that *NLRP3* and its connectors are important regulators of innate

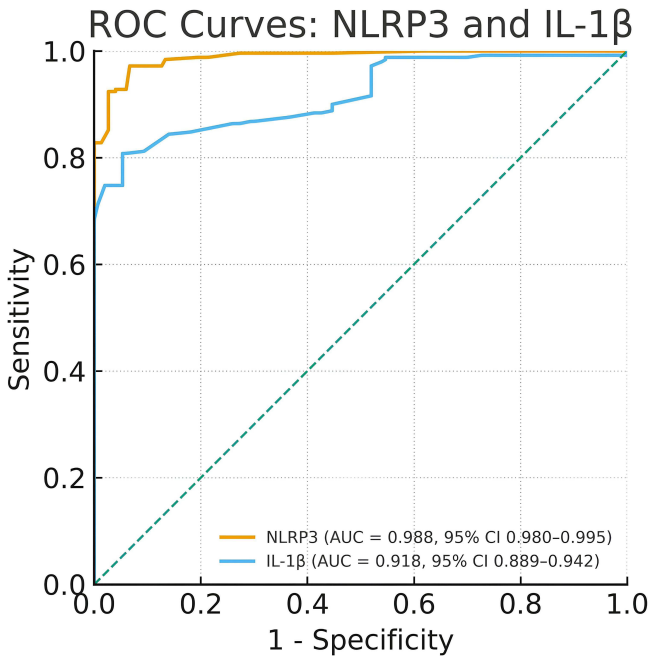


Figure 1 Receiver operating characteristic (ROC) curves exhibits the diagnostic performance of NLRP3 and IL-1β in separating patients from controls. Each biomarker's sensitivity and specificity are represented by the area under the curve (AUC).

