IL-1β, IL-10 and TNF-α polymorphisms may affect systemic lupus erythematosus risk and phenotype

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Abstract

Objective

Systemic lupus erythematosus (SLE) is an autoimmune disease, and IL-1β, IL-10, and TNF-α genes are important in the pathogenesis of this disease. We studied the impact of IL-1β-511, IL-1β +3953, IL-10 -592, IL-10 -1082, TNF-α -308, TNF-α -238, and TNF-α +489 polymorphisms on SLE risk and phenotype in SLE patients and healthy controls.

Methods

We genotyped SLE patients and healthy controls by real-time PCR on QuantStudio 5 (Applied Biosystems) and measured levels of cytokines by enzyme-linked immunosorbent assay (ELISA).

Results

We indicated that TNF-α -308, IL-10 -592, IL-10 -1082, IL-1β-511 and IL-1β +3953 polymorphisms affect SLE risk. Furthermore, we exposed that some of the TNF-α +489, TNF-α -238, IL-10 -1082 and IL-1β +3953 genotypes are connected with the SLE phenotype. Moreover, we discovered the linking between specific genotypes and the serum concentrations of TNF-α, IL-1β, and IL-10.

Conclusion

In conclusion, our study revealed that IL-1β-511, IL-1β +3953, IL-10 -592, IL-10 -1082, and TNF-α -308 polymorphisms may affect SLE risk and phenotype.

Key words

systemic lupus erythematosus, cytokines, pathogenesis, polymorphisms

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Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease that is problematic to diagnose and treat (1, 2). Activation of an immune system in SLE is described as a loss of immune tolerance against autoantigens as well as exaggerated responses of T cells and B cells (1). Cytokine and complement activation, circulation, and deposition of immune complexes in tissues as well as production and incorrect removal of antibodies contribute to clinical symptoms. Systemic lupus erythematosus, regardless of advances in treatment, is still linked with premature mortality (3, 4). Genome-wide association studies (GWAS) have revealed more than eighty SLE susceptibility loci (3).

Tumour necrosis factor α (TNF-α) is responsible for the co-stimulation of T cells and takes part in proliferation, differentiation, and activation as well as in the production of antibodies in B cells. TNF-α has a role in mediating organ damage and inflammation of tissues and contributing to immune dysfunction (5). TNF-α levels are higher in lupus than in rheumatoid arthritis (RA). Blockade of TNF-α may cause drug-induced lupus and autoantibodies production (6). TNF-α SNPs -238 and -308 were connected with SLE (7). Moreover, the TNF-α +489A allele also has a genetic contribution to SLE (8).

The activation and/or expansion of autoreactive lymphocytes are hindered by interleukin-10 (IL-10) (9). In autoimmune diseases, interleukin-10 is assumed as protective, because it impedes pathogenic inflammation and also indorses self-tolerance. However, in SLE, IL-10 has a pathogenic role and its neutralisation can be used in the disease treatment. IL-10 indorses survival of B cells, which are autoreactive and IL-10 works as differentiation and growth factor on B cells, and cytotoxic lymphocytes. Different immune cells produce IL-10 and they can have different functions, depending on the microenvironment or context. Specifically, $CD4^+$ T cells probably make a significant contribution to the abnormal production of interleukin 10. The single nucleotide polymorphisms (SNPs) within the IL-10 gene are connected with SLE risk (10, 11). IL-10 SNPs rs1800896 (-1082 T/C) and rs1800872 (-592 T/G) have been associated with SLE susceptibility (10, 12).

Interleukin-1β (IL-1β) is a member of the interleukin 1 (IL-1) family. IL-1β has a central role in rheumatic diseases with autoimmune compound (13). Overproduction of interleukin-1β is associated with the pathogenesis of autoimmune diseases, including SLE (5, 14). IL-1β is a pleiotropic and pro-inflammatory cytokine and is mostly produced by macrophages, monocytes, natural killer (NK) cells, B cells, and dendritic cells. Interleukin 1 beta stimulates proliferation and differentiation of B cells, activates NK cells as well as costimulates T cells (15). IL-1β -511C/T polymorphism is suggestively linked to SLE susceptibility (15, 16). Furthermore, in IL-1β+3953, allele T was protective for systemic lupus erythematosus (13).

This study aimed to check the influence of seven polymorphisms within IL-1β, IL-10, and TNF-α genes on susceptibility to SLE and the disease phenotype. Moreover, we studied the connection between particular genotypes within these genes and the concentration of cytokines.

Materials and methods

Study group

Two hundred and thirty SLE patients and three hundred sixty-one healthy controls were involved in this study. Patients with SLE were enrolled from the National Institute of Geriatrics, Rheumatology and Rehabilitation in Warsaw, Poland as well as from the Poznan University of Medical Sciences, Poland. All SLE patients met the classification criteria of the American College of Rheumatology (ACR) for SLE (at least 4 criteria). Safety of Estrogens in Lupus National Assessment-Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI) was used as the scale, which measures disease activity of SLE patients. Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) Damage Index (SDI) was used to score irreversible damage of SLE patients. The healthy subject

group did not show any clinical or laboratory signs of autoimmune diseases. All groups provided written informed consent for this study.

