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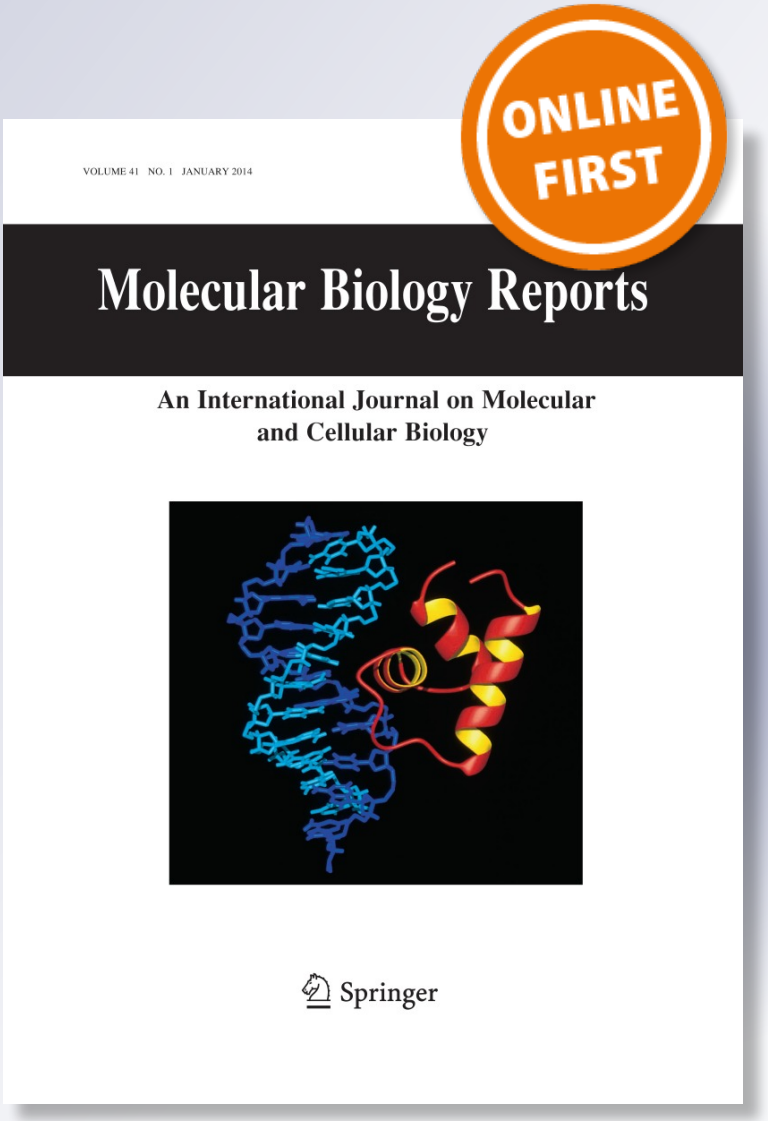

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Association between TNF promoter −308 G>A and LTA 252 A>G polymorphisms and systemic lupus erythematosus

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Abstract Tumor necrosis factor (TNF) and lymphotoxin alpha (LTA) are pivotal cytokines in the pathogenesis of systemic lupus erythematosus (SLE). To investigate the possible association of the polymorphism of the TNF promoter gene −308 and that of the LTA gene 252 with susceptibility to SLE and with phenotypic disease features in Egyptian patients. A case control study involving 100 SLE patients and 100 unrelated healthy controls. Polymerase chain reaction and restriction fragment length polymorphism methods were applied to detect genetic polymorphism. We found that TNF−308 genotype AA was significantly increase by 26 % in SLE patients compared to 10 % in the control group ($p = 0.003$; OR 3.16; CI 1.43–6.98) and the frequency of the A allele of the TNF promoter −308 was significantly higher in the SLE patients (42 %) than in the control subjects (24 %) ($p < 0.001$; OR 2.29; 95 % CI 1.49–3.52). Genotype LTA 252 GG showed a significant increase by 22 % in SLE patients compared to 6 % in the control group ($p = 0.001$; OR 4.42; 95 % CI 1.71–11.44), and the frequency of the G allele of the LTA was significantly higher in the SLE patients (38 %) than in the control subjects (21 %) ($p < 0.001$; OR 2.31; 95 % CI 1.48–3.6). Genotype (AA+GA) of TNF was significantly associated with clinical manifestations as malar rash, arthritis, oral ulcers, serositis and systemic lupus erythematosus disease activity index. Genotype (GG+GA) of LTA was significantly associated with arthritis. These results suggest that TNF and LTA genetic polymorphisms contribute to SLE susceptibility in the Egyptian population and are associated with disease characteristics. TNF−308 and LTA+252 polymorphic markers may be used for early diagnosis of SLE and early prediction of clinical manifestations, like arthritis.

