IL-6 and *TGF-β* gene polymorphisms, their serum levels, as well as HLA profile, in patients with systemic lupus erythematosus

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Abstract

Objective

The aim of the study was to explore whether TGF-β and IL-6 gene polymorphisms may be associated with SLE and assess the frequency of HLA-DRB1 alleles in Polish systemic lupus erythematosus (SLE) patients.

Methods

216 SLE patients and 552 healthy individuals were examined for TGF-β rs1800469 and rs1800470 by TaqMan SNP genotyping assay and for and IL-6(rs2069827 and rs1800795 using the PCR– RFLP method.

Results

*An increased frequency of TT genotype and T allele of the TGF β -509 C/T was found in SLE patients (p=0.02). The TGF- β 869 C allele was more frequent in SLE patients. The genotype-phenotype analysis showed association between the TGF β -509 C/T and mean value of CRP, ESR, haemoglobin, APTT, Pt and INR (p=0.05, p=0.03, p<0.001, p=0.03, p=0.03 and p=0.05, respectively) as well as anti-SSA and anti-Sm presence (p=0.04 and p=0.03, respectively); the TGF- β 869 T/C and mean value of APTT and INR (p=0.01 and p=0.05, respectively); the IL-6 -174 G/C and SLICC (p=0.05), anti-SSA (p=0.05) and anti-SSB (p=0.05). A higher TGF-β and IL-6 serum level were found in SLE patients compared to controls (both p<0.0001). In SLE patients with the TGF-β -509 TT genotype have shown positive association with the TGF-β serum levels. Polish SLE patients have strong positive association with HLA-DRB1*52.1, and negative with the HLA-DRB1*07:01 allele. HLA-DRB1*52.1 was also associated with higher TGF-β serum levels in the Polish population.*

Conclusion

Our results suggested that the TGF β -509 C/T variant may be considered as a genetic marker for SLE in the Polish population.

Key words

cytokine, gene, polymorphisms, systemic lupus erythematosus, pathogenesis

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Introduction

The interaction between genetic and environmental factors may lead to the development of systemic lupus erythematosus (SLE), which is a severe, chronic autoimmune disease caused by perturbations of the immune system (1, 2). Although human leukocyte antigen (HLA) Class I and Class II has been involved in SLE susceptibility since 1970, the specified alleles responsible for SLE risk have not been fully understood (3). From all known HLA alleles the strongest and most consistent genetic factors for SLE are alleles in HLA-DRB1 locus, which is characterised by allelic heterogeneity (4-6). HLA-DR together with several types of cells as well as cytokines actively regulated immune responses, immune homeostasis and self-tolerance in the course of SLE. The cytokines may exert their anti-inflammatory or pro-inflammatory effects, depending on specific local microenvironments (7). The abnormalities of cytokines gene and protein expression profiles may reflect the imbalance between pro-inflammatory and regulatory cells (Th17/Treg), local tissue injury as well as they may serve as biomarkers of disease severity and activity (8, 9). The manipulation of these cytokines function and expression not only provides a new approach to the SLE pathogenesis but also may become a potential candidate targets of novel biologic agents.

The pleiotropic cytokines with a wide range of biological activities, transforming growth factor (TGF)-β and interleukin-6 (IL-6), can interact with each other and forming a complex network especially involved in the regulation of CD4+T cell responses (10). TGF-β in the presence of IL-6 induces differentiation of pathogenic IL-17 producing T helper 17 (Th17) subset, which can promote inflammation and enhance autoimmune conditions. In contrast, TGF-β in the absence of IL-6 converts naïve Th17 cells to Foxp3-positive regulatory T cells (Tregs), playing an essential role in the suppression of the immune response (11-14). Both these cytokines through the control of autoimmunity as well as immunopathology of SLE may have a large role in mediating local inflammation and tissue damage (9, 15). In patients with SLE, accentuated IL-6 levels correlated with the anti-DNA levels and disease activity (8, 9), while reduced levels of TGF-β1 is associated with disease activity, susceptibility, and organ damage (16). In SLE patients, IL-6, as well as TGF-β may be useful biomarkers to monitor disease susceptibility and activity (9).

Because SLE is a disease with a strong genetic component and the cytokine production was found to be under genetic control where single nucleotide polymorphisms (SNP) may alter their production we decided to explore whether polymorphisms located in the *TGF-β* (rs1800469 (-509 C/T) and rs1800470 (869 T/C; Pro10/Leu10; codon10) as well as *IL-6* (rs2069827 (-136 G/T), rs1800795 (-174 G/C)) genes may be associated with SLE in a predominantly Polish population using case-control studies. Since the association between SLE and HLA-DR region is known, we also provide the data showing an association between study TGF-β and IL-6 genotypes and HLA-DRB1 alleles in our cohort.