SNP selection

Single nucleotide polymorphisms (SNPs) were selected after PubMed research in the public database (dbSNP database). We chose SNPs due to their probable clinical significance and connection with autoimmune diseases. Only SNPs with MAF (Minor Allele Frequency) above 5% (>0.05) were included in the study. We selected seven SNPs: IL-1β (rs16944, rs1143634), IL-10 (rs1800872, rs1800896) and TNF-α (rs1800629, rs361525, rs1800610).

DNA isolation

We collected whole blood samples gained from healthy controls and SLE patients in the EDTA tubes. DNA was obtained from the peripheral blood using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany).

Genotyping

We performed SNPs genotyping assays on QuantStudio 5 (Applied Biosystems, Forester City, CA, USA). To genotype our samples we used Taqman Genotyping Master Mix (Applied Biosystems, Foster City, CA, USA) and TaqMan SNP genotyping assays (Applied Biosystems, Foster City, CA, USA) to genotype SNPs: rs16944– C_1839943_10 (IL-1β), rs1143634– C_9546517_10 (IL-1β), rs1800872– C_1747363_10 (IL-10), rs1800896– C_1747360_10 (IL-10) and rs1800629– C_7514879_10 (TNF-α), rs361525– C_2215707_10 (TNF-α) and rs1800610– C_11918224_10 (TNF-α).

Reactions were performed according to manufacturer protocol in 10 ul volumes: 5 ul of TaqMan Genotyping Master Mix (2x), 0.5 ul of TaqMan genotyping assay mix (20x), 4.5 ul of DNA. The amplification protocol was: pre-read stage at 60°C for 30 s, denaturation at 95°C for 10 min, 40 cycles of denaturation at 95°C for 15 s as well as annealing and extension at 60°C for 1 min, then post-read stage at 65°C for 30 s.

Enzyme-linked immunosorbent assay

The concentrations of IL-1β, IL-10 and TNF-α in serum in SLE patients and healthy controls were quantified by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (DRG International, Inc., Springfield, NJ, USA). The ELISA detection limits were 0.35 pg/ml for IL-1β, 1.6 pg/ml for IL-10 and 0.7 pg/ml for TNF-α.

Statistical analysis

The laboratory variables and clinical data were described as mean ± standard deviation. The analysis was performed under four genetic models (recessive, dominant, codominant, and overdominant) and the odds ratios (OR) were calculated. Connections between SNPs and clinical parameters were calculated using the Mann-Whitney and χ^2 tests. In addition to the χ^2 test, the χ^2 test with Yates' correction was used, when expected values were less than 10 and sample size greater than 40 or expected values greater than 5, but sample size lower than 40 Fisher's exact test was used for the smaller sample. Hardy-Weinberg equilibrium (HWE) was measured using an online calculator created by Michael H. Court. Only outcomes with a *p*-value lower than 0.05 ($p<0.05$) were considered statistically significant.

Results

Patients' characteristics

The clinical characteristics of SLE patients are presented in Table I. Patients with SLE were in the active stage of disease with a mean disease duration of 10 years. We observed that the most common autoantibody presented in SLE patients was anti-dsDNA (74%), while the less frequent autoantibody was anti-CEN (3%). Furthermore, 63% of our patients had symptoms like arthritis, 21% antiphospholipid syndrome (APS), and 16% Sjögren's syndrome

Minor allele frequency (MAF) for SNPs of IL-1β, IL-10 and TNF-α

We calculated the minor allele frequency for all SNPs for SLE patients and controls. Then, we compared the outcomes with MAF from data for the European population in the 1000Genomes database. The MAF of seven chosen SNPs: IL-1β (rs16944, rs1143634), IL-10 (rs1800872, rs1800896) and TNF-α (rs1800629, rs361525, rs1800610) is presented in Table S1 in the Supplementary files.

Hardy-Weinberg Equilibrium of IL-1β, IL-10 and TNF-α genotypes

We calculated the Hardy-Weinberg Equilibrium (HWE) for genotypes in SLE patients and healthy controls. The HWE for seven chosen SNPs: IL-1β-511, IL-1β +3953, IL-10 -592, IL-10 -1082, TNF-α -308, TNF-α -238, and TNF-α +489 for both SLE patients and controls are presented in Supplementary Table S2. Only IL-10 -1082 genotypes are not consistent with HWE ($p=0.004$) in SLE patients. Moreover, IL-1β-511 genotypes are not consistent with HWE ($p=0.003$) in healthy controls.

IL-1β, IL-10 and TNF-α polymorphisms and SLE susceptibility

First, we examined the frequency of genotypes in IL-1β-511, IL-1β +3953, IL-10 -592, IL-10 -1082, TNF-α -308, TNF-α -238, and TNF-α +489 in SLE patients and healthy controls. The distribution of genotypes and allele frequencies of IL-1β, IL-10, and TNF-α among SLE patients and healthy controls (HCs) with their associations with the risk of SLE is presented in Table II.