Keywords SLE · TNF · LTA · Arthritis and SLEDAI

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Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease. While the etiology of SLE remains unclear, data from several studies have demonstrated an underlying genetic basis. A variety of candidate genes have been identified including the tumor necrosis factor (TNF) and the lymphotoxin alpha (LTA) genes, both located on chromosome 6, within the MHC class III region [1].

Tumor necrosis factor is a cytokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction.

Lymphotoxin is a lymphokine cytokine. It is a protein that is produced by Th1 type T-cells and induces vascular endothelial cells to change their surface adhesion molecules to allow phagocytic cells to bind to them. Lymphotoxin alpha, a member of the tumor necrosis factor family, is a cytokine produced by lymphocytes [2].

TNF and LTA are pivotal cytokines in the pathogenesis of several inflammatory diseases, including SLE. TNF levels are higher in active lupus and correlate with disease activity [3]. Up-regulated TNF expression was demonstrated in lupus nephritis [4], and short-term therapeutic benefit of TNF blockade was shown in refractory renal disease [5]. LTA is another member of the TNF

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superfamily with similar biologic activity. This cytokine also plays an important role in the formation of secondary lymphoid organs during development [6], which could be of potential relevance for SLE onset and progression. In the context of the regulation of TNF expression, the single nucleotide polymorphism (SNP) located on the gene promoter at position −308 (transition G/A) is of particular interest. In fact, this SNP is functionally relevant, and the TNF−308 A allele is related to higher transcriptional activity [7]. Interestingly, the A allele has been associated with the risk of SLE [8]. On the other hand, the role of the LTA mutation at the intron 1 (transition A/G at position +252) in lupus susceptibility has been less extensively investigated and published studies provided inconsistent results; allelic variation at LTA 252 was associated with SLE in German [9], and Asian patients [10], but this association was not confirmed in lupus patients from North America [11].

Santos et al. [12] investigated the possible association of tumor necrosis factor (TNF) promoter gene at position −308 and lymphotoxin alpha (LTA) gene at position 252 polymorphisms with susceptibility to SLE and with phenotypic disease features in Portuguese Caucasian patients. Their results showed that no significant differences in genotype or allele frequencies were identified between SLE cases and controls. Nevertheless, these polymorphisms appear to associate with the risk of renal Lupus and distinct immunological features.

Aim of the work

The aim of this study was to investigate the association of genetic polymorphism TNFpromoter −308 and LTA+252 with susceptibility to SLE and disease characteristics in Egyptian patients.

Subjects and methods

This case control study was conducted in the Medical Biochemistry Department, Faculty of Medicine, Cairo University in the period between September 2012 and August 2013. This study was approved by the Ethical Committee of Kasr Al Ainy Medical Hospital and was in accordance with the principles of Helsinki Declaration, and all participants provided written informed consent.

Two hundred Egyptian subjects participated in the present study. One hundred consecutive patients with the diagnosis of SLE (90 females/10 males; mean age 31.62 ± 8.27 years, mean disease duration 6.19 ± 3.84 years) attending El Kasr Al Ainy Hospital, Rheumatology Department and 100 unrelated healthy controls with similar demographic characteristics (88 females/12 males, mean age 30.28 ± 9.02 years). SLE patients were diagnosed according to the revised American College of Rheumatology classification criteria for diagnosis of SLE [13]. For each patient, we obtained detailed information regarding demographic characteristics, age at diagnosis, SLE clinical manifestations, presence of antinuclear antibodies (ANA), anti double stranded DNA (anti-dsDNA), disease activity, cumulative damage, and current medication. SLE disease activity was evaluated using the SLEDAI-2K [14].