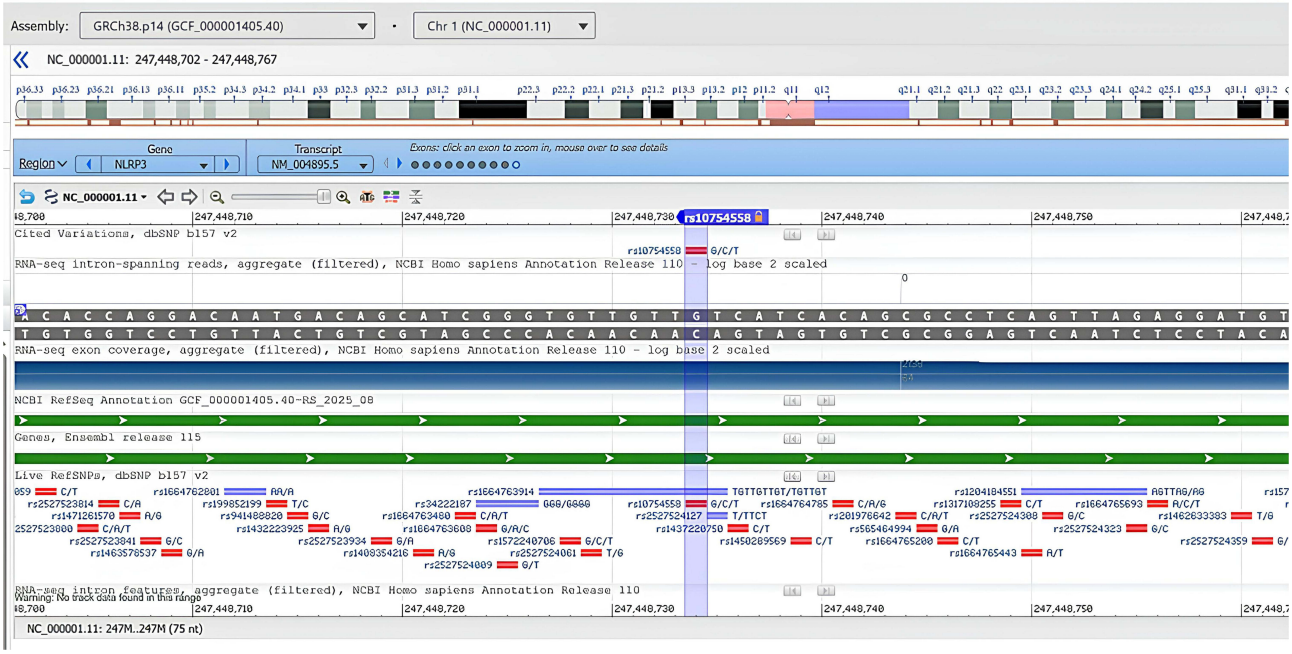


Figure 2 Genomic context of the *NLRP3* rs10754558 variant acquired from the NCBI Genome Data Viewer (GRCh38.p14). The variation can be found in the NLRP3 gene's 3′ untranslated region (3′UTR) on chromosome 1 (chr1:247,448,739, plus strand). The polymorphic seat is shown by the blue band.

immunity (Figure 4). Immunological roles of *NLRP3*-associated proteins are appeared in the enrichment in processes involving cytokine production, inflammasome complex assembly, and regulation of apoptotic signaling, which was further confirmed with complementary Gene Ontology (GO) biological process evaluation (Figure 5).

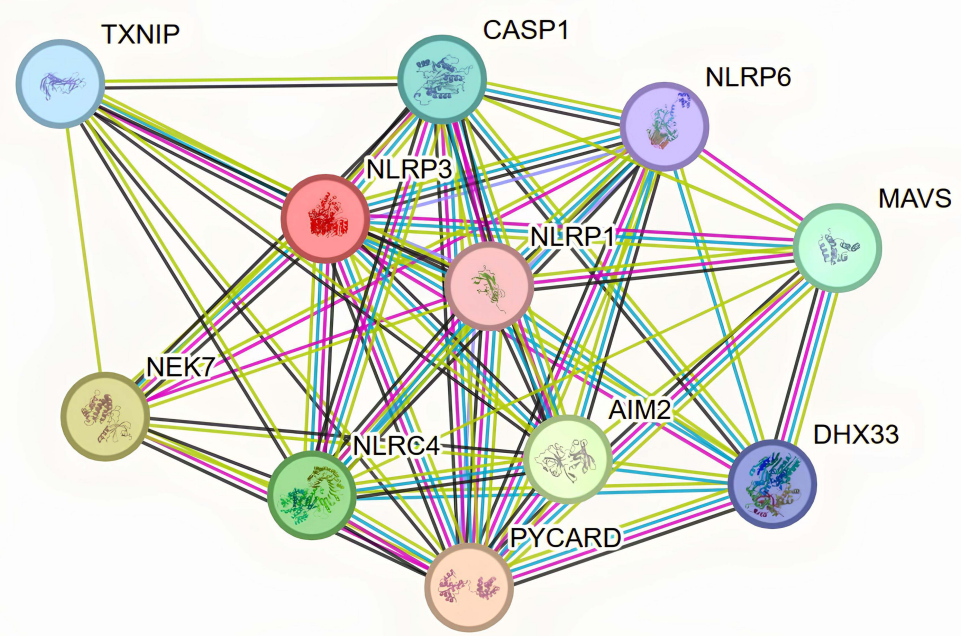


Figure 3 NLRP3 is a central hub in a protein–protein interaction (PPI) network involving inflammasome-related proteins such as CASP1, PYCARD, NEK7, TXNIP, NLRC4, AIM2, and MEFV. The STRING-generated network illustrates these associations with varying edge thickness based on the reliability of the data sources. This highlights NLRP3's role in regulating immune responses and inflammasome assembly.