Materials and methods

Patients and controls

There were 768 Polish subjects recruited to the present study, including 216 patients with SLE and 552 unrelated healthy controls. SLE patients were recruited from the Connective Tissue Diseases Department of the National Institute of Geriatrics, Rheumatology and Rehabilitation in Warsaw, Poland. The diagnosis of all patients with SLE was based on the presence of the combination of at least four criteria of 1982 revised American College of Rheumatology (ACR) classification criteria for SLE. The control group consisted of healthy volunteers who showed no clinical or laboratory signs of any autoimmune disease. They were selected randomly from blood bank donors.

All subjects provided written informed consent for genetic studies and consented to sample collection that was approved by the Research Ethics Committee of the National Institute of Geriatrics, Rheumatology and Rehabilitation in Warsaw and by the Research Ethics Committee of the Poznan Uni-

versity of Medical Sciences. This study conducted in accordance with the ethical standards of our Institute and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards

DNA extraction

Whole blood samples obtained from the SLE patients and controls were collected in EDTA tubes. Total genomic DNA was isolated using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany).

Genotyping analysis. Genotyping of *TGF-β* (rs1800469 (-509 C/T) and rs1800470 (869 T/C; Leu10/Pro10; codon10) genetic variants was analysed using TaqMan SNP Genotyping Assays (C_8708473_10 and C_22272997_10, respectively; Applied Biosystems, Forester City, CA, USA). The reaction was performed in 10 ul volumes on StepOne real-time PCR system following the manufacturer's protocol with the following amplification protocol: denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 92°C for 15 s, and annealing and extension at 60°C for 1 min. Allelic discrimination was conducted in a QuantStudio 5 (Applied Biosystems, Forester City, CA, USA).

The polymerase chain reaction-restriction fragment-length polymorphism (PCR-RFLP) method was applied to genotype the *IL-6* rs2069827 (-136 G/T) and rs1800795 (-174 G/C) gene SNPs using the following primers: -174 G/C rs1800795 forward 5'-TGA CTT CAG CTT TAC TCT TTG T-3' and reverse 5'-CTG ATT GGA AAC CTT ATTAAG-3', to generated a 168 bp product; -136 G/T (rs2069827) forward 5′-CGGGTCCTGAAATGT-TAT-3' and reverse 5′-GTTGTCC-CTCCAGTCTCC-3′, to generated a 222 bp product. The genotyping was performed on the SensQuest Labcycler (SensoQuest GmbH, Göttingen, Germany) in a 50-µl reaction system which contains 200 ng of genomic DNA, 10 pmol of each primer, 0,25 mM each deoxyribonucleoside triphosphate (dNTP), 1 U HotStar Taq polymerase and ×1 PCR buffer (containing 1·5 µM magnesium chloride). 10µl of the amplification products, were digested with 1 µl of *TaqI* (Fermentas, Burlington, Canada) for the -136 G/T (rs2069827) at 65°C, and with *NlaIII* (Fermentas, Burlington, Canada) for the -174 G/C rs1800795 at 37°C and separated by size on agarose gel.

Table I. Clinical characteristics of patients with SLE.

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)
SLICC	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)
Creatinin	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	60	50 ± 54.36 (1 – 317)
AST	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-Scl7	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)
Sjögren'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: SLE disease activity index; BMI: body mass index; PLT: platelets; APTT: activated partial thromboplastin time; PT: prothrombin time; INR: international normalised ratio; ESR: erythrocyte sedimentation 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.

Genotyping of HLA-DRB1

Typing of HLA-DRB1 alleles was performed as we described previously (17). The primer sequences and PCR product length are shown in Supplementary Table S1 in the Supplementary file.

Cytokine measurements

Randomly 101 samples of SLE patients and 124 controls were chosen to test the serum concentration of TGF-β and IL-6. They were quantitatively determined using enzyme-linked immunosorbent assay kits (IL-6 and TGF-β from DRG Instruments GmbH, Germany) according to the manufacturer's instructions.

Statistical analysis

The results are presented as a percentage for categorical variables, mean with 1 standard deviation for normally distributed continuous variables, or median (range) for non-normally distributed continuous variables as tested by the Shapiro-Wilk test. A *p*-value of

Table II. Distribution of genotypes and allele frequencies of TGF-β and IL-6 SNPs among patients with SLE and healthy subjects