Patients exclusion criteria were as follows: diabetes, neoplasia, cigarette smoking and other autoimmune diseases e.g. rheumatoid arthritis. All subjects were subjected to the following:- Complete blood picture (CBC), Erythrocyte sedimentation rate (ESR), serum Creatinine , ANA , Anti ds DNA and serum Complements 3 and 4 (C3 & C4).

Methods

Five mL of blood was collected in EDTA–K3 tubes. Blood samples were preserved at −80 °C until genotyping. Additional blood and urine tests were performed in SLE patients in order to determine disease activity (complete blood count, creatinine, ESR, anti-DNA antibody, antinuclear antibody, C3 and C4 level, and urine analysis).

TNF and LTA genotyping

DNA was extracted from whole blood using QIA amp DNA Blood Mini Kit supplied by Qiagen GmbH, (Cat No. 51104, Hilden Germany), according to manufacturer's instructions.

TNF−308 (G/A) and LTA 252 (A/G) genotypes were identified by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) methods. PCR was accomplished by Taq PCR Master Mix (Qiagen, Valencia, CA, USA). The primers for the detection of TNF−308 G>A polymorphism were designed forward primer 5′-AGGCAATAGGTTTTGAGGGCCAT-3′ and the reverse primer 5′-TCCTCCCTGCTCCGATTCCG-3′ (UniSTS: 155846), and the forward primer 5′-CTCC TGCACCTGCTGCCTGGATC-3′ and the reverse primer 5′-GAAGAGACGTTCAGGTGGTGTCAT-3′ (UniSTS: 273125) for the detection of LTA 252 A>G polymorphism, in a total volume of 100 µL containing 400 ng DNA, 0.2 µM each primer, 1 × QIAGEN PCR Buffer (200 µM of each dNTP, 2.5 U Taq DNA polymerase and 1.5 mM $MgCl_2$). The reaction was carried out under the following conditions: an initial denaturation for 3 min at 94 °C, samples were subjected to 35 cycles of amplification, consisting of a 45 s denaturing phase at 94 °C, a 45 s annealing phase at (60 °C for TNF & 65 °C for LTA) and a 45 s extension phase at 72 °C. A 10 min 72 °C hold was the final step of the program.

Amplified products (8 μL) were loaded onto 2 % agarose gels previously stained with 0.5 μg/mL ethidium bromide, electrophoresed at 100 V for 30 min and then visualized by UV transilluminator. PCR products (10 μL) of TNF & LTA genes were digested in a 30 μL reaction volume for 10 min with 1 U of Nco I restriction endonuclease FastDigest (Fermentas, St. Leon-Rot, Germany) at 37 °C. The digested PCR product of TNF was separated on 3.5 % agarose gel and then visualized by UV transilluminator. The genotypes of TNF were homozygous GG wild genotype, with the presence of the Nco I restriction site (87, 20 bp), homozygous mutant AA genotype, lacking the Nco I restriction site (107 bp band) and heterozygous GA genotype (107, 87 and 20) (Fig. 1).

The digested PCR product of LTA was separated on 2 % agarose gel and then visualized by UV transilluminator. The genotypes of LTA were homozygous AA wild genotype, lacking the Nco I restriction site (368 bp), homozygous GG mutant genotype, with the presence of the Nco I restriction site (235 and 133 bp) and heterozygous GA genotype, (368, 235 and 133 bp) (Fig. 2a, b).

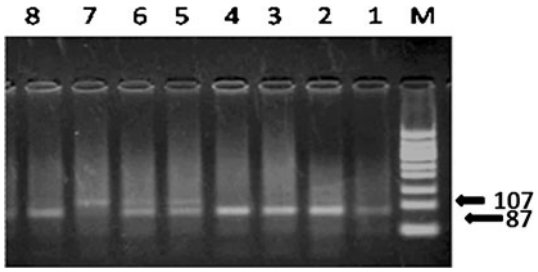

Fig. 1 TNF−308 promoter genotypes by gel electrophoresis. *M*: 50–500 *ladder size marker*. *Lanes 1–4* and *8*: TNF−308 promoter genotypes GG (wild type) showing 87 bp *band*. *Lane 7*: TNF−308 promoter genotypes AA (mutant) homotype showing 107 bp *band*. *Lanes 5, 6*: TNF−308 promoter genotypes GA heterotype showing 107 and 87 bp *bands*

