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Association between Toll-Like Receptor 4 Genetic Variants and Hyperlipidemia Risk in Palestinian Adults: A Case-Control Study

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Abstract

Hyperlipidemia and hypercholesterolemia are metabolic conditions that elevate cardiovascular disease risk and frequently co-occur with obesity, insulin resistance, and other metabolic disturbances. Toll-like receptor 4 (TLR4), a key mediator of innate immune responses, has been proposed as a link between lipid metabolism and inflammatory pathways; however, the extent to which TLR4 genetic variation influences hyperlipidemia susceptibility remains inadequately defined. This investigation examined whether the TLR4 polymorphisms rs4986790 (Asp299Gly) and rs1927914 are associated with hyperlipidemia risk in a Palestinian cohort. The study employed a case-control design comprising 55 diagnosed hyperlipidemia/hypercholesterolemia patients and 50 age- and sex-matched healthy individuals. Genomic DNA was extracted from peripheral blood, and TLR4 variants were genotyped using PCR-RFLP with *BccI* and *SphI* enzymes. Genotype and allele frequency comparisons were performed using chi-square analysis, with associations quantified through odds ratios (ORs) and 95% confidence intervals (CIs). No statistically significant relationship emerged between rs4986790 and hyperlipidemia susceptibility ($P > 0.05$). In contrast, rs1927914 demonstrated a robust association with disease risk. Relative to the TT genotype, TC genotype carriers exhibited elevated susceptibility (OR = 17.63, 95% CI: 3.85–81.20, $P < 0.001$), and the CC genotype likewise conferred increased risk (OR = 10.06, 95% CI: 1.15–88.40, $P = 0.03$). Dominant model analysis and allele-based comparisons confirmed a significant relationship between the C allele and hyperlipidemia ($P < 0.001$). These observations suggest that TLR4 rs1927914, but not rs4986790, may influence genetic predisposition to hyperlipidemia among Palestinians and could potentially serve as a biomarker for identifying individuals at heightened disease risk.

Keywords: Hyperlipidemia, Hypercholesterolemia, Toll-like receptor 4, TLR4 polymorphism, rs1927914, Palestinian population

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Introduction

Dyslipidemia is one of the most common metabolic disorders worldwide and remains a major public health concern due to its strong association with cardiovascular disease (CVD), atherosclerosis, and premature death (Abera *et al*., 2024; Ballena-Caicedo *et al*., 2025). It is characterized by abnormal plasma lipid profiles, including elevated levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG), and decreased levels of high-density lipoprotein cholesterol (HDL-C). Beyond these quantitative abnormalities, dyslipidemia is also associated with qualitative alterations in lipoprotein composition, such as an increased proportion of small dense LDL particles, triglyceride-enriched LDL, and more electronegative LDL particles, all of which possess enhanced atherogenic potential. These lipid abnormalities promote endothelial dysfunction, oxidative stress, chronic vascular inflammation, and lipid deposition in the arterial wall, thereby accelerating the initiation and progression of atherosclerosis and increasing the risk of adverse cardiovascular outcomes (Owens *et al*., 2014; Libby, 2021). The burden of dyslipidemia is particularly pronounced in the Middle East, where rapid urbanization, westernized dietary habits, physical inactivity, and rising obesity rates have contributed to a substantial increase in hyperlipidemia, metabolic disorders, type 2 diabetes mellitus (T2DM), and other cardiovascular disease risks, making dyslipidemia an important and growing public health concern across the region (Motlagh *et al*., 2009; Kargar & Ansari, 2023; Al-Ashwal *et al*., 2024).

The burden of dyslipidemia is significantly increasing in Palestine. According to the Palestinian Ministry of Health (2024) and the World Health Organization STEPS survey reported by Massad *et al*. (2025), non-communicable diseases, particularly CVD and diabetes, remain the leading causes of morbidity and mortality among Palestinian adults. Studies conducted in the West Bank have further shown that metabolic syndrome affects nearly one-third of the adult population and is frequently accompanied by dyslipidemia, obesity, hypertension, and impaired glucose

metabolism (El Bilbeisi *et al*., 2017; Damiri *et al*., 2022). These health challenges are compounded by socioeconomic hardship, limited healthcare resources, and the prolonged effects of regional conflict, which hinder effective prevention and management of chronic diseases (Marzouk *et al*., 2023). Together, these observations emphasize the importance of identifying genetic as well as environmental factors that influence susceptibility to dyslipidemia, particularly genes involved in inflammatory pathways that may contribute to inter-individual variation in disease risk within the Palestinian population.

The innate immune system serves as the body's primary defense against invading pathogens and endogenous danger signals by utilizing pattern-recognition receptors (PRRs). Among these, Toll-like receptors (TLRs) constitute the most extensively investigated receptor family. Through the detection of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), TLRs trigger intracellular signaling cascades that activate nuclear factor-κB (NF-κB) and interferon regulatory factors, thereby orchestrating inflammatory responses that are crucial for effective host defense and the maintenance of tissue homeostasis (Medzhitov, 2001; Akira, 2003; Beutler & Rietschel, 2003; Akira & Takeda, 2004; Bhardwai *et al.*, 2024). Structural and functional studies have further clarified the ligand recognition mechanisms of TLRs and their broader roles in immunity, inflammation, and chronic diseases (Gay & Gangloff, 2007; Jin & Lee, 2008; Kumar *et al*., 2009; Sameer & Nissar, 2021; Kawai *et al*., 2024).

Within the Toll-like receptor family, TLR4 plays a pivotal role in connecting innate immune responses with metabolic dysfunction. In addition to detecting bacterial lipopolysaccharide (LPS), TLR4 responds to endogenous mediators produced during metabolic stress, such as saturated fatty acids, oxidized low-density lipoprotein (oxLDL), heat-shock proteins, and other damage-associated molecules. Its activation initiates MyD88- and TRIF-mediated signaling cascades that stimulate NF-κB and promote persistent production of pro-inflammatory cytokines, thereby driving chronic metabolic inflammation (Akira & Takeda, 2004; Takeda & Akira, 2015; Kawai *et al*., 2024).