In the case of IL-1β-511, genotypes in three models were statistically significant in comparison patients with SLE with healthy subjects. The first is the CT genotype in the codominant model, which occurs more often in SLE patients (44.78%) than in control group (24.78%) with the odds ratio (OR)=2.669 ($p=0.0002$). The second is the CT+TT genotype in the dominant model, which occurs more often in SLE patients (55.65%) than in the control group (34.51%) with OR=2.381 ($p=0.0003$). The third is the CT genotype in the overdominant model, which occurs more often in SLE patients (44.78%) than in the control group (24.78%) with OR=2.462 ($p=0.0004$). Furthermore, allele T was significant ($p=0.003$).

In the case of IL-1β +3953, genotypes in three models were statistically sig-

Table I. Clinical characteristics of SLE patients.

Characteristics	SLE patients	
	N*	mean values ±SD (range)
Age (years)	100	44.59±14.17 (21–87)
Disease duration (years)	63	10.08±10.08 (0–43)
SELENA_SLEDAI	63	6±5.82 (0–26)
SDI	63	1.17±1.26 (0–5)
APTT	49	35.34±16.52 (21–127)
ESR (mm/h)	59	42.80±28.86 (5–114)
CRP (mg/L)	58	33.79±68.92 (1–395)
Haemoglobin (g/dL)	59	11.97±2.17 (4.90–16)
Pt	48	16.72±17.86 (8.60–100)
INR	48	1.23±0.79 (0.70–5.40)
Urea	51	41.65±38.72 (16.70–296)
PLT (x10^3/mm^3)	59	242.14±123.86 (38–598)
Creatinine	56	0.83±0.22 (0.50–1.50)
C3	55	76.29±29.07 (33–162)
C4	54	14.05±13.49 (3.09–102)
ALT (U/L)	60	50±54.36 (1–317)
AST (U/L)	59	51.25±108.82 (14–850)
	N*	n** (%)
anti-dsDNA	93	69 (74)
anti-SSA	59	25 (43)
anti-SSB	58	5 (9)
anti-SM	68	20 (29)
anti-Rib	57	4 (7)
anti-Scl70	78	21 (27)
anti-CEN	58	2 (3)
anti-U1RNP	73	30 (41)
anti-IgM	59	6 (10)
anti-IgG	60	17 (28)
LAC	59	16 (27)
Sjogren's syndrome	63	10 (16)
Facial erythema	63	35 (56)
Arthritis	75	47 (63)
Lung fibrosis	63	3 (5)
Leucopenia	63	23 (37)
APS	52	11 (21)

N: number of patients with clinical information; n: number of patients with positive clinical manifestation; SELENA-SLEDAI :Safety of Estrogens in Lupus National Assessment - Systemic Lupus Erythematosus Disease Activity Index; SDI: Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) Damage Index; PLT: platelets; APTT: activated partial thromboplastin time; PT: prothrombin time; INR - international normalised ratio; ESR: erythrocyte s:imentation ratio; CRP: C-reactive protein; C3 and C4: complement; LAC: lupus anticoagulant; ALT: glutamic pyruvic transferase; AST: glutamic oxoloacetic transaminase; ALP: alkaline phosphatase; TG: triglyceride; LDL: low-density lipoprotein; HDL: high-density lipoprotein; APS :antiphospholipid syndrome.

nificant in comparison patients with SLE with healthy subjects. The first is the CT genotype in the codominant model, which occurs more often in SLE patients (41.03%) than in the control group (21.24%) with OR=2.636 (p=0.005). The second is the CT+TT genotype in the dominant model, which occurs more often in SLE patients (44.87%) than in the control group (24.78%) with OR=2.471 (p=0.006). The third is the CT genotype in the overdominant model, which occurs more often in SLE patients (41.03%) than in the control group (21.24%) with OR=2.580 (p=0.005). Furthermore, allele T was more frequently observed in SLE patients than in healthy subjects (p=0.017).

In the case of IL-10 -592, three genotypes in three models were statistically significant in comparison patients with SLE with healthy subjects. The first is the CA genotype in the codominant model, which occurs more often in SLE patients (39.34%) than in the control group (28.25%) with OR=1.735 (p=0.004). The second is the CA+AA genotype in the dominant model, which occurs more often in SLE patients (45.97%) than in the control group (32.69%) with OR=1.752 (p=0.002). The third is the CA genotype in the overdominant model, which occurs more often in SLE patients (39.34%) than in the control group (28.25%) with OR=1.647 (p=0.005). Furthermore, allele A was more frequently observed in SLE patients than in healthy subjects (p=0.003).