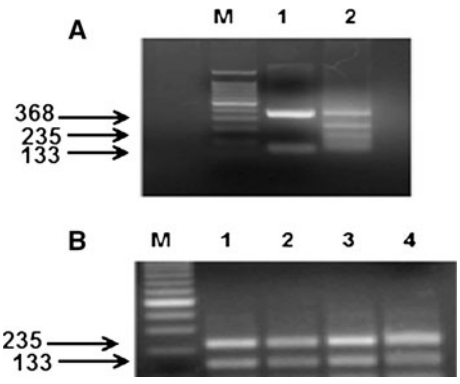

Fig. 2 **a** LTA 252 genotypes by gel electrophoresis. *M*: 100–1000 *ladder size marker*. *Lane 1:* LTA+252 AA genotype (wild type) showing a 368 bp *band*. *Lane 2*: LTA+252 GA heterotype showing 133, 235 and 368 bp *bands*. **b** LTA 252 genotypes by gel electrophoresis. *M:* 100–1000 Ladder size marker. *Lanes 1–4:* LTA252 GG homotype showing 133 and 235 bp *bands*

Statistical analysis

Data were coded and entered using the statistical package SPSS version 17. Data was summarized using mean and standard deviation for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. Genotype and allele frequencies were compared between the disease and the control groups using Chi square tests. Odds ratio (OR) with 95 % confidence intervals was calculated. Association of SLE clinical manifestations with genotype was performed using binary logistic regression and incorporating gender, age at first diagnosis, and disease duration as covariates. The nonparametric Mann–Whitney *U* test was used to compare numeric variables between genotype groups and between cases and control. p value < 0.05 was considered as statistically significant.

Results

As regards the clinical and immunological features. The majority of the patients (64 %) suffered from nephritis followed by arthritis (56 %), malar rash (28 %) and haematological disorders (28 %). Eighty-four patients (84 %) had antinuclear antibodies (ANA) while anti-double stranded DNA (anti-dsDNA) were found in (74 %) of patients.

The comparison between demographic and laboratory data of the SLE patients and control subjects are shown in Table (1)

There was a significant increase in the mean levels of WBCS, ESR and creatinine in SLE patients compared to the control group ($p < 0.001$). There was a significant decrease in the mean levels of Hb, platelets, C3 and C4 in SLE patients compared to the control group ($p < 0.001$). There were no significant differences between SLE patients and control group as regards age and sex ($p > 0.05$).

The genotype distribution of TNF promoter −308 G>A and LTA 252 A>G genotype distribution and allele frequency in SLE patients and control subjects are demonstrated in Table (2)

The frequencies of GG, GA and AA genotypes of the TNF promoter −308 were 42, 32 and 26 %, respectively in SLE patients. The frequencies of GG, GA and AA genotypes of the TNF promoter −308 were 62, 28 and 10 %,

respectively in control subjects. There was a significant difference in the GG (42 %) and AA (26 %) genotypes in SLE patients compared to the control subjects (62 %) and (10 %), respectively. The frequency of the A allele of the TNF promoter −308 was significantly higher in the SLE patients (42 %) than in the control subjects (24 %) ($p < 0.001$; OR 2.29; 95 % CI 1.49–3.52). The frequencies of GG, GA and AA genotypes of the LTA 252 were 22, 32 and 46 %, respectively in SLE patients. The frequencies of GG, GA and AA genotypes of the LTA 252 were 6, 30 and 64 %, respectively in control subjects. There was a significant difference in the GA (22 %) and AA (46 %) genotypes in SLE patients compared to the control subjects (6 %) and (64 %), respectively. The frequency of the G allele of the LTA was significantly higher in the SLE patients (38 %) than in the control subjects (21 %) ($p < 0.001$; OR 2.31; 95 % CI 1.48–3.6).