A growing body of experimental evidence supports the involvement of TLR4 in lipid metabolism and cardiovascular disease. Saturated fatty acids activate TLR4-dependent inflammatory signaling and modulate TLR-mediated immune responses (Lee *et al*., 2001; Lee *et al*., 2003), whereas TLR4 activation has been implicated in insulin resistance, obesity-associated inflammation, and atherosclerosis (Shi *et al*., 2006; Rocha *et al*., 2016; Yang *et al*., 2016). Animal studies have further demonstrated that disruption of TLR4 signaling reduces atherosclerotic lesion formation (Michelsen *et al*., 2004), while reviews by Erridge (2009), Moore *et al*. (2013), and Libby (2021) established chronic TLR4-mediated inflammation as a major driver of vascular disease. Additional evidence indicates that TLR4 expression is associated with hyperlipidemia in humans (Zhu *et al*., 2015), and that interactions between TLR4 signaling, cholesterol metabolism, and innate immune activation contribute to vascular dysfunction (Zhu *et al*., 2010; Motkowski *et al*., 2022; de Oliveira *et al*., 2024; Bosco *et al*., 2025).

Genetic variation within the TLR4 gene may further influence susceptibility to inflammatory and metabolic disorders by altering receptor expression or downstream signaling. Lazarus *et al*. (2002) first highlighted the extensive genetic diversity within innate immunity genes and its potential contribution to complex diseases. Among the identified variants, rs4986790 (Asp299Gly) is one of the most extensively investigated functional polymorphisms. Functional studies demonstrated that this variant modifies TLR4 responsiveness to inflammatory stimuli (Ferwerda *et al*., 2008), while numerous association studies and meta-analyses have linked TLR4 polymorphisms to inflammatory, infectious, autoimmune, cardiovascular, and malignant diseases (Jing *et al*., 2012; Senhaji *et al*., 2014; Chrzęszczyk *et al*., 2015; Hu *et al*., 2016; Liu *et al*., 2016; Pellegrino *et al*., 2016; Chaiwiang & Poyomtip, 2019; Semlali *et al*., 2019; Tongtawee *et al*., 2019; Abida *et al*., 2020; Quirino *et al*., 2021; Mostafa *et al*., 2022; Jahromi *et al*., 2024). Despite growing interest in TLR4 genetics, relatively few studies have investigated the association between TLR4 polymorphisms and dyslipidemia. Moreover, the reported findings have been inconsistent across different ethnic populations, underscoring the need for further research in understudied populations such as Palestinians.

Evidence supporting the involvement of TLR4 polymorphisms in metabolic disease is emerging in the Palestinian population. Our recent work (Al-Razem *et al*., 2025) demonstrated significant associations between TLR4 polymorphisms and susceptibility to T2DM, suggesting that genetic variation within innate immune pathways contributes to metabolic disease risk among Palestinians. However, no previous study has investigated whether TLR4 polymorphisms influence susceptibility to hyperlipidemia or hypercholesterolemia in this population.

Therefore, this case-control study investigated the association of two TLR4 polymorphisms, rs4986790 (Asp299Gly) and rs1927914, with susceptibility to hyperlipidemia/hypercholesterolemia in Palestinian adults. Understanding the contribution of TLR4 genetic variation to dyslipidemia may improve our understanding of the inflammatory mechanisms underlying lipid disorders and provide a foundation for population-specific genetic risk assessment and future precision medicine strategies for preventing cardiovascular and metabolic diseases.

Materials and Methods

Study Design and Study Population

This case-control study investigated the association between two TLR4 gene polymorphisms and susceptibility to hyperlipidemia/hypercholesterolemia in Palestinian adults. A total of 105 unrelated participants of Palestinian origin were enrolled, including 55 patients with hyperlipidemia/hypercholesterolemia and 50 apparently healthy controls. Participants were recruited from hospitals, outpatient clinics, and primary healthcare centers in the Hebron Governorate, Palestine. Both men and women aged 30-74 years were included. The case and control groups were well matched for age and sex, with no statistically significant differences between them.

Clinical Evaluation and Participant Selection

Patients were diagnosed with hyperlipidemia/hypercholesterolemia by their treating physicians according to the Palestinian national clinical guidelines for the management of dyslipidemia. The diagnosis was based on the participants' clinical history (repeated measurements) together with fasting lipid profile results obtained during routine clinical care after an overnight fast of 10-12 hours. Laboratory investigations, including measurements of total cholesterol, triglycerides, and other lipid parameters, were performed by the participating healthcare centers as part of routine patient management. Measurements were all carried out by specialized health professionals and were not repeated by the research team.

Individuals were eligible for inclusion in the case group if they had a documented physician diagnosis of hyperlipidemia and/or hypercholesterolemia based on fasting lipid profile results recorded in their medical records. Three patients were also diagnosed with concomitant hyperglycemia according to their medical records and fasting plasma glucose measurements.

Healthy controls were recruited from the same geographical area and were frequency-matched to cases by age and sex. Control participants had no previous history of hyperlipidemia, hypercholesterolemia, diabetes mellitus, cardiovascular disease, or other major metabolic disorders. Their medical records indicated normal fasting lipid profiles according to the same national clinical guidelines. None of the healthy controls had documented hyperglycemia or diabetes mellitus.

Participants with incomplete clinical information or insufficient blood samples for DNA extraction were excluded from the study.

Ethical Approval and Blood Collection

The study protocol received ethical approval from the Research Ethics Committee of Palestine Polytechnic University and from the relevant ethics committees and administrative authorities of participating hospitals and healthcare centers in the Hebron Governorate, Palestine.

Peripheral venous blood samples (3-5 mL) were collected from each participant into sterile EDTA tubes. Samples were transported to the Molecular Biology Laboratory at Palestine Polytechnic University and stored at 4°C for a maximum of several days before genomic DNA extraction.