In the case of IL-10 -1082, two genotypes in three models were statistically significant in comparison patients with SLE with healthy subjects. The first is the AG genotype in the codominant model, which occurs less often in SLE patients (33.03%) than in the control group (43.92%) with OR=0.542 (p=0.018). The second is the AG+GG genotype in the dominant model, which occurs less often in SLE patients (51.38%) than in the control group (64.99%) with OR=0.569 (p=0.016). Furthermore, allele G was more frequently observed in healthy subjects than in SLE patients (p=0.039).

In the case of TNF-α -308, four genotypes in three models were statistically significant in comparison patients with SLE with healthy subjects. The first genotype in the codominant model is GA, which occurs less often in SLE patients (20.73%) than in the control group (30.27%) with OR=0.574 (p=0.011). The second genotype in the codominant model is the AA, which occurs less often in SLE patients (2.07%) than in the control group (5.04%) with OR=0.295 (p=0.049). The third is the GA+AA genotype in the dominant model, which occurs less often in SLE patients (22.8%) than in the control group (35.31%) with OR=0.541 (p=0.003). The fourth is the GA genotype in the overdominant model, which occurs less often in SLE patients (20.73%) than in the control group (30.27%) with OR=0.602 (p=0.021). Furthermore, allele A was more frequently observed in controls than in SLE patients (p=0.002).

In the case of TNF-α -238 and TNF-α +489, results were not statistically significant.

Table II. Distribution of genotypes and allele frequencies of SNPs among SLE patients and healthy subjects.

IL-1β-511		SLE n (%)	Controls n (%)**	OR (95% CI)	*p*-value
	genotype				
Codominant	CC	102 (44.35)	74 (65.49)	References	
	CT	103 (44.78)	28 (24.78)	2.669 (1.554–4.642)	**0.0002**
	TT	25 (10.87)	11 (9.73)	1.649 (0.726–3.948)	0.273
Dominant	CC	102 (44.35)	74 (65.49)	References	
	CT+TT	128 (55.65)	39 (34.51)	2.381 (1.457–3.914)	**0.0003**
Recessive	CC+CT	205 (89.13)	102 (90.27)	References	
	TT	25 (10.87)	11 (9.73)	1.3131 (0.512–2.651)	1.131
Overdominant	CC+TT	127 (55.22)	85 (75.22)	References	
	CT	103 (44.78)	28 (24.78)	2.462 (1.458–4.222)	**0.0004**
	alleles				
	C	307 (66.74)	176 (77.88)	References	
	T	153 (33.26)	50 (22.12)	1.754 (1.197–2.594)	**0.003**
IL-1β +3953		SLE n (%)	Controls n (%)**	OR (95% CI)	*p*-value
	genotype				
Codominant	CC	43 (55.13)	85 (75.22)	References	
	CT	32 (41.03)	24 (21.24)	2.636 (1.317–5.285)	**0.005**
	TT	3 (3.85)	4 (3.54)	1.483 (0.207–9.167)	0.689*
Dominant	CC	43 (55.13)	85 (75.22)	References	
	CT+TT	35 (44.87)	28 (24.78)	2.471 (1.272–4.803)	**0.006**
Recessive	CC+CT	75 (96.15)	109 (96.46)	References	
	TT	3 (3.85)	4 (3.54)	1.090 (0.155–6.642)	0.754*
Overdominant	CC+TT	46 (58.97)	89 (78.76)	References	
	CT	32 (41.03)	24 (21.24)	2.580 (1.299–5.138)	**0.005**
	alleles				
	C	118 (75.64)	194 (85.84)	References	
	T	38 (24.36)	32 (14.16)	1.952 (1.119–3.413)	**0.017**
IL-10 -592		SLE n (%)	Controls n (%)**	OR (95% CI)	*p*-value
	genotype				
Codominant	CC	114 (54.03)	243 (67.31)	References	
	CA	83 (39.34)	102 (28.25)	1.735 (1.183–2.539)	**0.004**
	AA	14 (6.64)	16 (4.43)	1.865 (0.811–4.229)	0.153
Dominant	CC	114 (54.03)	243 (67.31)	References	
	CA+AA	97 (45.97)	118 (32.69)	1.752 (1.217–2.520)	**0.002**
Recessive	CC+CA	197 (93.36)	345 (95.57)	References	
	AA	14 (6.64)	16 (4.43)	1.532 (0.676–3.428)	0.343
Overdominant	CC+AA	128 (60.66)	259 (71.75)	References	
	CA	83 (39.34)	102 (28.25)	1.647 (1.130–2.394)	**0.008**
	alleles				
	C	311 (73.7)	588 (81.44)	References	
	A	111 (26.3)	134 (18.56)	1.566 (1.163–2.106)	**0.003**
IL-10 -1082		SLE n (%)	Controls n (%)**	OR (95% CI)	*p*-value
	genotype				
Codominant	AA	53 (48.62)	118 (35.01)	References	
	AG	36 (33.03)	148 (43.92)	0.542 (0.322–0.906)	**0.018**
	GG	20 (18.35)	71 (21.07)	0.627 (0.328–1.172)	0.158
Dominant	AA	53 (48.62)	118 (35.01)	References	
	AG+GG	56 (51.38)	219 (64.99)	0.569 (0.359–0.904)	**0.016**
Recessive	AA+AG	89 (81.65)	266 (78.93)	References	
	GG	20 (18.35)	71 (21.07)	0.842 (0.459–1.494)	0.642
Overdominant	AA+GG	73 (66.97)	189 (56.08)	References	
	AG	36 (33.03)	148 (43.92)	0.630 (0.388–1.012)	0.057
	alleles				
	A	142 (65.14)	384 (56.97)	References	
	G	76 (34.86)	290 (43.03)	0.709 (0.528–0.984)	**0.039**

