Genetic Polymorphism of TNF-α -308 G/A (rs1800629) Associated with Systemic Lupus Syndrome in Al Najaf Province

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Abstract
Systemic lupus erythematosus (SLE) is a complicated autoimmune illness that affects several systems. It is multifaceted, encompassing epigenetic, genetic, ecological, and environmental components. Primarily, it activates both innate and adaptive immunity. This study examined the relationship of TNF-α-308 G/A (rs1800629) polymorphisms in SLE patients. The study involves 100 participants, grouped into two primary groups: 50 RA sufferers and 50 healthy controls. All subjects were genotyped for TNF–α rs1800629 (G/A) using allele-specific PCR(ASP). Genotype and allele frequency results of TNF-α -308 G/A (rs1800629) gene polymorphisms were examined under codominant, dominant, and recessive models. The SLE patients with heterozygous genotype (G/A) those of the (GA + AA) genotypes and under dominant pattern, SLE patients had substantially greater odds than those in the control group (OR: 2.49, CI 95%: (1.01-6.12), P<0.037) and (OR: 2.79, CI 95%: (1.22-6.39), P<0.012), respectively. The recessive model was found to exhibit no significant variation (OR: 2.55, CI 95%: (0.62-10.49), P< 0.69). The allele (A) frequency was observed to be significantly (OR: 2.41, CI 95%: (1.24-4.69) P< 0.0072) higher in SLE patients (33%) in comparison with the control group (17%). The results of this study suggest that the 'A' allele of the TNF-α (rs1800629) G/A polymorphism is significantly associated with an increased risk of SLE in the studied population.

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1. Introduction

The chronic autoimmune disease known as systemic lupus erythematosus (SLE) is typified by the generation of autoantibodies and inflammation of several systems (Ameer *et al*., 2022) [8]. It affects multiple organs such as serous membranes, the kidney, skin, joints, the central nervous system, and hematological, gastrointestinal, and cardiovascular systems (Neamah *et al*.,2024) [10]. There are significant differences in incidence and prevalence by sex, age, and ethnicity. Women are almost nine times more likely than males to have it, and more cases are diagnosed in those aged 15 to 44 (Bitencourt *et al*., 2020) [11]. Because of its extreme heterogeneity, patients display a wide range of symptom combinations and laboratory characteristics. Humoral autoimmune is a characteristic of SLE, and many patients develop circulating autoantibodies against minor nuclear RNA-binding

proteins (e.g.,
anti-Ro, anti-La, anti-Sm, and anti-RNP) and/or double-stranded DNA (anti-ds-DNA). Compagno (2015) [14]. SLE pathogenesis generally involves the immune system's innate and adaptive mechanisms. A component of the innate immune system, the activation of toll-like receptor (TLR) initiates the downstream pathway that produces pro-inflammatory mediators like interferon (IFN). The neutrophil extracellular trap generation program, known as NETosis, is another innate immune system component that contributes to the pathophysiology of SLE. The role of T- and B-lymphocytes in adaptive immune systems is distinct from that of innate immune systems. In SLE, T-cells produce a variety of cytokines due to aberrant gene expression. T-cells cause autoreactive B-cells to become activated, and the cytokine synthesis that results in autoantibody formation is a characteristic of SLE. B-cells also behave as antigen-presenting cells, triggering T-cell activation and resulting in autoimmunity. (Graßhoff *et al*., 2021) [12].