DNA Extraction and TLR4 Genotyping

Genomic DNA was isolated from blood leukocytes using the Macherey-Nagel Genomic DNA Isolation Kit (Macherey-Nagel, Germany) according to the manufacturer's instructions. The TLR4 polymorphisms rs4986790 (Asp299Gly) and rs1927914 were amplified by polymerase chain reaction (PCR) using sequence-specific primers **(Table 1)**. PCR amplification was performed with a commercial PCR Master Mix (New England Biolabs, USA) according to the manufacturer's protocol and as previously described by Al-Razem *et al.* (2025).

Table 1. The primers and PCR-RFLP analysis of the TLR4 genetic polymorphisms used in the study.

SNP ID	Primers (5‘-3‘)	Restriction enzyme	RFLP Product size
rs4986790	Forward: CTGCTCTAGAGGGCCTGTG Reverse: TTCAATAGTCACACTCACCAG	*Bcc1*	AA = 140
			AG = 140, 77, 63
			GG = 77, 63
rs1927914	Forward: ACAAAATGGTCCCTCACAGC Reverse: TGGAAAGTAGCAAGTGCAATG	*Sph1*	TT = 157
			TC = 157, 90, 67
			CC = 90, 67

Genotyping was performed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. PCR products were digested with the restriction enzymes *BccI* for rs4986790 and *SphI* for rs1927914 **(Table 1)**. Each digestion reaction contained 1 µL of restriction enzyme, 5 µL of the appropriate reaction buffer, 2.5 µL of PCR product, and 41.5 µL of nuclease-free water, yielding a final volume of 50 µL. Reactions were incubated at 37°C for 60 minutes, followed by enzyme inactivation at 65°C for 20 minutes.

The resulting restriction fragments were separated by electrophoresis on 2% agarose gels stained with ethidium bromide and visualized using a Bio-Rad Gel Documentation System. Genotypes were determined based on the characteristic restriction fragment patterns. All molecular analyses were performed at the Palestine-Korea Biotechnology Center, Palestine Polytechnic University.

Statistical Analysis

All statistical analyses were conducted using IBM SPSS Statistics (IBM Corp., Armonk, NY, USA). Continuous variables are reported as mean ± standard deviation (SD), whereas categorical variables are summarized as numbers and percentages.

Baseline demographic characteristics were compared between the hyperlipidemia and control groups using the independent-samples Student's *t*-test for continuous variables and Pearson's chi-square (χ^2) test for categorical variables. The distribution of each TLR4 genotype among control participants was evaluated for conformity with Hardy-Weinberg equilibrium (HWE) using the chi-square goodness-of-fit test.

Differences in genotype and allele frequencies between cases and controls were assessed using Pearson's chi-square test or Fisher's exact test when expected cell counts were small. The strength of the association between TLR4 polymorphisms and hyperlipidemia risk was quantified by calculating odds ratios (ORs) with corresponding 95% confidence intervals (95% CIs) under codominant, dominant, and allelic inheritance models. All statistical tests were two-sided, and statistical significance was defined as a P value < 0.05.

Results and Discussion

Demographic and Clinical Characteristics of the Study Population

The study cohort comprised 105 unrelated adults of Palestinian origin, including 55 individuals with clinically diagnosed hyperlipidemia/hypercholesterolemia and 50 apparently healthy control subjects. The demographic and clinical characteristics of the participants are presented in **Table 2**.

Table 2. Demographic and clinical characteristics of the study participants

Characteristic	Cases (Hyperlipidemia/ Hypercholesterolemia) (n = 55)	Healthy Controls (n = 50)
Age group, n (%):		
<40	5(9.1)	3(6.0)
40-49	7(12.7)	11(22.0)
50-59	21(38.2)	17(34.0)
>60	22(40.0)	19(38.0)
Sex:		
Male	31 (56.4)	28 (56.0)
Female	24 (43.6)	22 (44.0)
Clinical inclusion criteria:		
Total cholesterol (mg/dL)	≥240	<200
Triglycerides (mg/dL)	≥200	<150
Fasting glucose (mg/dL)	≥126	<100
HbA1c	>6.5%	<5.7%
Hyperglycemia, n (%)	3 (5.5)	0 (0.0)

The case and control groups showed similar demographic profiles. Patients with hyperlipidemia included 31 men (56.4%) and 24 women (43.6%), while the control group comprised 28 men (56.0%) and 22 women (44.0%). Statistical analysis revealed no significant differences between the two groups with respect to age or sex, indicating that the controls were appropriately matched to the patient group for these baseline characteristics.

All patients in the case group had a documented physician diagnosis of hyperlipidemia/hypercholesterolemia based on their history of fasting lipid profiles obtained during routine clinical evaluation according to the Palestinian national clinical guidelines. In contrast, healthy controls had normal fasting lipid profiles and no previous history of hyperlipidemia or cardiovascular disease. Three patients (5.5%) in the hyperlipidemia group had concomitant hyperglycemia, whereas none of the healthy controls had documented hyperglycemia.

Association between the TLR4 rs4986790 Polymorphism and Hyperlipidemia

The distribution of TLR4 rs4986790 (Asp299Gly) genotypes and alleles in patients and controls is summarized in **Table 3**, and the corresponding risk estimates are presented in **Table 4**. Among patients with hyperlipidemia/hypercholesterolemia, the frequencies of the AA, AG, and GG genotypes were 78.2%, 12.7%, and 9.1%, respectively. In the control group, the corresponding frequencies were 82.0%, 10.0%, and 8.0%. Comparison of genotype distributions showed no significant difference between cases and controls ($\chi^2 = 0.22$, df = 2, $P = 0.89$).

Table 3. Genotype and allele frequencies of the TLR4 rs4986790 (Asp299Gly) polymorphism in hyperlipidemic patients and healthy controls Data are presented as number (percentage).

Variable	Cases (n = 55) n (%)	Controls (n = 50) n (%)	χ^2	*P-value*
Genotypes				
AA	43 (78.2)	41 (82.0)		
AG	7 (12.7)	5 (10.0)		
GG	5 (9.1)	4 (8.0)	0.22	0.89
Alleles				
A	93 (84.5)	87 (87.0)		
G	17 (15.5)	13 (13.0)	0.25*	0.62*

Using the AA genotype as the reference category, neither the AG genotype (OR = 1.34, 95% CI: 0.39–4.65, $P = 0.64$) nor the GG genotype (OR = 1.19, 95% CI: 0.30–4.79, $P = 0.81$) was associated with a significant change in the likelihood of hyperlipidemia **(Table 4)**.