TNF-α -308		SLE n (%)	Controls n (%)**	OR (95% CI)	*p*-value
	genotype				
Codominant	GG	149 (77.2)	218 (64.69)	References	
	GA	40 (20.73)	102 (30.27)	0.574 (0.366–0.889)	**0.011**
	AA	4 (2.07)	17 (5.04)	0.295 (0.196–0.440)	**0.049***
Dominant	GG	149 (77.2)	218 (64.69)	References	
	GA+AA	44 (22.8)	119 (35.31)	0.541 (0.352–0.823)	**0.003**
Recessive	GG+GA	189 (97.93)	320 (94.96)	References	
	AA	4 (2.07)	17 (5.04)	0.398 (0.096–1.248)	0.107*
Overdominant	GG+AA	153 (79.27)	235 (69.73)	References	
	GA	40 (20.73)	102 (30.27)	0.602 (0.386–0.931)	**0.021**
	alleles				
	G	338 (87.56)	538 (79.82)	References	
	A	48 (12.44)	136 (20.18)	0.562 (0.385–0.811)	**0.002**
TNF-α -238		SLE n (%)	Controls n (%)**	OR (95% CI)	*p*-value
	genotype				
Codominant	GG	188 (93.53)	323 (95.56)	References	
	GA	12 (5.97)	15 (4.44)	1.374 (0.574–3.219)	0.544
	AA	1 (0.5)	0 (0)	-	-
Dominant	GG	188 (93.53)	323 (95.56)	References	
	GA+AA	13 (6.47)	15 (4.44)	1.489 (0.637–3.434)	0.406
Recessive	GG+GA	200 (99.5)	338 (100)	References	
	AA	1 (0.5)	0 (0)	-	
Overdominant	GG+AA	189 (94.03)	323 (95.56)	References	
	GA	12 (5.97)	15 (4.44)	1.367 (0.571–3.202)	0.553
	alleles				
	G	388 (96.52)	661 (97.78)	References	
	A	14 (3.48)	15 (2.22)	1.590 (0.703–3.574)	0.296
TNF-α +489		SLE n (%)	Controls n (%)**	OR (95% CI)	*p*-value
	genotype				
Codominant	CC	132 (70.97)	226 (72.67)	References	
	CT	49 (26.34)	83 (26.69)	1.011 (0.652–1.557)	1.000
	TT	5 (2.69)	2 (0.64)	4.280 (0.686–45.36)	0.108*
Dominant	CC	132 (70.97)	226 (72.67)	References	
	CT+TT	54 (29.03)	85 (27.33)	1.088 (0.710–1.657)	0.757
Recessive	CC+CT	181 (97.31)	309 (99.36)	References	
	TT	5 (2.69)	2 (0.64)	4.268 (0.688–45.12)	0.109*
Overdominant	CC+TT	137 (73.66)	228 (73.31)	References	
	CT	49 (26.34)	83 (26.69)	0.983 (0.635–1.511)	1.000
	alleles				
	C	313 (84.14)	535 (86.01)	References	
	T	59 (15.86)	87 (13.99)	1.159 (0.794–1.683)	0.473

p-χ^2 test; *p**-Fisher test; $p<0.05$ was considered significant.

SNPs and phenotype

Next, we examined the connection between laboratory parameters and the disease activity in relation to IL-1β +3953 CC genotype *versus* IL-1β +3953 CT+TT genotype, which is shown in Suppl. Table S3. The only significant result is that SLE patients with the CC genotype have significantly lower complement 3 (C3) levels than those patients with the CT+TT genotype (68.16 *vs*. 88.97; $p=0.006$) (Table III).

Then, we examined the connection between laboratory parameters and the disease activity in relation to IL-10 -592 CC genotype *versus* IL-10 -592 CA+AA genotype, which is shown in Suppl. Table S4. The results were not significant.

Afterwards, we observed the connection between laboratory parameters and the disease activity in relation to IL-10 -1082 AA genotype *versus* IL-10 -1082 AG+GG genotype, which is shown in Suppl. Table S5. The only significant result is that SLE patients with the AA genotype have significantly greater complement 4 (C4) levels than those patients with the AG+GG genotype (14.2 *vs*. 8.4; $p=0.042$) (Table III).

Next, we checked the connection between laboratory parameters and the disease activity in relation to TNF-α -308 GG genotype *versus* TNF-α -308 GA+AA genotype, which is shown in Suppl. Table S6. The results were not significant.