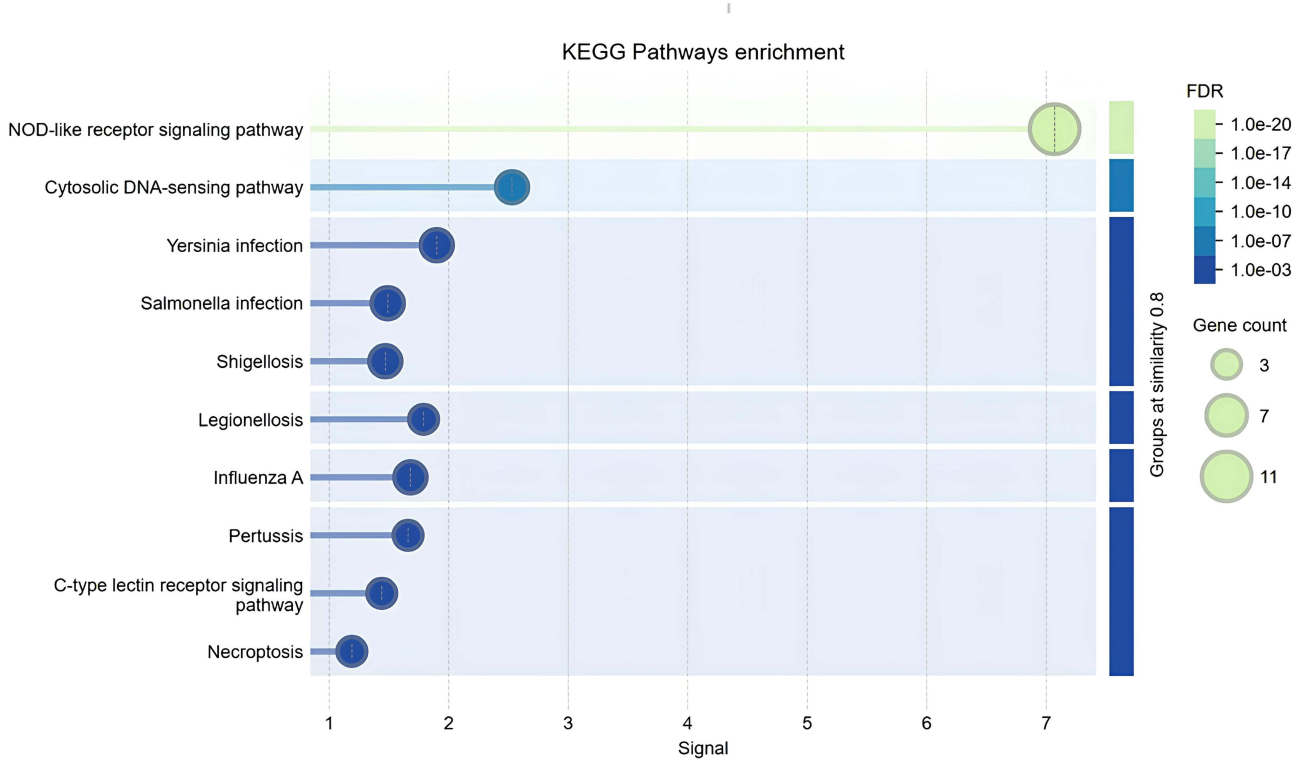


Figure 4 KEGG pathway enrichment of NLRP3-interacting proteins. Bubble plot of significantly enriched pathways based on KEGG analysis. The x-axis: enrichment score, y-axis: top pathways. Bubble size corresponds to the number of associated genes, and bubble color represents the false discovery rate (FDR) (from 1e−03 to 1e−29).