Table 4. Association between the TLR4 rs4986790 (Asp299Gly) polymorphism and susceptibility to hyperlipidemia

Genetic Model	Comparison	OR	95% CI	P-value
Codominant	AG vs AA	1.34	0.39-4.65	0.64
	GG vs AA	1.19	0.30-4.79	0.81
Dominant	AG + GG vs AA	1.27	0.48-3.37	0.63
Allelic	G vs A	1.22	0.56-2.68	0.62

Reference genotype: AA.
Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using the AA genotype as the reference.

Evaluation of the dominant inheritance model (AG + GG vs. AA) likewise showed no evidence of an association. Although carriers of the G allele had slightly higher estimated odds of hyperlipidemia than individuals with the AA genotype, the difference did not reach statistical significance (OR = 1.27, 95% CI: 0.48–3.37, $P = 0.63$). Analysis at the allele level produced similar results. The G allele accounted for 15.5% of alleles in the patient group and 13.0% in the control group, yielding an OR of 1.22 (95% CI: 0.56–2.68, $P = 0.62$).

Taken together, these findings provide no evidence that the TLR4 rs4986790 (Asp299Gly) polymorphism is associated with susceptibility to hyperlipidemia/hypercholesterolemia in this Palestinian cohort.

Association between the TLR4 rs1927914 Polymorphism and Hyperlipidemia

The distribution of TLR4 rs1927914 genotypes and alleles among patients and controls is presented in **Table 5**, while the corresponding measures of association are shown in **Table 6**.

Table 5. Genotype and allele frequencies of the TLR4 rs1927914 polymorphism in hyperlipidemic patients and healthy controls

Variable	Cases (n = 55) n (%)	Controls (n = 50) n (%)	χ^2	*P-value*
Genotypes				

TT	28 (50.9)	47 (94.0)		
TC	21 (38.2)	2 (4.0)		
CC	6 (10.9)	1 (2.0)	31.4	<0.001
Alleles				
T	77 (70.0)	96 (96.0)		
C	33 (30.0)	4 (4.0)	34.2	<0.001

Table 6. Association between the TLR4 rs1927914 polymorphism and susceptibility to hyperlipidemia

Genetic Model	Comparison	OR	95% CI	*P-value*
Codominant	TC vs TT	17.63	3.85-81.20	<0.001
	CC vs TT	10.06	1.15-88.40	0.03
Dominant	TC + CC vs TT	15.11	4.21-54.40	<0.001
Allelic	C vs T	10.29	3.50-30.30	<0.001

Reference genotype: TT.
Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using the TT genotype as the reference.

A pronounced difference in genotype frequencies was observed between the two study groups. The TT genotype was the predominant genotype in healthy controls, occurring in 47 individuals (94.0%), but was identified in only 28 patients (50.9%). In contrast, the TC genotype was present in 21 patients (38.2%) compared with only two controls (4.0%), whereas the CC genotype was detected in six patients (10.9%) and one control (2.0%). Overall, the genotype distributions differed significantly between cases and controls ($\chi^2 = 31.43$, df = 2, $P < 0.001$).

Using TT homozygotes as the reference group, carriers of the TC genotype had a markedly greater likelihood of hyperlipidemia (OR = 17.63, 95% CI: 3.85–81.20, $P < 0.001$). A significant association was also observed for individuals with the CC genotype, who exhibited an approximately tenfold higher risk than TT homozygotes (OR = 10.06, 95% CI: 1.15–88.40, $P = 0.03$).

Evaluation of the dominant genetic model further supported these findings. Participants carrying at least one C allele (TC or CC) showed significantly higher odds of hyperlipidemia than those with the TT genotype (OR = 15.11, 95% CI: 4.21–54.40, $P < 0.001$) **(Table 6)**.

The allele-based analysis produced consistent results. The C allele frequency was substantially higher in patients than in controls (30.0% vs. 4.0%), corresponding to an OR of 10.29 (95% CI: 3.50–30.30, $P < 0.001$).

Overall, the results identify a strong relationship between the TLR4 rs1927914 polymorphism and susceptibility to hyperlipidemia/hypercholesterolemia in this Palestinian population. The presence of the C allele, particularly in individuals with the TC genotype, was associated with a markedly increased risk of hyperlipidemia compared with the TT genotype.

Hyperlipidemia and hypercholesterolemia remain among the most important modifiable risk factors contributing to cardiovascular disease (CVD) worldwide. Although disturbances in lipid metabolism have traditionally been attributed to dietary, metabolic, and genetic factors, increasing evidence indicates that chronic low-grade inflammation plays a fundamental role in the initiation and progression of dyslipidemia-associated vascular complications. Innate immune mechanisms, particularly Toll-like receptor (TLR)-mediated signaling, have emerged as important regulators linking metabolic stress, inflammation, and cardiovascular pathology (Garcia *et al*., 2020). Toll-like receptor 4 (TLR4) recognizes both pathogen-related molecular patterns, such as lipopolysaccharide, and endogenous metabolic danger signals, including saturated fatty acids and modified lipids, resulting in activation of downstream inflammatory pathways, particularly nuclear factor-κB (NF-κB), and the subsequent release of pro-inflammatory cytokines (Akira, 2003; Lee *et al*., 2003; Akira & Takeda, 2004). Persistent activation of TLR4 signaling contributes to macrophage activation, foam cell formation, insulin resistance, and atherosclerotic progression (Erridge, 2009; Moore *et al*., 2013; Libby, 2021; Mulyani *et al*., 2026).