Next, we examined the connection between laboratory parameters and the disease activity in relation to TNF-α -238 GG genotype *versus* TNF-α -238 GA+AA genotype, which is shown in

Table III. The disease activity and laboratory parameters in relation to IL-1β, IL-10 and TNF-α polymorphisms, dominant models.

SNP	Mean ± SD	*p*-value
IL-1β +3953		
C3		
CC	68.16 ± 23.30	
CT+TT	88.97 ± 27.25	0.006
SNP	Median (range)	*p*-value
IL-10 -1082		
C4		
AA	14.2 (3.16–31)	
AG+GG	8.4 (5.58–12.8)	0.042
TNF-α -238		
SDI		
GG	1 (0–5)	
GA+AA	3.5 (3–4)	0.028
TNF-α +489		
Pt		
CC	11.4 (8.7–100)	
CT+TT	10.1 (8.6–12.8)	0.003
INR		
CC	1.03 (0.7–5.4)	
CT+TT	0.9 (0.8–1.2)	0.004

$p<0.05$ was considered significant.

Suppl. Table S7. The only significant result is that SLE patients with the GG genotype have significantly lower the Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) Damage Index (SDI) value than those patients with the GA+AA genotype (1 *vs*. 3.5; $p=0.028$) (Table III).

Then, we studied the connection between laboratory parameters and the disease activity in relation to TNF-α +489 CC genotype *versus* TNF-α +489 CT+TT genotype, which is shown in Suppl. Table S8. SLE patients with the CC genotype have significantly greater prothrombin time (Pt) value than those patients with the CT+TT genotype (11.4 *vs*. 10.1; $p=0.003$). Moreover, SLE patients with the CC genotype have also a significantly greater international normalised ratio (INR) value than those patients with the CT+TT genotype (1.03 *vs*. 0.9; $p=0.004$) (Table III).

Variations in average levels of cytokines in SLE and control group in relation to in IL-1β, IL-10 and TNF-α genotypes

Afterwards, we examined the variations in average levels of cytokines in patients with SLE and healthy subjects in relation to IL-1β, IL-10, and TNF-α genotypes, which is shown in Table IV. The outcomes are presented as median (IQR: interquartile range).

We can see that CC, CT, TT genotypes in IL-1β -511 in SLE were significantly higher than in HCs with the same IL-1β -511 genotypes ($p<0.00001$; $p=0.003$ and $p=0.0005$, respectively). In the case of IL-1β +3953, CC and CT genotypes in SLE were significantly higher than in healthy subjects with the same IL-1β +3953 genotypes ($p<0.00001$ and $p=0.001$, respectively). In the case of IL-10 -592, only the CC genotype in SLE was significantly higher than in healthy controls with the same IL-10 -592 genotype ($p<0.00001$). In the case of IL-10 -1082, only the AA genotype in SLE was significantly lower than in healthy subjects with the same IL-10 -1082 genotype (p=0.0009). In the case of TNF-α -308, GG, GA, AA genotypes in SLE were significantly higher than in HCs with the same TNF-α -308 genotypes ($p<0.00001$; $p=0.026$ and $p=0.037$, respectively). In the case of TNF-α -238, only GG genotype in SLE was significantly higher than in healthy controls with the same TNF-α -238 genotype ($p<0.00001$). In the case of TNF-α +489, CC and CT genotypes in SLE were significantly higher than in controls with the same TNF-α +489 genotypes ($p<0.00001$ and $p=0.0008$, respectively).

Discussion

In this study, we investigated the influence of polymorphisms within IL-1β, IL-10, and TNF-α genes on SLE susceptibility and phenotype.

In the present study, we observed that CT in the codominant model, CT+TT in the dominant model and CT genotypes in the overdominant model as well as T allele in IL-1β-511 polymorphism have revealed significant associations with SLE risk. The remark that allele T is a risk allele was similarly made by Parks *et al*., who established that carriers of the allele T in IL-1β -511 have an increased risk of SLE. However, this observation was made in African Americans (16). Also, Wang *et al*. revealed in the meta-analysis the connection between IL-1β-511 SNP and SLE risk, however, the association exists only for TT *versus* CT+CC genotypes in Asians (17). On contrary, Mohammadoo-Khorasani *et al*. did not find an association between IL-1β -511 C/T polymorphism and SLE, but the carriage of allele T was associated with malar rash in SLE patients (18). Moreover, Song *et al*. in the meta-analysis did not find any link between this SNP and SLE risk (19). In IL-1β +3953 polymorphism, the higher risk of SLE is carried by CT in the codominant model, CT+TT in the dominant model and CT genotypes in the overdominant model as well as T allele. Results from other scientists are contradictory. Camargo *et al*. revealed that allele T in IL-1β +3953 polymorphism was protective for SLE. The explanation can be that under inflammatory conditions IL-1β +3953T allele suppresses the production of IL-1β (13). Nevertheless, in their meta-analysis, Song *et al*. did not find any connections between IL-1β -3953 C/T polymorphism and SLE (19), nor did Wang *et al*. (17) and Parks *et al*. (16). However, in some studies in other autoimmune disorders, like RA in Caucasians and Asians (20), RA in Turkish patients (21), multiple sclerosis (MS) in the Iranian population (22) and Hashimoto's thyroiditis in

Table IV. Variations in average levels of cytokines in patients with SLE and healthy subjects in relation to IL-1β, IL-10 and TNF-α genotypes.