https://doi.org/10.2147/DMSO.S586787

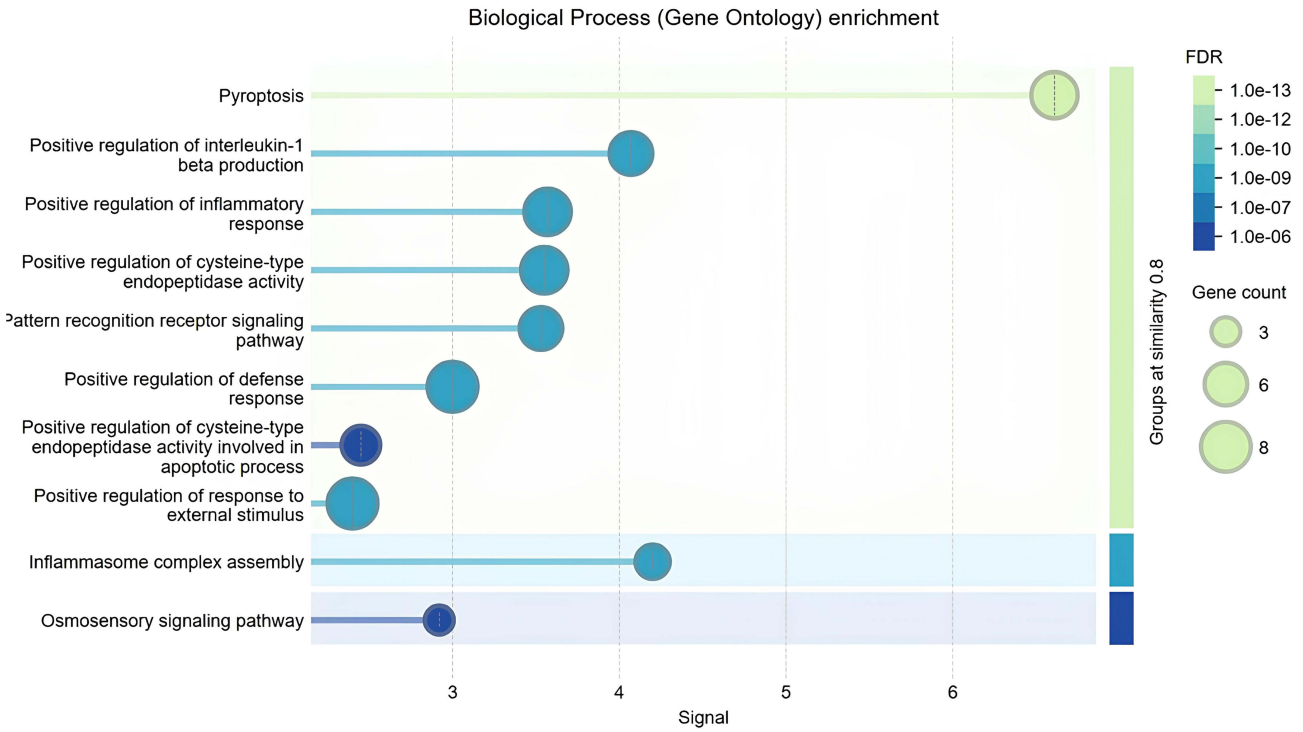


Figure 5 Biological process Gene Ontology enrichment analysis of *NLRP3* interactors. Bubble size denotes gene counts, and bubble color indicates the FDR value (scale from 1.0e−10 to 1.0e−24). Horizontal bands separate functional categories from different annotation databases. Larger and greener bubbles located toward the right side of the plot denote the strongest enrichment signals.