The results of this work support the growing body of evidence indicating that dyslipidemia should be viewed not only as a disorder of lipid synthesis and transport but also as a chronic inflammatory condition. Increasing evidence suggests that persistent low-grade inflammation contributes to both the initiation and progression of metabolic disorders by disrupting lipid homeostasis and promoting vascular injury. In this regard, Robinson *et al*. (2023) proposed that sustained activation of innate immune pathways drives the development of hyperlipidemia, T2DM, and their cardiovascular complications involving mechanisms of trained immunity and prolonged inflammatory activation. Likewise, Campos-Bayardo *et al*. (2025) highlighted the pivotal role of innate immune signaling in linking obesity, insulin resistance, dyslipidemia, and chronic metabolic inflammation. These observations provide a biologically plausible framework for the present results and further support the concept that genetic variation within innate immune genes, including TLR4, may influence individual susceptibility to dyslipidemia by modulating inflammatory responses to metabolic stress.

The present study investigated the association between two TLR4 polymorphisms, rs4986790 (Asp299Gly) and rs1927914, and susceptibility to hyperlipidemia/hypercholesterolemia in a Palestinian population. The demographic characteristics of cases and controls were comparable, with no significant differences in age or sex distribution, reducing the likelihood that these variables influenced the observed genetic associations. Furthermore, recruiting participants from the same geographical region helped minimize potential confounding caused by population genetic variation.

Analysis of the TLR4 rs4986790 polymorphism showed no evidence of an association with hyperlipidemia under the codominant, dominant, or allelic genetic models **(Table 4)**. Although the AG genotype and the G allele were observed slightly more frequently in patients than in healthy controls **(Table 3)**, the differences were not statistically significant. These results indicate that the rs4986790 variant is unlikely to play a major role in genetic susceptibility to hyperlipidemia in the Palestinian population studied.

The absence of an association between rs4986790 and hyperlipidemia is consistent with previous reports demonstrating that the functional effects of this polymorphism vary considerably among different populations and disease conditions, as previously noted in other Middle Eastern cohorts (Ferwerda *et al*., 2008; Semlali *et al*., 2019).

The Asp299Gly substitution occurs within the extracellular domain of TLR4 and may influence receptor responsiveness to inflammatory stimuli. Functional studies have suggested that this variant can alter TLR4-mediated signaling; however, epidemiological studies have reported inconsistent associations with inflammatory and metabolic disorders (Ferwerda *et al*., 2008). Such discrepancies may result from differences in allele frequencies, environmental exposures, linkage disequilibrium patterns, and sample size. The relatively low frequency of the minor G allele in our study population may have reduced the statistical power to detect a modest association with hyperlipidemia **(Table 3)**.

In contrast, the TLR4 rs1927914 polymorphism demonstrated a significant association with hyperlipidemia. Individuals carrying the TC genotype showed increased disease susceptibility compared with TT homozygotes, and the C allele was associated with increased risk under dominant and allelic models **(Table 5)**. The observed association across multiple genetic models supports the possibility that rs1927914 contributes to genetic susceptibility to hyperlipidemia in the Palestinian population.

Although rs1927914 is not a coding variant and does not directly influence the amino acid primary structure of TLR4, regulatory polymorphisms located within non-coding regions may influence gene expression, transcriptional activity, mRNA stability, or interactions with regulatory elements. Altered TLR4 expression could modify the magnitude of inflammatory responses induced by endogenous metabolic ligands. Previous studies have demonstrated that increased TLR4 activity promotes inflammatory responses in adipose tissue, liver, and vascular cells, contributing to impaired insulin signaling, altered lipid metabolism, and atherosclerosis development (Shi *et al*., 2006; Libby, 2021). Therefore, the association identified between rs1927914 and hyperlipidemia may reflect an effect on TLR4 regulation rather than receptor structure.

The present findings are biologically plausible given experimental evidence linking TLR4 to lipid-mediated inflammatory responses. Previous studies have shown that saturated fatty acids activate TLR4 signaling, leading to NF-κB activation and the subsequent production of pro-inflammatory mediators (Lee *et al*., 2003; Rocha *et al*., 2016). In addition, animal studies have demonstrated that disruption of TLR4 signaling reduces atherosclerotic lesion formation and alters plaque characteristics, highlighting the importance of TLR4-mediated inflammation in lipid-associated vascular disease (Michelsen *et al*., 2004). Activation of macrophages through TLR4 signaling also contributes to cholesterol accumulation and foam cell formation, which are key events in atherosclerosis development (Moore *et al*., 2013).

The identification of rs1927914 as a potential genetic marker for hyperlipidemia is particularly relevant in the Palestinian population, where non-communicable diseases represent an increasing public health challenge. Studies from Palestine and neighboring Middle Eastern populations have reported a substantial burden of metabolic syndrome, dyslipidemia, obesity, and cardiovascular risk factors (El Bilbeisi *et al*., 2017; Damiri *et al*., 2022; Massad *et al*., 2025). Genetic variations affecting inflammatory pathways may contribute to differences in individual susceptibility to metabolic disorders among populations exposed to similar environmental and lifestyle pressures.

The present study has several notable strengths. To our knowledge, it is among the first to investigate the association between TLR4 polymorphisms and hyperlipidemia in a Palestinian population. The study design incorporated well-matched control subjects, evaluated the data under multiple genetic inheritance models, and employed PCR-RFLP for genotype determination, thereby enhancing the robustness of the findings. In addition, the consistent association observed for rs1927914 across different analytical models increases confidence in the reliability of the reported results.

Future studies involving larger multicenter Palestinian cohorts, integration of genetic data with metabolic and lifestyle parameters, and functional analyses assessing the impact of rs1927914 on TLR4 expression and downstream inflammatory pathways are needed. Such investigations may improve understanding of the contribution of innate immune regulation to lipid disorders and may support the development of population-specific approaches for cardiovascular risk prediction and personalized prevention strategies.

Conclusion

In summary, the present study investigated whether two TLR4 polymorphisms influence susceptibility to hyperlipidemia/hypercholesterolemia in a Palestinian population. The findings showed no evidence of an association between the rs4986790 (Asp299Gly) variant and disease risk under the codominant, dominant, or allelic genetic models examined. In contrast, the rs1927914 polymorphism demonstrated a strong and consistent association with hyperlipidemia, with carriers of the C allele exhibiting significantly increased odds of disease compared with TT homozygotes.