Table 1 Comparison between demographic and laboratory data of the SLE patients and control subjects

	SLE patients ($N = 100$)	Controls ($N = 100$)	p value
Sex(M/F)	10/90	12/88	0.65
Age(years)	31.62 ± 8.27	30.28 ± 9.02	0.14
Hb(g/dL)	10.112 ± 2.26	11.8 ± 1.04	<0.001
WBC(10^3/μL)	7.7(5.1)*	6.36 ± 1.51	<0.001
PLT(10^3/μL)	287.66 ± 86.18	328.48 ± 66.74	<0.001
ESR(mm/h)	62(55)*	7.78 ± 2.29	<0.001
Creatinine(mg/dL)	0.8(0.9)*	0.62 ± 0.11	<0.001
C3(mg/dL)	80(39)*	117.44 ± 17.66	<0.001
C4(mg/dL)	24(11)*	30.02 ± 5.69	<0.001

Hb hemoglobin, *WBĆs* white blood cells, *PLT* platelets, *ESR* erythrocyte sedimentation rate in the first hour, *C3* complement component 3, *C4* complement component 4

Data were presented as mean ± SD

* Median (interquartile range)

The association of the TNF promoter −308 genotypes with the clinical manifestations in SLE patients was shown in Table (3)

Patients carrying the GA+AA genotypes showed a significant association with serositis ($p = 0.027$; OR 3.36; 95 % CI 1.15–9.83), arthritis ($p = 0.004$; OR 3.86; 95 % CI 1.53–9.74), oral ulcer ($p = 0.017$; OR 6.83; 95 % CI 32.89) and malar rash ($p = 0.04$; OR 2.87; 95 % CI 1.05–7.86). Other clinical manifestations were similar in both groups.

The association of the LTA 252 genotypes with the clinical manifestations in SLE patients was shown in Table (4)

Except for the significant association ($p = 0.036$; OR 2.54; 95 % CI 1.06–6.08) of arthritis with the GA+GG genotypes, clinical manifestations were similar in both groups. A trend for higher prevalence of discoid rash in the GA+GG genotypes was observed but the difference didn't reach statistical difference ($p = 0.05$; OR 4.56; 95 % CI 1–20.82).

The association of the TNF promoter −308 genotypes with the demographic data, laboratory investigations and SLEDAI in SLE patients was shown in Table (5)

SLE disease activity measured by the SLEDAI, was significantly higher ($p < 0.01$) among patients carrying the TNF−308 GA+AA genotypes. Except for disease activity,

Table 2 TNF promoter −308 G>A and LTA+ 252 A>G genotype distribution and allele frequency in SLE patients and control subjects

	SLE patients ($N = 100$) N (%)	Control group ($N = 100$) N (%)	P value	OR (95 % CI)
Genotypes				
TNF−308 GG	42 (42)	62 (62)	0.005	0.44 (0.25–0.78
TNF−308 GA	32 (32)	28 (28)	0.537	1.21 (0.66–2.22)
TNF−308 AA	26 (26)	10 (10)	0.003	3.16 (1.43–6.98)
LTA252 AA	46 (46)	64 (64)	0.011	0.48 (0.27–0.85)
LTA252 GG	22 (22)	6 (6)	0.001	4.42 (1.71–11.44)
LTA252 AG	32 (32)	30 (30)	0.76	1.1 (0.6–2)
Alleles				
TNF−308 G	116 (58)	152 (76)		
TNF−308 A	84 (42)	48 (24)	<0.001	2.29 (1.49–3.52)
LTA 252 A	124 (62)	158 (79)		
LTA 252 G	76 (38)	42 (21)	<0.001	2.31 (1.48–3.6)

OR (95 % CI) = odds ratio (95 % confidence interval)

Table 3 Association of the TNF promoter −308 genotypes with the clinical manifestations in SLE patients