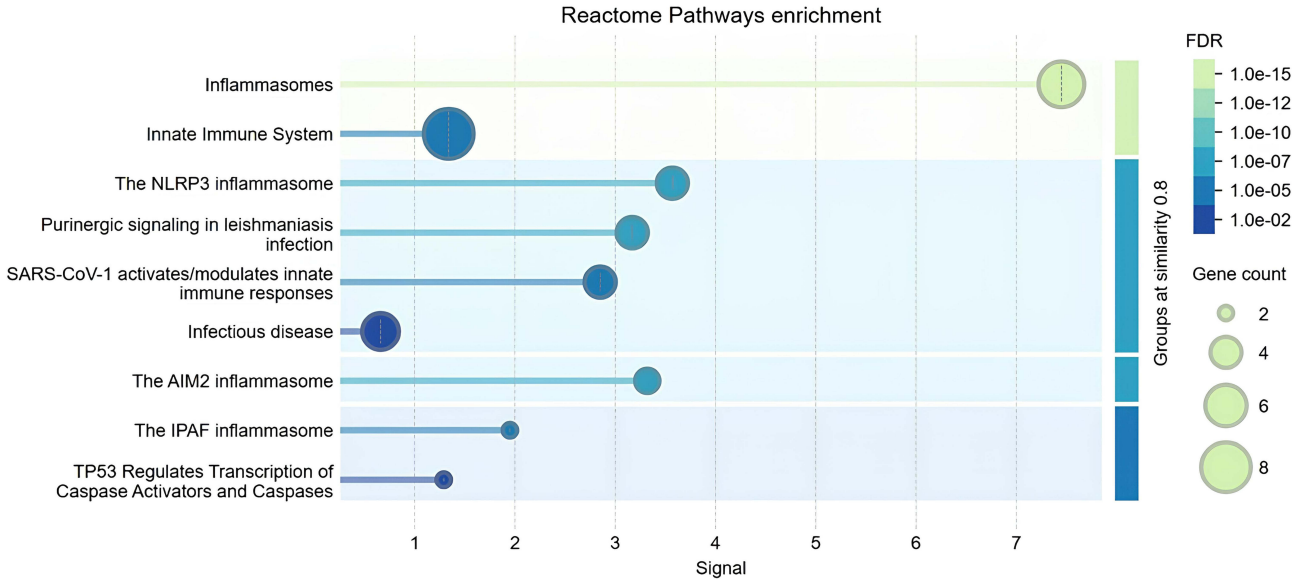


Figure 6 Reactome pathways enrichment analysis of NLRP3-associated genes is illustrated through a bubble plot, where color gradient represents significance (FDR) and bubble size indicates gene count. The upper right quadrant displays pathways with higher enrichment scores and larger gene counts, notably highlighting significant enrichment in interleukin-1 processing, the NLRP3 inflammasome, and innate immune system activation.

Figure 6 shows reactome pathway analysis highlighted the downstream inflammatory consequences of *NLRP3* activation via showing enrichment pathways related to interleukin-1 processing, innate immune system activation, and caspase-mediated cell death. Each of these outcomes demonstrates the essential role of *NLRP3* complex network of inflammatory signaling pathways, and that variation at rs10754558 could influence these pathways through altering post-

transcriptional regulation. This systems-level architecture underscores how *NLRP3* coordinates the cellular response to metabolic "danger signals," such as hyperglycemia and lipotoxicity, which are prevalent in T2DM.

Discussion

Numerous complications can affect patients with T2DM, including microvascular and macrovascular complications. T2DM could be linked with hundreds of genetic variations and polymorphisms that may contribute to the risk of developing diabetes.[19]

The association between the *NLRP3* inflammasome variant rs10754558 in the current study and T2DM, as well as its complications, among Saudi patients, was assessed, along with the protein levels of *NLRP3, IL-1β*, glycemic, and lipid profiles. The most striking results were the significant association between the *NLRP3* inflammasome variant rs10754558 and T2DM, along with disease complications, particularly nephropathy and CVD. The significant association between T2DM and the *NLRP3* inflammasome rs10754558 gene polymorphism agrees with previous findings.[9–11,20–22] The *NLRP3* inflammasome functions as a molecular sensor, initiating the release of *IL-1β* and *IL-18* upon activation, thereby propagating inflammation that contributes to the development of metabolic disorders.[23,24] The *NLRP3–IL-1β* axis plays a central role in the onset and development of T2DM. A recent review emphasizes that metabolic stressors (obesity, hyperglycemia) could lead to overactivation of this mechanism, resulting in insulin dysfunction through systemic inflammation.[5] *NLRP3* could contribute to glycemic dysregulation and exacerbate micro/macrovascular complications through endothelial dysfunction, a hallmark of vascular pathology in diabetes, which is intensified with persistent *NLRP3* activation, leading to elevated atherosclerosis and CVD.[7]