These findings suggest that genetic variation within the TLR4 gene may contribute to susceptibility to hyperlipidemia, although the effect appears to be polymorphism-specific. The observed association between rs1927914 and hyperlipidemia supports the growing evidence that innate immune signaling and chronic inflammation participate in the pathogenesis of lipid disorders.

To the best of our knowledge, this is the first study to examine the association between TLR4 polymorphisms and hyperlipidemia in a Palestinian population. Additional studies involving larger, independent Palestinian cohorts with broader demographic representation are needed to confirm these findings. Future research should also incorporate functional analyses to clarify how rs1927914 influences TLR4 expression and activity and to elucidate its potential biological role in lipid metabolism and inflammation. Such work could improve our understanding of the

genetic basis of dyslipidemia and support the development of more accurate approaches for assessing cardiovascular disease risk.

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Conflict of interest: None

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Ethics statement: The study protocol was reviewed and approved by the Scientific Committee of the Deanship of Scientific Research and Higher Studies at Palestine Polytechnic University (Approval No. 06, session dated 02 February 2023).

References

Abera, A., Worede, A., Hirigo, A.T., Alemayehu, R., Ambachew, S. (2024). Dyslipidemia and associated factors among adult cardiac patients: a hospital-based comparative cross-sectional study. *European Journal of Medical Research*, *29*(1), 237. doi: 10.1186/s40001-024-01802-x.

Abida, O., Bahloul, E., Elloumi, N., Toumi, A., Tahri, S., Ben Jmaa, M., Fakhfakh, R., Mahfoudh, N., Turki, H., & Masmoudi, H. (2020). Toll-like receptor gene polymorphisms in Tunisian endemic pemphigus foliaceus. *BioMed Research International, 2020*, 6541761. doi:10.1155/2020/6541761

Akira, S. (2003). Mammalian Toll-like receptors. *Current Opinion in Immunology, 15*(1), 5–11.

Akira, S., & Takeda, K. (2004). Toll-like receptor signalling. *Nature Reviews Immunology, 4*, 499–511. doi:10.1038/nri1391

Al-Ashwal, A., Alsagheir, A., Al Dubayee, M., Al-Khnifsawi, M., Al-Sarraf, A., Awan, Z., Ben-Omran, T., Al-Yaarubi, S., Almutair, A., Habeb, A., et al. (2024). Modern approaches to the management of homozygous familial hypercholesterolemia in the Middle East and North Africa. *Journal of Clinical Lipidology, 18*(2), e132–e141. doi:10.1016/j.jacl.2023.12.003

Al-Razem, F., Iqnaibi, E. B., Abu Rmeileh, R. A., & Ideis, L. I. (2025). Association of Toll-like receptor 4 gene polymorphisms with type 2 diabetes patients in the Palestinian population. *Endocrine, Metabolic & Immune Disorders - Drug Targets, 25*(9), 675–681. doi:10.2174/0118715303301789240826113528

Ballena-Caicedo, J., Zuzunaga-Montoya, F. E., Loayza-Castro, J. A., Vásquez-Romero, L. E. M., Tapia-Limonchi, R., De Carrillo, C. I. G., & Vera-Ponce, V. J. (2025). Global prevalence of dyslipidemias in the general adult population: A systematic review and meta-analysis. *Journal of Health, Population and Nutrition, 44*(1), 308. doi:10.1186/s41043-025-01054-3

Beutler, B., & Rietschel, E. T. (2003). Innate immune sensing and its roots: The story of endotoxin. *Nature Reviews Immunology, 3*, 169–176. doi:10.1038/nri1004

Bhardwaj, A., Prasad, D., & Mukherjee, S. (2024). Role of Toll-like receptor in the pathogenesis of oral cancer. *Cell Biochemistry and Biophysics, 82*(1), 91–105. https://doi.org/10.1007/s12013-023-01191-8

Bosco, G., Di Giacomo Barbagallo, F., Di Marco, M., Scilletta, S., Miano, N., Capuccio, S., Musmeci, M., Di Mauro, S., Filippello, A., Scamporrino, A., et al. (2025). Evaluations of metabolic and innate immunity profiles in subjects with familial hypercholesterolemia with or without subclinical atherosclerosis. *European Journal of Internal Medicine, 132*, 118–126. doi:10.1016/j.ejim.2024.12.002

Campos-Bayardo, T. I., Román-Rojas, D., García-Sánchez, A., Cardona-Muñoz, E. G., Sánchez-Lozano, D. I., Totsuka-Sutto, S., Gómez-Hermosillo, L. F., Casillas-Moreno, J., Andrade-Sierra, J., Pazarín-Villaseñor, L., et al. (2025). The role of TLRs in obesity and its related metabolic disorders. *International Journal of Molecular Sciences, 26*(5), 2229. doi:10.3390/ijms26052229

Chaiwiang, N., & Poyomtip, T. (2019). The association of toll-like receptor 4 gene polymorphisms with primary open-angle glaucoma susceptibility: A meta-analysis. *Bioscience Reports, 39*(4), BSR20190029. doi:10.1042/BSR20190029

Chrzęszczyk, D., Konopka, T., & Ziętek, M. (2015). Polymorphisms of Toll-like receptor 4 as a risk factor for periodontitis: Meta-analysis. *Advances in Clinical and Experimental Medicine, 24*(6), 1059–1070. doi:10.17219/acem/47394

Damiri, B., Badran, L., Safadi, D., Sawalha, A., Yasin, Y., Sawalha, M., & Amir, M. (2022). Metabolic syndrome and related risk factors among adults in the northern West Bank: A cross-sectional study. *International Health, 14*(4), 339–345. doi:10.1093/inthealth/ihz093

de Oliveira, A. A., Elder, E., Spaans, F., Graton, M. E., Quon, A., Kirschenman, R., Wooldridge, A. L., Cooke, C. M., & Davidge, S. T. (2024). Excessive hypercholesterolemia in pregnancy impairs rat uterine artery function via activation of Toll-like receptor 4. *Clinical Science, 138*(4), 137–151. doi:10.1042/CS20231442