IL-1β -511	SLE median (IQR)	Controls median (IQR)	*p*-value
CC	770.559±129.795 806.575 (184.643)	552.226±214.679 573.457 (287.614)	**<0.00001**
CT	708.414±154.588 723.543 (252.787)	543.745±234.883 557.211 (300.803)	**0.003**
TT	756.661±110.423 743.392 (190.24)	480.941±164.907 440.25 (304.65)	**0.0005**
IL-1β +3953	SLE median (IQR)	Controls median (IQR)	*p*-value
CC	757.755±126.656 790.143 (213.485)	533.896±223.917 556.833 (313.814)	**<0.00001**
CT	732.005±154.759 721.408 (260.592)	558.453±198.505 560.402 (229.768)	**0.001**
TT	701.917±32.149 701.917 (45.465)	630.244±97.175 658.056 (121.016)	0.389
IL-10 -592	SLE median (IQR)	Controls median (IQR)	*p*-value
CC	746.87±132.987 745.22 (198.657)	538.317±212.272 558.084 (302.797)	**<0.00001**
CA	770.825±129.797 786.705 (164.658)	770.984±257.097 770.984 (363.59)	0.999
AA	715.231±79.68 711.581 (53.381)	-	-
IL-10 -1082	SLE median (IQR)	Controls median (IQR)	*p*-value
AA	23±198.657 744.686 (722.436)	587.396±201.387 580.993 (207.713)	**0.0009**
AG	-	508.524±226.662 550.477 (356.08)	-
GG	1±0 462.878 (462.878)	456.138±205.127 468.51 (345.309)	-
TNF-α -308	SLE median (IQR)	Controls median (IQR)	*p*-value
GG	35.611 (33.571)	15.895 (15.494)	**<0.00001**
GA	36.691 (101.378)	16.73 (10.786)	**0.026**
AA	37.835 (64.344)	12.355 (4.786)	**0.037**
TNF-α -238	SLE median (IQR)	Controls median (IQR)	p-value
GG	35.923 (39.508)	15.895 (13.465)	**<0.00001**
GA	23.012 (14.085)	-	-
AA	-	-	-
TNF-α +489	SLE median (IQR)	Controls median (IQR)	*p*-value
CC	36.131 (44.268)	16.084 (12.386)	**<0.00001**
CT	36.659 (40.675)	12.928 (16.557)	**0.0008**
TT	28.954 (12.333)	-	-

IQR: interquartile range; $p<0.05$ was considered significant.

the Polish population (23), researchers showed the association between IL-1β -3953 C/T polymorphism and the disease susceptibility.

We observed that CA genotype and A allele of the IL-10 -592 C/A polymorphism may be SLE risk factor. Nonetheless, Liu *et al*. showed in the meta-analysis that IL-10 -592 C/A polymorphism is associated with decreased SLE risk in the case of IL-10 -592A and under CC+CA *vs*. AA genetic model in Asians (24). Mohammadi *et al*. revealed that AA genotype and A allele in the IL-10 -592 C/A polymorphism are connected with lower SLE risk in the Iranian population (25). Also, Yuan *et al*. found that IL-10 -592 C/A polymorphism is linked with reduced SLE risk (26). On the other hand, Guarnizo-Zuccardi *et al*. did not show the connection between this polymorphism and SLE susceptibility in Colombians (27). In IL-10 -1082 polymorphism, AG in the codominant model and AG+GG genotypes in the dominant model, as well as G allele, have a lower SLE risk, which suggests that they can be protective for SLE. Similar to us, Zhou *et al*. showed the association between allele G and SLE risk in the case of IL-10 1082 G/A polymorphism, however, in their study allele G was connected with the increased SLE risk (28). On the other hand, the increased IL-10 -1082G allele and GG genotype is connected with SLE susceptibility in the Iranian population (25). Nevertheless, Talaat *et al*. showed that AA and GG against GA genotypes in the IL-10 -1082 polymorphism are more common in SLE patients than in healthy controls in the Egyptian population (29). Also, Song *et al*. (30) and Yuan *et al*. (26) showed in the meta-analysis that in European populations IL-10 1082 G/A polymorphism is linked with a higher SLE risk. Liu et al. exposed in the meta-analysis that IL-10 -1082 G/A polymorphism among Caucasians and Asians is linked with SLE susceptibility (24). Similarly, Nath *et al*. showed that the G allele of the IL-10 -1082 G/A polymorphism was related to SLE in Asians (31).