Mechanistically, the C allele at rs10754558 is associated with increased NLRP3 expression and inflammasome activation, leading to higher IL-1β release, which impairs β-cell function and increases insulin resistance.[8] This study found the CC genotype linked to nephropathy and cardiovascular disease (CVD) at 83.3%, while GC and GG genotypes showed no such connection. Individuals with the CC genotype also had a higher glycemic profile, elevated NLRP3 and IL-1β levels, and significant differences in outcomes. The C allele at rs10754558 may predispose individuals to severe metabolic inflammatory defects in type 2 diabetes mellitus (T2DM) and is connected to a higher risk of diabetes-related complications, while the GG genotype exhibited the lowest rates of nephropathy and CVD, emphasizing the role of NLRP3-mediated inflammation in diabetic complications.[9] Moreover, the study highlights the diagnostic potential of NLRP3 and IL-1β as biomarkers for early detection and management of T2DM complications. Their strong correlation with UACR suggests that the NLRP3–IL-1β axis could serve as a sensitive indicator of early-stage diabetic nephropathy, identifying risks prior to significant clinical decline through traditional glycemic markers.

These outcomes reflect recent emerging evidence from studies in the Saudi population, which indicates that glycemic control and inflammation modulate *NLRP3* levels. Alfadul et al[25] show that in prediabetic Saudi subjects, *NLRP3*, linked to interleukins (eg, *IL-1α, IL-33*), is significantly affected by lifestyle modifications and glycemic status (reduced *NLRP3* after 6 months of improvement). Furthermore, increased *IL-1β* and its regulatory inflammasome could elevate diabetic complications.

Furthermore, recent research in adult Chinese populations[26] has shown that *NLRP3* polymorphisms are associated with the risk and inflammatory dysregulation in hypertension, a finding that contrasts with our study. However, the synergistic influence of genetic factors and metabolic stressors may also be applicable in our cohort, considering the increased BMI and systolic and diastolic blood pressure among cases.

When comparing our findings with existing data from the 1000 Genomes Project, the frequency of the C allele in Saudi patients in the current study is 20.8%, which is slightly higher than that recorded in Europeans (17%) and lower than in East Asians (25%).[27] There are historical factors, such as genetic drift, that might have influenced the frequencies of some alleles in East Asian populations more than in European populations. The NLRP3 rs10754558 variation has been linked to metabolic and inflammatory characteristics in a variety of ethnic groups, according to research mostly conducted on a Saudi population. Replication studies in a variety of groups are necessary to validate outcomes since reported associations are affected by variations in allele frequencies and effect sizes.

Our in-silico analysis showed that rs10754558 alters the *NLRP3* 3′UTR miRNA–mRNA landscape, causing both gain and loss of binding sites. While sepsis studies with luciferase assays indicated that the C allele increases miR-146a

binding and reduces *NLRP3* expression, our data from Saudi T2DM patients revealed that the CC genotype is linked to higher *NLRP3* expression and *IL-1β* levels.[28] This discrepancy probably likely reflects unique disease contexts: chronic low-grade inflammation in T2DM, when miR-146a is often dysregulated, and acute inflammation in sepsis, when the loss of other inhibitory miRNAs (eg, miR-3065-5p, miR-425-5p) can result in excessive expression. The idea that *NLRP3* regulation is heavily dependent on miRNAs and context is further reinforced by research on preeclampsia, when miR-223-3p inhibits *NLRP3* inflammasome activation.[29] Taken into account, our findings emphasize it is important to interpret regulatory polymorphisms in frameworks which are applicable to both populations and diseases.

Beyond analyzing a single gene, STRING-based protein–protein interaction (PPI) networks positioned *NLRP3* at the center of an inflammasome regulatory module, interacting with *CASP1, ASC, IL-1β*, and *IL-18*. NOD-like receptor signaling and IL-1 signaling were significantly prominent, as determined by pathway enrichment analysis using KEGG and Reactome. These findings reveal how *NLRP3* dysregulation associates innate immunity and metabolic disease from a systems-level viewpoint.