SLE is a complex disease, and the irreversible loss of immunologic self-tolerance that defines it can be linked to the interaction of several environmental variables and genetic risk factors. Women are more likely than men to have SLE, especially during the childbearing years (9:1), but people of all ages, genders, and ancestries are at risk (Ghodke-Puranik & Niewold, 2015) [7]. The host's defence against infection includes the production of the cytokine tumour necrosis factor (TNF). This cytokine contributes to various immunological and inflammatory reactions and the aetiology of numerous viral and autoimmune disorders. In the class III area of the major histocompatibility complex (MHC), on chromosome 6, the TNF gene is flanked by the genes for lymphotoxins "a" and "b." It has been found that the TNF genes, HLA class I (HLA-B), and class II (HLA-DR) are closely related (Kumar *et al*., 2007) [1]. The transcription of the TNF gene is closely controlled (Tsytsykova *et al*., 2002) [2], and rs1800629 has been linked to several autoimmune and inflammatory illnesses (Wang *et al*., 2024) [3]. According to Qidwai and Khan (2011) [4], the rs1800629 polymorphism favours RA and is in charge of elevated TNF production. Several studies and meta-analyses have investigated the association between the TNF-α-308 G/A polymorphism and the likelihood of having SLE (Padhi *et al*., 2022) [21].

2. Materials and methods

2.1 Patient and control

A case-control study was based on two groups. The first group consisted of fifty samples of SLE patients collected from Al-Sader Medical City, Al-Najaf province. They are diagnosed according to the laboratory findings and clinical examinations made by the specialist doctor in the hospital, based on the patient's initial symptoms and signs. The second group is fifty control samples; 2 ml was collected from both patients and controls and distributed in EDTA tubes for allele-specific PCR.

DNA extraction kit

FavorprepTM Genomic DNA Extraction Mini Kit for Blood/Cultured Cells from FAVORGEN BIOTECH CORPORATION, established in Australia-Taiwan

2.3 Ethical Approval

The Medical College Ethical Committee approved the Medical and Pharmaceutical Science study protocol at Jabir Ibn Hayyan University. Informed consent has been obtained from all study participants

2.4 Estimation of DNA purity

The UV/visible spectrophotometer was used to determine the purity of human DNA. The absorbance ratio of DNA purity was determined at wavelengths of 260/280 nm. Per the extraction kit's recommendations, the optimal absorbance ratio for pure DNA is between 1.7 and 2.1, producing roughly 4–10 µg/ml of DNA.

2.5 PCR mixture for TNF–α (rs1800629) snp detection

GoTaq® Green Master Mix (Promega-USA) was used to prepare the PCR mixture according to the DNA type and target region. Table 1 lists the components, volumes, and concentrations of the PCR mixture used to amplify the IL21 G and/or A allele (s) for allele-specific PCR-PCR SNP detection. Regarding the polymorphism TNF–α rs1800629, TNF-α Primer sequences were as follows:

5′-ATAGGTTTTGAGGGGCATGG-3′ (allele G forward)

5′-AATAGGTTTTGAGGGGCATGA-3′ (allele A forward) and

5′-TCT CGG TTT CTT CTC CAT CG-3′ (reverse)

Table 1: Mixture of allele-specific PCR for TNF G/A SNP detection

Mixture Solution	Volume	Concentration
Master mix	12.5 µl	1X
Target DNA	5 µl	--
Left-1 primer (G allele forward) for each tube added only with right primer	1.5 µl	10 pmol/µl
Left-2 primer (A allele forward) for each tube added only with right primer	1.5 µl	10 pmol/µl
Right primer (reverse) added for each L-1 tube mixture and L-2 tube mixture	1.5 µl	10 pmol/µl
Nuclease free water	4.5 µl	--
Total Volume	25 µl	--

2.6. PCR program for TNF–α (rs1800629) SNP detection

The steps, temperatures, times and number of cycles to detect TNF-α-308 G/A (rs1800629) were detailed in Table 2.

Table 2: Amplification conditions of TNF–α (rs1800629) SNP

Steps	Temperature	Time	No. of cycle
Initial denaturation	95 °C	5 minute	1
Denaturation	95 °C	30 second	35
Annealing	60 °C	30 second	
Elongation	72 °C	30 second	
Final elongation	72 °C	5 minute	I
Hold	4 °C	30minute	1

2.7 PCR product electrophoresis

The electrophoresis method of agar gel was used to examine the PCR results by
to the following steps:

2.7.1 Preparation of TBE solution

About 1X Tris-borate EDTA (TBE) buffer was made from 10X TBE buffer
(one volume) Moreover, distilled water (9 volumes).