	TNF promotor −308 genotypes			
	GG ($N = 42$) N (%)	GA+AA ($N = 58$) N (%)	p value	OR^ (95 % CI)
Haematological disorder	10 (23.8)	18 (31)	0.51	1.36 (0.54–3.4)
Nephritis	26 (61.9)	38 (65.5)	0.41	1.45 (0.6–3.46)
Serositis	6 (14.3)	20 (34.5)	0.027	3.36 (1.15–9.83)
Myositis	8 (19)	8 (13.8)	0.61	0.75 (0.25–2.26)
Arthritis	12 (28.6)	31 (53.4)	0.004	3.86 (1.53–9.74)
Neurological disorder	6 (14.3)	10 (17.2)	0.96	0.97 (0.31–3.08)
Fever	8 (19)	14 (24.1)	0.36	1.65 (0.56–4.88)
Oral ulcer	2 (4.8)	14 (24.1)	0.02	6.83 (1.42–32.89)
Malar rash	8 (19)	20 (34.5)	0.04	2.87 (1.05–7.86)
Alopecia	6 (14.3)	6 (10.3)	0.61	0.72 (0.21–2.51)
Discoid rash	4 (9.5)	10 (17.2)	0.39	1.76 (0.48–6.45)

OR^ adjusted odds ratio (adjusted for gender, age at first diagnosis, and disease duration)

Table 4 Association of the LTA+252 genotypes with the clinical manifestations in SLE patients

	LTA+252 genotypes			
	AA ($N = 46$) N (%)	AG+GG ($N = 54$) N (%)	p value	OR^ (95 % CI)
Haematological disorder	14 (30.4)	14 (25.9)	0.65	0.81 (0.32–2.02)
Nephritis	30 (65.2)	34 (63)	0.87	0.93 (0.38–2.24)
Serositis	10 (21.7)	16 (29.6)	0.18	1.98 (0.72–5.41)
Myositis	6 (13)	10 (18.5)	0.54	1.44 (0.45–4.66)
Arthritis	14 (30.4)	29 (53.7)	0.04	2.54 (1.06–6.08)
Neurological disorder	6 (13)	10 (18.5)	0.28	1.94 (0.58–6.46)
Fever	8 (17.4)	14 (25.9)	0.19	2.12 (0.7–6.42)
Oral ulcer	6 (13)	10 (18.5)	0.61	1.35 (0.42–4.36)
Malar rash	8 (17.4)	20 (37)	0.10	2.26 (0.85–6.02)
Alopecia	6 (13)	6 (11.1)	0.85	0.89 (0.25–3.14)
Discoid rash	4 (8.7)	10 (18.5)	0.05	4.56 (1–20.82)

OR^ adjusted odds ratio (adjusted for gender, age at first diagnosis, and disease duration)

demographic data and laboratory investigations were similar in both groups.

The association of the LTA 252 genotypes with the demographic data, laboratory investigations and SLEDAI in SLE patients was shown in Table (6)

Patients carrying the GA+GG genotypes had a significantly lower age ($p = 0.009$) and a significantly higher WBCs count ($p = 0.001$). All other demographic data and laboratory investigations were similar in both groups. A trend for higher activity of disease SLEDAI in the GA+GG genotypes was observed but the difference didn't reach statistical difference ($p = 0.05$).

The comparison of autoantibodies between different genotypes

No significant association was seen between TNF−308 genotypes GA+AA and the ANA ($p = 0.743$; OR 0.83; 95 % CI 0.27–2.55) or anti-dsDNA ($p = 0.121$; OR 2.07; 95 %CI 0.83–5.18).

No significant association was seen between LTA genotypes GA+GG and the ANA ($p = 0.493$; OR 1.47; 95 % CI 0.49–4.44) or anti-dsDNA ($p = 0.967$; OR 0.98; 95 % CI 0.39–2.47).

Discussion

SLE is a multifactorial autoimmune disease characterized by chronic inflammation in many organs [15]. Several studies examined the association of TNFα−308 and LT α+252 genetic polymorphisms with SLE. Nevertheless, the results were controversial [8–12, 16].

The aim of this study was to investigate the association of genetic polymorphism TNFα−308 and LT α+252 with susceptibility to SLE and clinical manifestations in Egyptian patients. To achieve this aim, TNFα−308 and LT α+252 genotyping was performed by PCR-RFLP technique.