Clinically, nephropathy and cardiovascular disease are two main causes of mortality and morbidity in people with T2DM, were closely associated with the CC genotype in our participants. Carriers of rs10754558 exhibit greater inflammasome activity, which contributes to vascular damage and β-cell dysfunction, based on our integrated genetic data, functional identification, and pathway analysis. The NLRP3–IL-1β axis is a prospective therapeutic target, based on these findings. In fact, NLRP3-specific inhibitors and IL-1 antagonists such anakinra and canakinumab are currently in clinical studies to treat cardiovascular and metabolic disease. According to our findings, genetic stratification may be useful in identifying patients who stand to gain the most. Considering their connection with nephropathy, cardiovascular disease, and glycemic indices, NLRP3 and IL-1β represent promising inflammatory biomarkers in T2DM. They could enhance traditional metabolic evaluations, enabling early risk classification; however, further research is required for defined thresholds and prediction validity.

Environmental and lifestyle factors, such as nutrition, inactivity, and obesity, affect genetic vulnerability to T2DM and its consequences by promoting inflammation and metabolic stress. Insulin resistance is aggravated by a high-fat diet and oxidative stress, both enhance NLRP3 signaling and IL-1β. Future research should examine how genetic and lifestyle factors interact.

Chronic metabolic stress in metabolic diseases affects vascular health via the NLRP3 inflammasome, which interacts with GSDMD and CASP1 leading to endothelial dysfunction and related complications. Hyperglycemia activates the NOD-like receptor pathway, heightening IL-1β secretion and worsening diabetes-related issues, particularly in carriers of the rs10754558 C allele.

This study is the first comprehensive assessment of rs10754558 in Saudi patients, combining genetic, biochemical, and bioinformatic evidence. Its strengths include using clinical markers, inflammatory mediators, and computational predictions to gain mechanistic insights. Limitations include a small sample size, especially for the rare CC genotype, and a case–control design that limits causal conclusions. Larger, comprehensive cohorts, gene–gene interactions such as CARD8, and environmental factors like dietary habits and obesity should all be addressed in future research. Validation of the SNP's regulatory function may also be improved by conducting functional investigations to confirm miRNA and NLRP3 interactions. The low frequency of the rs10754558 CC genotype, which reduces statistical power for CC carrier analysis. It limited power for alone CC comparisons, while it was well powered for GC+CC pairings. Results should be interpreted carefully, emphasizing the need of further extensive, multicenter research on uncommon genotypes.

In order to enhance early identification of high-risk individuals and focused interventions for T2DM and address the complex interactions among metabolic, inflammatory, and hereditary variables in diabetes mellitus,[30] future research should integrate genetic risk scores (GRS) with continuous glucose monitoring (CGM) data to improve risk prediction and tailor management techniques.

Conclusion

This study highlights a significant link between NLRP3 rs10754558 and susceptibility to T2DM and its vascular complications within the Saudi population. The variant is associated with heightened inflammasome activity and poorer glycemic profiles. While these results support the biological plausibility of the NLRP3–IL-1β axis in the development of

diabetes, they should be regarded as preliminary until confirmed through experimental validation. Additional multi-ethnic and functional research is necessary to establish the regulatory role of this variant and its potential translational relevance. Future targeted anti-inflammatory treatments may be guided by NLRP3 rs10754558, a genetic marker for T2DM risk and vascular consequences.

Data Sharing Statement

Data used in this study are available upon request from the corresponding author, and the data are not public for privacy of participants.

Acknowledgment

The authors would like to thanks Taibah University for support.

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All authors have agreed on the journal to which the article will be submitted, have agreed on the final version of the article for publication, and have agreed to take responsibility and be accountable for the contents of the article.

Funding

No funding was received.

Disclosure

The authors declare there were no conflicts of interest.

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