2.7.2 Preparation of agarose gel and detection

About 1.5 grams of agarose were put into a beaker containing 1X TBE buffer (100 ml) to obtain a 1.5 % agarose solution concentration. After this, the solution was melted by a Microwave device to dissolve the agarose and become transparent after adding five µl (10 mg/ml) of ethidium bromide. The tops of the gel tray were closed with tape, and the comb was situated. After carefully pouring the agarose solution into the tray, it was left to solidify at ambient temperature. The comb was removed courteously. The samples were then put into each well (5 µl). Ultimately, the electrophoresis was set for 80 minutes at 75 volts. The Agarose gel was exposed to a UV beam on the gel documentation's UV transilluminator for detection, and pictures were taken with a camera.

3. Statistical Analysis

The data was analysed using the statistical program of social sciences (SPSS, version 23) software. The frequencies of the investigated genotypic and allelic polymorphisms among patients and controls were compared using Fisher's exact method and odds ratio (OR) with a 95% confidence interval. The genotyping data was also analysed. Conformance to the Hardy-Weinberg rule of genetic equilibrium was investigated by comparing the measured and predicted frequency of genotypes in the case and control groups using a chi-square test. For statistical significance, a p-value of less than 0.05 was considered the lowest possible criterion.

4. Results

4.1. Detection of TNF–α (rs1800629) SNP

Allele-specific PCR(AS-PCR) was used to detect the product of the TNF–α (rs1800629) gene, which used three primers: a reverse primer and two forward primers (F1 for amplifying allele G and F2 for amplifying allele A). A heterozygous G/A genotype pattern was obtained when a set of reverse and two (F1 and F2) primers were used to amplify the G and the A allele. Homozygous G/G genotype pattern resulted when primer pair of reverse and (F1) were used to amplify allele G. Homozygous A/A genotype pattern resulted when primer pair of reverse and (F2) were used to amplify allele A. PCR product with 184 bp indicated successful amplification based on the presence or absence of a band at 184 bp, figure 1 illustrate the results of TNF–α rs1800629 genotyping.

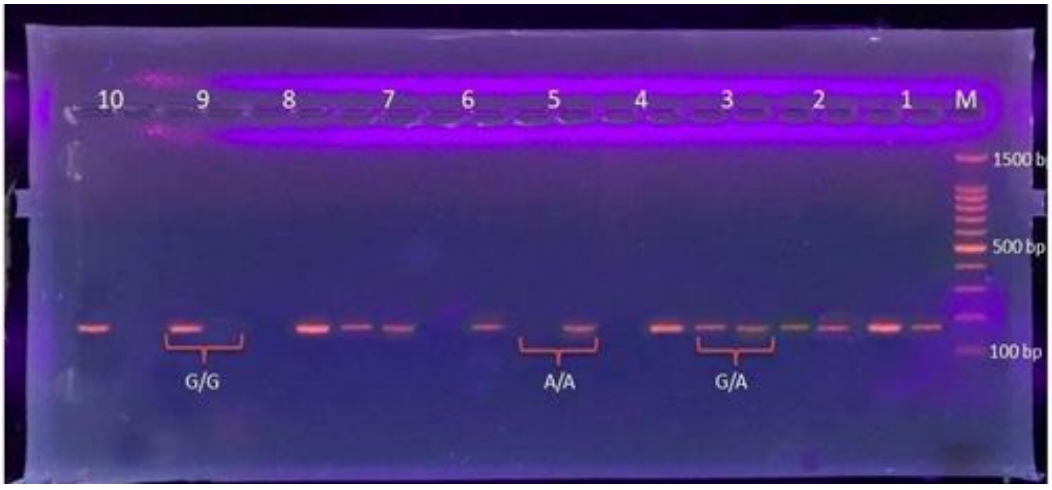

Fig 1: Agarose gel electrophoresis image using AS-PCR to detect TNF-α-308 G/A (rs1800629) locus genotyping. Electrophoresis conditions were (1.5% agarose gel stained with 5µl(10mg/ml) ethidium bromide (80 min, 75 Volts, 1X TBE buffer).M: marker (100bp). PCR amplicon for G and A allele is (184bp).