Our results have also shown that TNF-α -308 GA genotype and A allele may be associated with a lower SLE risk, which suggests that they can be protective for SLE. However, Rood *et al*. (32) and Guarnizo-Zuccardi *et al*. in Colombian patients (27) showed that allele A in TNF-α -308 polymorphism is a risk factor for SLE. Corresponding-

ly, in Indian SLE patients TNF-α -308A was considered as a risk factor for disease susceptibility and was linked to enlarged serum levels of TNF-α (33). Lee *et al*. revealed that TNF-α-308 A/G polymorphism can confer susceptibility to SLE (34). However, Rudwaleit *et al*. (35), D'Alfonso *et al*. (36), Zúñiga *et al*. (37) and Santos *et al*. (38) did not notice that TNF-α -308 A/G polymorphism may confer susceptibility to SLE. Also, Lin *et al*. showed that the genotype and allele frequencies of TNF-α -308 A/G polymorphism did not differ significantly between SLE and controls. However, TNF-α -308A was meaningfully enlarged in patients with photosensitivity, serositis, oral ulcers, and discoid as well as a malar rash (8).

Discrepancies in the observed links between IL-1β, IL-10 and TNF-α genes polymorphisms and SLE susceptibility may be caused by different origin, causing, *i.e*. exposure to various environmental factors. Other factors may be dissimilar clinical manifestations of the groups, different sample size as well as methodology. Furthermore, the inclusion and exclusion criteria for SLE patients and healthy controls are also relevant.

We noticed some associations between particular genotypes and clinical manifestations. We investigated that C3 level is significantly higher in SLE patients with IL-1β +3953 CT+TT genotype. We also observed that patients with IL-10 -1082 AA genotype have significantly higher C4 levels. C3 and C4 levels are widely used as a disease activity marker. The activation of the complement system is the key factor in SLE pathogenesis. Autoantibodies, which form the immune complexes with the autoantigens activate the complement by the immune complexes. The products of such activation trigger tissue injury and disease (39). Furthermore, TNF-α -238 GA+AA genotype carriers have revealed significantly higher SDI value, and hence they can have a more advanced form of the disease. However, the increasing of SDI value can be also observed at the beginning of the SLE in the newly-diagnosed patients. Interestingly, Mahto *et al*. observed that TNF-α -238A allele and GA genotype were meaningfully linked with lupus nephritis (LN), a severe and common manifestation of systemic lupus erythematosus (40). What is more, we noticed that patients with TNF-α +489 CC genotype have significantly greater Pt and INR values.

Additionally, we noted that some genotypes were linked with the higher serum levels of the cytokines. Moreover, GG, GA, and AA genotypes in TNF-α -308 were associated with a higher concentration of the cytokines in SLE patients. GG genotype in TNF-α -238 and CC and CT genotypes in TNF-α +489 were connected with enlarged concentration of the cytokines in SLE. In another study, plasma TNF-α -308 and TNF-α -238 levels were higher in SLE patients than in healthy controls (40). We investigated that CC, CT and TT genotypes in IL-1β -511 and CC and CT genotypes in IL-1β +3953 were related with the greater concentration of the cytokines in SLE patients than in healthy controls. We also revealed that the CC genotype in IL-10 -592 was connected with the enlarged concentration of the cytokines in SLE patients in comparison to healthy controls. Contrary to the above-mentioned results, the AA genotype in IL-10 -1082 was linked to the lower concentration of the cytokine in SLE patients than in controls. Our results contradict the outcomes of Zhou *et al*. study showing that serum IL-10 was higher in SLE patients with GA and AA genotypes However, this study was made in the Egyptian population, different from ours, Polish population (28). On the other hand, our results confirm the study by Mohammadi *et al*., who showed that plasma levels of IL-10 were higher in carriers of CC genotype in IL-10 -592 and lower in carriers of AA genotype in IL-10 -1082 (25).

Our study has some limitations. First of all, we studied a narrow number of polymorphisms. Second, two polymorphisms, one in SLE (rs1800896) and one in healthy subjects (rs16944) were not consistent with HWE. The possible explanation of this inconsistency is too small sample size in both groups or the selection of groups with inaccurate randomisation. Insufficient sample size can also cause a lack of observations of some relationships. Further studies should have a larger sample size and improved the project of the study. Nonetheless, our samples were derived from a mono-ethnic population. Furthermore, they were carefully chosen owing to clinical phenotype.

In conclusion, our study indicated that IL-1β-511, IL-1β +3953, IL-10 -592, IL-10 -1082, and TNF-α -308 polymorphisms affect SLE risk. However, two SNPs-SLE risk connections from our study contrast with outcomes from other researchers. It seems reasonable to perform further studies from different populations with larger sample sizes to draw more precise conclusions about the influence of those genotypes on SLE susceptibility. Moreover, we showed that some of the genotypes within IL-1β +3953, IL-10 -1082, TNF-α -238, TNF-α +489 SNPs are associated with the disease phenotype. Additionally, we revealed the connection between particular genotypes and the concentrations of IL-1β, IL-10, and TNF-α.

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