El Bilbeisi, A. H., Shab-Bidar, S., Jackson, D., & Djafarian, K. (2017). The prevalence of metabolic syndrome and its related factors among adults in Palestine: A meta-analysis. *Ethiopian Journal of Health Sciences, 27*(1), 77–84. doi:10.4314/ejhs.v27i1.10

Erridge, C. (2009). The roles of Toll-like receptors in atherosclerosis. *Journal of Innate Immunity, 1*(4), 340–349. doi:10.1159/000191413

Ferwerda, B., McCall, M. B., Verheijen, K., Kullberg, B. J., van der Ven, A. J., van der Meer, J. W. M., & Netea, M. G. (2008). Functional consequences of Toll-like receptor 4 polymorphisms. *Molecular Medicine, 14*(5–6), 346–352. doi:10.2119/2007-00135.Ferwerda

Garcia, M. M., Goicoechea, C., Molina-Álvarez, M., & Pascual, D. (2020). Toll-like receptor 4: A promising crossroads in the diagnosis and treatment of several pathologies. *European Journal of Pharmacology, 874*, 172975. doi:10.1016/j.ejphar.2020.172975

Gay, N. J., & Gangloff, M. (2007). Structure and function of Toll

receptors and their ligands. *Annual Review of Biochemistry, 76*, 141–165. doi:10.1146/annurev.biochem.76.060305.151318

Hu, W., Wu, S., Zhang, Y., Sigdel, K. R., Lin, Y., & Zhong, H. (2016). Association between Toll-like receptor 4 polymorphisms and systemic lupus erythematosus susceptibility: A meta-analysis. *BioMed Research International, 2016*, 7842587. doi:10.1155/2016/7842587

Jahromi, A. S., Erfanian, S., Safavi, S., & Roustazadeh, A. (2024). Association of Toll-like receptor 4 gene polymorphism with multiple sclerosis in Iranian patients. *Acta Neurologica Taiwanica, 33*(1), 1–6.

Jin, M. S., & Lee, J. O. (2008). Structures of the Toll-like receptor family and its ligand complexes. *Immunity, 29*(2), 182–191. doi:10.1016/j.immuni.2008.07.007

Jing, J. J., Li, M., & Yuan, Y. (2012). Toll-like receptor 4 Asp299Gly and Thr399Ile polymorphisms in cancer: A meta-analysis. *Gene, 499*(2), 237–242. doi:10.1016/j.gene.2012.03.045

Kargar, S., & Ansari, H. (2023). Prevalence of dyslipidemias in the Middle East region: A systematic review and meta-analysis. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews, 17*(11), 102870. doi:10.1016/j.dsx.2023.102870

Kawai, T., Ikegawa, M., Ori, D., & Akira, S. (2024). Decoding Toll-like receptors: Recent insights and perspectives in innate immunity. *Immunity, 57*(4), 649–673. doi:10.1016/j.immuni.2024.03.004

Kumar, H., Kawai, T., & Akira, S. (2009). Toll-like receptors and innate immunity. *Biochemical and Biophysical Research Communications, 388*(4), 621–625. doi:10.1016/j.bbrc.2009.08.062

Lazarus, R., Vercelli, D., Palmer, L. J., Klimecki, W. J., Silverman, E. K., Richter, B., Riva, A., Ramoni, M., Martinez, F. D., Weiss, S. T., et al. (2002). Single nucleotide polymorphisms in innate immunity genes: Abundant variation and potential role in complex human disease. *Immunological Reviews, 190*(1), 9–25. doi:10.1034/j.1600-065X.2002.19002.x

Lee, J. Y., Sohn, K. H., Rhee, S. H., & Hwang, D. (2001). Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through Toll-like receptor 4. *Journal of Biological Chemistry, 276*(20), 16683–16689. doi:10.1074/jbc.M011695200

Lee, J. Y., Zhao, L., & Hwang, D. H. (2003). Modulation of Toll-like receptor-mediated signaling by fatty acids. *Journal of Lipid Research, 44*(3), 479–486. doi:10.1194/jlr.R200012-JLR200

Libby, P. (2021). The changing landscape of atherosclerosis. *Nature, 592*(7855), 524–533. doi:10.1038/s41586-021-03392-8

Liu, R., Mo, Y. Y., Wang, H. L., Tan, Y., Wen, X. J., Deng, M. J., Yan, H., & Li, L. (2016). The relationship between Toll-like receptor 4 gene rs4986790 and rs4986791 polymorphisms and sepsis susceptibility: A meta-analysis. *Scientific Reports, 6*, 38947. doi:10.1038/srep38947

Marzouk, M., Durrance-Bagale, A., Lam, S. T., Nagashima-Hayashi, M., Ung, M., Aribou, Z. M., Zaseela, A., Ibrahim, N. M., Agarwal, S., Omar, M., et al. (2023). Health system evaluation in conflict-affected countries: A scoping review of approaches and methods. *Conflict and Health, 17*(1), 30. doi:10.1186/s13031-023-00526-9

Massad, S., Isbeih, M., Owess, M., Fouad, H., Saman, K. A., Issawi, S., Barkley, S., Falah, N., Savin, S., Rarau, P., et al. (2025). Noncommunicable diseases: A silent epidemic in occupied Palestine: Results from the World Health Organization STEPS Survey 2022. *BMC Public Health, 25*(1), 2726. doi:10.1186/s12889-025-23880-0

Medzhitov, R. (2001). Toll-like receptors and innate immunity. *Nature Reviews Immunology, 1*(2), 135–145. doi:10.1038/35100529

Michelsen, K. S., Wong, M. H., Shah, P. K., Zhang, W., Yano, J., Doherty, T. M., Akira, S., Rajavashisth, T. B., & Arditi, M. (2004). Lack of Toll-like receptor 4 or myeloid differentiation factor 88 reduces atherosclerosis and alters plaque phenotype in mice deficient in apolipoprotein E. *Proceedings of the National Academy of Sciences of the United States of America, 101*(29), 10679–10684. doi:10.1073/pnas.0403249101

Moore, K. J., Sheedy, F. J., & Fisher, E. A. (2013). Macrophages in atherosclerosis: A dynamic balance. *Nature Reviews Immunology, 13*(10), 709–721. doi:10.1038/nri3520