4.2. Genotype and allele frequencies analysis of TNF-α (rs1800629) G/A gene polymorphisms

Multinomial logistic regression was used to analyse the genotype and allele frequency results of TNF-α (rs1800629) G/A gene polymorphisms under codominant, dominant, and recessive models. Table 3 and Figure 2 show the results for SLE patients and healthy people. When the codominant model is used, the SLE patients with heterozygous genotype (G/A) seem to be significant variation in the comparison with those of the wild type of the control group (OR: 2.49, CI 95%:

(1.01-6.12), P< 0.037) higher in SLE patients concerning those of the control group. However, such variation is changed when the analysis is directed towards the homozygous genotype (A/A) variant (OR: 3.5, CI 95 (0.82-14.89), P< 0.077). Under the dominant pattern, those of the GA+AA genotypes were indicated to be significant (OR: 2.79, CI 95%: (1.22-6.39), P< 0.012), higher in SLE patients compared to those of the control group. The recessive model was found to exhibit no significant variation (OR: 2.55, CI 95%: (0.62-10.49), P< 0.69). The allele (A) frequency was observed to be significantly (OR: 2.41, CI 95%: (1.24-4.69) P< 0.0072) higher in SLE patients (33%) in comparison with control group (17%) whereas the allele (G) frequency observed to be less than (67%) in respect with control group (83%).

Table 3: Allele frequencies and genotypes of TNF-α -308 G/A (rs1800629) gene polymorphisms

	Control group (N= 50)		Patient group (N= 50)		OR (CI %) 184 bp	P value
	NO.	%	NO.	%		
Genotyping						
Codominant						
GG	36	72	24	48	Reference group	
GA	11	22	19	38	2.49 (1.01-6.12)	0.037*
AA	3	6	7	14	3.5 (0.82-14.89)	0.077
Dominant						
GG	36	72	24	48	Reference group	
AA + GA	14	28	26	52	2.79 (1.22-6.39)	0.012*
Recessive						
GG + GA	47	94	43	86	Reference group	
AA	3	6	7	14	2.55 (0.62-10.49)	0.16
Frequency						
G	83	83	67	67	Reference group	
A	17	17	33	33	2.41 (1.24-4.69)	0.007*
	100		100		200	