Table 5 Association of the TNF promoter −308 genotypes with the demographic data, laboratory investigations and SLEDAI in SLE patients

	TNF promotor −308 genotypes		
	GG ($N = 42$) Median (interquartile range)	GA+AA ($N = 58$) Median (interquartile range)	p value
Age (years)	30 (10)	31 (13)	0.11
Age of onset (years)	21 (8.9)	23 (11)	0.06
Disease duration (years)	4 (5.8)	5 (6.3)	0.37
Hb (g/dL)	10.6 (3.5)	10 (3.6)	1
WBCs (10^3/μL)	7.4 (4.2)	8 (6.1)	0.95
PLT (10^3/μL)	299 (80)	278 (130)	0.55
ESR (mm/h)	60 (39)	72 (59)	0.27
Creatinine (mg/dL)	0.8 (0.8)	0.8 (0.9)	0.32
C_3 (mg/dL)	78 (30)	83 (38)	0.60
C_4 (mg/dL)	19 (16)	24 (11)	0.38
24 h U.Pr. (gm/L)	1.2 (3)	0.48 (3)	0.40
Disease activity (SLEDAI-2 K)	11 (11)	12 (9)	0.01

Hb hemoglobin, *WBC's white blood cells, PLT platelets, ESR erythrocyte sedimentation rate in the first hour, C3* complement component 3, *C4* complement component 4, *24 h U.Pr* 24 h urinary protein

Table 6 Association of the LTA+252 genotypes with the demographic data, laboratory investigations and SLEDAI in SLE patients

	LTA+252 genotypes		p value
	AA ($N = 46$) Median (Interquartile range)	AG+GG ($N = 54$) Median (Interquartile range)	
Age (years)	32 (13)	30 (12)	0.009
Age of onset (years)	23 (11)	21 (10)	0.32
Disease duration (years)	5 (9)	5 (5)	0.13
Hb (g/dL)	9.8 (3.6)	10.6 (3.7)	0.94
WBCs (10^3/μL)	6.4 (4)	10 (4.8)	0.001
PLT (10^3/μL)	299 (127)	283 (99)	0.84
ESR (mm/h)	63 (49)	57.5 (65)	0.30
Creatinine (mg/dL)	0.8 (1)	0.8 (0.9)	0.22
C_3 (mg/dL)	79 (45)	82 (37)	0.55
C_4 (mg/dL)	19 (15)	26 (12)	0.07
24 h U.Pr. (gm/L)	1.2 (3)	0.48 (3)	0.38
Disease activity (SLEDAI-2 K)	11 (12)	13 (8)	0.05

Hb hemoglobin, *WBĆs* white blood cells, *PLT* platelets, *ESR* erythrocyte sedimentation rate in the first hour, *C3* complement component 3, *C4* complement component 4, *24 h U.Pr* 24 h urinary protein

SLE patients showed a significant decrease in the mean level of Hb than control group. This may be due to the poor general condition and anorexia leading to inadequate feeding or the cytotoxic therapy that may suppress erythroid precursors in the bone marrow.

The mean ESR was significantly higher in SLE patients than control group ($p < 0.001$) which correlates with Steinberg [17]. This is due to the presence of inflammatory changes in SLE.

SLE patients showed a significant decrease in the mean level of platelets than control group. This correlates with Ziakas et al. [18] who stated that immunologically mediated thrombocytopenia is a frequent clinical manifestation in patients with SLE and explained this by the presence of autoantibodies targeting platelet membrane glycoproteins leading to peripheral platelet destruction.

ANA was positive in all 84 % of SLE patients and negative in all controls. Anti ds-DNA was positive in 74 % of patients but negative in all controls. This correlates with Dean et al. [19] who reported that ANA was positive in 98 % and anti ds-DNA was positive in 50–80 % of patients.

The mean C3 and C4 level were significantly lower in SLE patients than control subjects. These results agree with Sturfelt et al. [20] who explained this by the consumption of the complement C3 and C4 in immune complex formation and by reduced synthesis.

TNF α genotyping in SLE patients revealed that TNFα AA mutant genotype was significantly higher than controls and TNFαGG wild genotype was significantly higher in the controls compared to SLE patients. A higher frequency of the TNF−308 A allele (42 %) was reported in SLE patients. This was in accordance with, the European study in patients with SLE, which reported higher frequency of the TNF−308 A allele (up to 48 %) [21], and a meta-analysis confirmed the association of the A/A risk genotype (recessive model) and G/A+A/A genotypes (dominant model) with SLE susceptibility in North Europeans [8]. However, in Asians [8], Black Africans [22], African Americans [3], Mexican Mestizo [23], in Italian patients [24], Thai patients [25] and in Portuguese [12], the TNF−308 A allele was not associated with SLE.