Mostafa, M., Elwasify, M., Fathy, A. A., & Abdelsalam, M. (2022). Toll-like receptor 4 gene polymorphisms and susceptibility to schizophrenia: A case-control study. *Immunological Investigations, 51*(7), 2009–2024. doi:10.1080/08820139.2022.2093118

Motkowski, R., Alifier, M., Abramowicz, P., Konstantynowicz, J., Mikołuć, B., & Stasiak-Barmuta, A. (2022). Innate and acquired cellular immunity in children with familial hypercholesterolemia treated with simvastatin. *Journal of Clinical Medicine, 11*(10), 2924. doi:10.3390/jcm11102924

Motlagh, B., O'Donnell, M., & Yusuf, S. (2009). Prevalence of cardiovascular risk factors in the Middle East: A systematic review. *European Journal of Cardiovascular Prevention & Rehabilitation, 16*(3), 268–280. doi:10.1097/HJR.0b013e328322ca1b

Mulyani, R., Suharjono, S., Suprapti, B., Hermansyah, A., & Purnama, S. (2026). Diabetes distress and associated factors in type 2 diabetes at an Indonesian hospital: A cross-sectional study. *Journal of Advanced Pharmacy Education and Research, 16*(2), 1–9. doi:10.51847/7y6OKqnuGm

Owens, A. P., III, Byrnes, J. R., & Mackman, N. (2014). Hyperlipidemia, tissue factor, coagulation, and simvastatin. *Trends in Cardiovascular Medicine, 24*(3), 95–98. doi:10.1016/j.tcm.2013.07.003

Palestinian Ministry of Health. (2024). *Annual health report 2024*. Palestinian Ministry of Health.

Pellegrino, P., Falvella, F. S., Cheli, S., Perrotta, C., Clementi, E., & Radice, S. (2016). The role of Toll-like receptor 4 polymorphisms in vaccine immune response. *The Pharmacogenomics Journal, 16*(1), 96–101. doi:10.1038/tpj.2015.21

Quirino, M. G., Macedo, L. C., Pagnano, K. B. B., Pagliarini-E-Silva, S., Sell, A. M., & Visentainer, J. E. L. (2021). Toll-like receptor gene polymorphisms in patients with myeloproliferative neoplasms. *Molecular Biology Reports, 48*(6), 4995–5001. doi:10.1007/s11033-021-06238-8

Robinson, K. A., Akbar, N., Baidžajevas, K., & Choudhury, R. P. (2023). Trained immunity in diabetes and hyperlipidemia: Emerging opportunities to target cardiovascular complications and design new therapies. *The FASEB Journal, 37*(11), e23231. doi:10.1096/fj.202301078R

Rocha, D. M., Caldas, A. P., Oliveira, L. L., Bressan, J., & Hermsdorff, H. H. (2016). Saturated fatty acids trigger TLR4-mediated inflammatory response. *Atherosclerosis, 244*, 211–215. doi:10.1016/j.atherosclerosis.2015.11.015

Sameer, A. S., & Nissar, S. (2021). Toll-like receptors (TLRs): Structure, functions, signaling, and role of their polymorphisms in colorectal cancer susceptibility. *BioMed Research International, 2021*, 1157023. doi:10.1155/2021/1157023

Semlali, A., Al Mutairi, M., Oqla Alanazi, I., Awad Aljohi, H., Reddy Parine, N., Alhadheq, A., Al-Jafari, A. A., Mobeirek, A. F., Al Amri, A., Shaik, J. P., et al. (2019). Toll-like receptor 4 polymorphisms in Saudi population with cardiovascular diseases. *Molecular Genetics & Genomic Medicine, 7*(9), e852. doi:10.1002/mgg3.852

Senhaji, N., Diakité, B., Serbati, N., Zaid, Y., Badre, W., & Nadifi, S. (2014). Toll-like receptor 4 Asp299Gly and Thr399Ile polymorphisms: New data and a meta-analysis. *BMC Gastroenterology, 14*, 206. doi:10.1186/s12876-014-0206-x

Shi, H., Kokoeva, M. V., Inouye, K., Tzameli, I., Yin, H., & Flier, J. S. (2006). TLR4 links innate immunity and fatty acid-induced insulin resistance. *The Journal of Clinical Investigation, 116*(11), 3015–3025. doi:10.1172/JCI28898

Takeda, K., & Akira, S. (2015). Toll-like receptors. *Current Protocols in Immunology, 109*, 14.12.1–14.12.10. doi:10.1002/0471142735.im1412s109

Tongtawee, T., Simawaranon, T., Wattanawongdon, W., Dechsukhum, C., & Leeanansaksiri, W. (2019). Toll-like receptor 2 and 4 polymorphisms associated with *Helicobacter pylori* susceptibility and gastric cancer. *Turkish Journal of Gastroenterology, 30*(1), 15–20. doi:10.5152/tjg.2018.17461

Yang, W., Cao, M., Mao, X., Wei, X., Li, X., Chen, G., Zhang, J., Wang, Z., Shi, J., Huang, H., et al. (2016). Alternate-day fasting protects the livers of mice against high-fat diet-induced inflammation associated with the suppression of Toll-like receptor 4/nuclear factor κB signaling. *Nutrition Research, 36*(6), 586–593. doi:10.1016/j.nutres.2016.02.001

Zhu, X., Owen, J. S., Wilson, M. D., Li, H., Griffiths, G. L., Thomas, M. J., Hiltbold, E. M., Fessler, M. B., & Parks, J. S. (2010). Macrophage ABCA1 reduces MyD88-dependent Toll-like receptor trafficking to lipid rafts by reduction of lipid raft cholesterol. *Journal of Lipid Research, 51*(11), 3196–3206. doi:10.1194/jlr.M006486

Zhu, Y. J., Wang, C., Song, G., Zang, S. S., Liu, Y. X., & Li, L. (2015). Toll-like receptor-2 and -4 are associated with hyperlipidemia. *Molecular Medicine Reports, 12*(6), 8241–8246. doi:10.3892/mmr.2015.4465