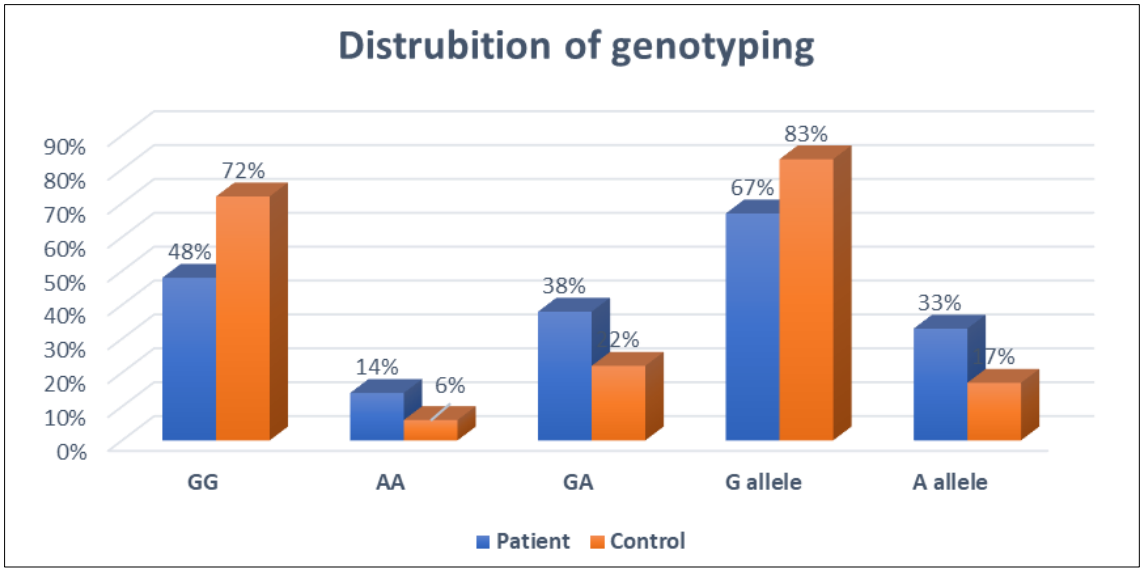

Fig 2: Distribution of genotyping and allele frequencies

5. Discussion

The -308 G/A polymorphism in the promoter region of the TNF-α gene may affect transcription and, hence, the production of TNF-α proteins.The current study used various genetic models to assess the relationship between systemic lupus erythematosus (SLE) susceptibility and the TNF-α promoter polymorphism (rs1800629 G/A). The results show that the A allele and its associated genotypes are linked to an increased risk of SLE, which is in line with other findings that suggest that a pro-inflammatory genetic background influences autoimmunity.

In the codominant model, those with the heterozygous G/A genotype had a significantly higher risk of SLE than those with the wild-type G/G genotype (P < 0.037). These results are consistent with new research that found that the TNF-α-308 G/A variation, which has been found in most populations, including Taiwanese patients, is linked to SLE (Lin *et al*., 2009; Manolova *et al*., 2018) [16, 17]. In North Indian people with type 1 diabetes, the rs1800629 G/A and A/A genotypes significantly increased (Martínez-Ramírez *et al*., 2021) [5]. A meta-analysis by Zhang *et al*. (2014) revealed that the -308A allele was strongly associated with an increased risk of SLE in the general population, particularly in Asian and Caucasian groups.

On the other hand, new research indicates that Iranian SLE patients did not differ substantially from controls regarding

genotypes or allele frequencies of the re1800629 polymorphism at position 308 G/A (Mashayekh *et al*., 2022) [15]. Based on this finding, a single A allele could be enough to alter immunological function or gene expression, increasing the disease risk. As observed in earlier studies with limited A/A frequencies, the homozygous A/A genotype, although exhibiting an increased frequency, did not approach statistical significance (P = 0.077). This is probably because of the genotype's small sample size (Sharma *et al*., 2016; Mok *et al*., 2003).

A substantial connection with SLE was further corroborated by analysis under the dominant model (G/A + A/A vs. G/G) (P < 0.012). This model suggested that even a single copy can raise the risk of sickness, offering more proof of the A allele's function. These findings align with those of Manolova *et al*. (2018) [17] and Bayram *et al*. (2012), who discovered that bearers of the TNF-α-308A allele were more prone to SLE..Due to the low frequency of the A/A genotype, the recessive model (A/A vs. G/G + G/A) did not demonstrate a statistically significant correlation (P = 0.69). When homozygous minor alleles are uncommon in the population, this lack of importance is frequently observed in genetic investigations of SLE (Cuchacovich *et al*., 2004).

Additionally, the A allele was substantially more common in SLE patients (33%) than in controls (17%), according to allele frequency analysis (P < 0.0072), suggesting that it may be a risk allele. This result is consistent with earlier research indicating that the -308A mutation is linked to elevated TNF-α production, which could exacerbate the chronic inflammation seen in SLE. In 2017, Umare *et al*., and in 2019, Mahto *et al*.Increased TNF-α levels have been linked to the pathophysiology of SLE, including tissue damage, immune cell activation, and the generation of autoantibodies.

6. Conclusion:

The TNF-α-308 G/A (rs1800629) genetic polymorphism, particularly the -308A allele, appears to be associated with an increased risk of Systemic Lupus Erythematosus in several populations. This polymorphism may influence SLE susceptibility by affecting TNF-α production, a key cytokine involved in the inflammatory processes of the disease. It is important to note that genetic association studies often yield varying results across different populations due to genetic heterogeneity and environmental factors. Further research with larger sample sizes and diverse ethnic groups is warranted to confirm these findings and fully elucidate this polymorphism's role in SLE pathogenesis.

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