The TNF−308 minor allele A is in strong linkage with HLA-DR3 [26], and while some studies have reported the association with SLE susceptibility to be dependent on the presence of DR3 [23], others concluded that TNF and HLA are independent susceptibility factors [27].

In the present study, the association between the clinical manifestations of SLE patients with each TNF-α genetic polymorphism was also investigated. We found that patients carrying TNF−308 (GA+AA) genotypes were

more susceptible to serositis, oral ulcers and malar rash than those carrying TNF−308 GG genotype.

This concurs with the study done by Lin et al. [28] which reported that the A allele of the polymorphism at −308 was significantly increased in patients with malar rash, discoid rash, photosensitivity, oral ulcers and serositis. While, the study done by Santos et al. [12] reported that patients carrying the −308 A allele were almost threefold more likely to have nephritis compared to those with the G/G genotype, explaining these by non significantly higher serum TNF levels in SLE patients carrying the A allele, which may have contributed to the observed higher disease activity and greater prevalence of lupus nephritis.

We found that patients carrying TNF −308 (GA+AA) genotypes were more susceptible to arthritis than those carrying TNF −308 GG genotype. These results are in accordance with previous studies of Portuguese patients with inflammatory rheumatic diseases, which found the TNF polymorphism −308 to influence rheumatoid arthritis activity and severity [29], juvenile idiopathic arthritis inflammatory activity [30], some clinical features of ankylosing spondylitis [31], but not to influence the susceptibility to these diseases and also meta-analysis done by Erik et al. [32] which proved that the TNFA −308 G>A promoter polymorphism is associated with joint damage in patients with rheumatoid arthritis, and this is not mediated by differences in TNF gene expression between genotypes.

We proved also significantly higher systemic lupus activity index SLEDAI in patients carrying TNF −308 (GA+AA) than those carrying TNF −308 GG genotype, these coincide with Santos et al. [12].

There was strong evidence that *TNF* −308A allele enhances transcriptional activation [26], and TNF-α production [33], which may have contributed to the observed higher disease activity in patients carrying A allele LTα genotyping in SLE patients revealed that LTα GG mutant genotype was significantly higher than controls and LTα AA wild genotype was significantly higher in the controls compared to SLE patients. This was in accordance with the study in German patients, where the LTA 252 polymorphism was linked to SLE susceptibility [34], this association could not be reported in Caucasian or African American patients [11].

In the current work, a higher frequency of the LTα G allele (38 %) was found in SLE patients which was similar to results found in Portuguese SLE patients (36.2 %) [12], and in other European populations (35.5 %) [35].

In the present study, the association between the clinical manifestations of SLE patients with each LT α+252 genetic polymorphism was also investigated. We reported that patients carrying LT α (GA+GG) genotypes were more susceptible to arthritis than those carrying LTα AA genotype. While, the study done by Santos et al. [12] reported that lupus nephritis was more frequent among LTA 252 A/G and G/G genotypes.

Messer first reported the existence of a SNP in the intron of LT-α at position 252 (G>A), which is associated with overexpression of LT-α [36]. Calmon-Hamaty et al. [37] found that LTα is as effective as TNF α in stimulating fibroblast-like synoviocytes (FLS), and blocking both cytokines might allow a better control of inflammation and synovial proliferation in rheumatoid arthritis (RA).

Higher systemic lupus activity index SLEDAI was found in patients carrying LT α (GA+GG) than those carrying LT α AA genotype, these coincide with Santos et al. [12].

Conclusion

Our findings strongly suggest an association between TNFα−308 and LTα+252 genetic polymorphisms and SLE disease susceptibility in the Egyptian population and associated with disease characteristics. TNF−308 and LTA+252 polymorphic markers may be used for early diagnosis of SLE and early prediction of clinical manifestations, like arthritis. A possible association of lupus arthritis and lupus inflammatory disease activity with these polymorphisms needs further investigation.

Conflict of interest The authors declare no conflicts of interest with respect to the authorship and/or publication of this article.

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