SNP/genetic model	Genotype/Alleles	SLE n (%)	Controls n (%)	OR (95% CI)	*p*-value
TGF β rs1800469 (-509 C/T)					
	Genotype				
Codominant	TT	24 (16.44)	55 (9.96)	1.983 (1.134 – 3.467)	**0.019**
	CT	65 (44.52)	238 (43.12)	1.241 (0.835 – 1.845)	0.313
	CC	57 (39.04)	259 (46.92)	Reference	
Recessive	TT	24 (16.44)	55 (9.96)	1.778 (1.058 – 2.986)	**0.039**
	CT+CC	122 (83.56)	497 (90.04)	Reference	
Dominant	TT+CT	89 (60.96)	293 (53.08)	1.380 (0.951 – 2.003)	**0.093**
	CC	57 (39.04)	259 (46.92)	Reference	
Overdominant	TT+CC	81 (55.48)	314 (56.88)	Reference	
	CT	65 (44.52)	238 (43.12)	1.059 (0.733 – 1.529)	0.779
	Alleles				
	T	113 (38.7)	348 (31.52)	1.374 (1.050 – 1.792)	**0.025**
	C	179 (61.3)	756 (68.48)	Reference	
TFG β rs1800470 (869 T/C)					
	Genotype				
Codominant	TT	40 (27.03)	202 (36.66)	Reference	
	TC	82 (55.41)	255 (46.28)	1.624 (1.066 – 2.473)	**0.023**
	CC	26 (17.57)	94 (17.06)	1.397 (0.716 – 2.424)	0.249
Dominant	TT	40 (27.03)	202 (36.66)	Reference	
	TC+CC	108 (72.97)	349 (63.34)	1.563 (1.045 – 2.336)	**0.032**
Recessive	TT+TC	122 (82.43)	457 (82.94)	Reference	
	CC	26 (17.57)	94 (17.06)	1.036 (0.642 – 1.671)	0.902
Overdominant	TT+CC	66 (44.59)	296 (53.72)	Reference	
	TC	82 (55.41)	255 (46.28)	1.442 (1.00 – 2.077)	**0.052**
	Alleles				
	T	162 (54.73)	659 (59.8)	Reference	
	C	134 (45.27)	443 (40.2)	1.230 (0.950 – 1.594)	0.126
IL-6 rs1800795 (-174G/C)					
	Genotype				
Codominant	GG	95 (43.98)	166 (41.09)	Reference	
	GC	104 (48.15)	209 (51.73)	0.874 (0.619 – 1.224)	0.481
	CC	17 (7.87)	29 (7.18)	1.024 (0.535 – 1.961)	1.000
Dominant	GG	95 (43.98)	166 (41.09)	Reference	
	GC+CC	121 (56.02)	238 (58.91)	0.888 (0.636 – 1.241)	0.496
Recessive	GG+GC	199 (92.13)	375 (92.82)	Reference	
	CC	17 (7.87)	29 (7.18)	1.105 (0.593 – 2.059)	0.750
Overdominant	CC+GG	112 (51.85)	195 (48.27)	Reference	
	GC	104 (48.15)	209 (51.73)	0.866 (0.623 – 1.206)	0.401
	Alleles				
	G	294 (68.06)	541 (66.96)	Reference	
	C	138 (31.94)	267 (33.04)	0.951 (0.741 – 1.221)	0.704
IL-6 rs2069827 (-136G/T)					
	Genotype				
Codominant	GG	87 (74.36)	371 (77.13)	Reference	
	GT	28 (23.93)	101 (21)	1.182 (0.732 – 1.909)	0.530
	TT	2 (1.71)	9 (1.87)	0.948 (0.201 – 4.464)	1.000
Dominant	GG	87 (74.36)	371 (77.13)	Reference	
	GT+TT	30 (25.64)	110 (22.87)	1.163 (0.730 – 1.854)	0.544
Recessive	GG+GT	115 (98.29)	472 (98.13)	Reference	
	TT	2 (1.71)	9 (1.87)	0.912 (0.194 – 4.279)	1.000
Overdominant	GG+TT	89 (76.07)	380 (79)	Reference	
	GT	28 (23.93)	101 (21)	1.184 (0.734 – 1.909)	0.531
	Alleles				
	G	202 (86.32)	843 (87.63)	Reference	
	T	32 (13.68)	119 (12.37)	1.122 (0.734 – 1.707)	0.584

less than 0.05 was considered significant. All polymorphisms were tested for deviation from Hardy-Weinberg equilibrium using the Hardy Weinberg Simulator software (available at Institute of Human Genetics, Helmholtz Zentrum Munchen, Germany). The Fisher exact probability test or chi-square test were used to evaluate differences in genotype and allele prevalence between the examined groups. The Fisher exact test was used for power analysis. The association between SNPs and clinical/serological parameters were compared by the Mann-Whitney test, *t*-test, or

Cochran-Cox test for continuous variables. Statistical analysis was performed using Graph-Pad InStat 3.10, 32 bit for Windows (GraphPad Software, Inc., San Diego, California, United States), CytelStudio version 10.0 (CytelStudio Software Corporation, Cambridge, Massachusetts, United States), and Statistica v. 10, 2011 (Stat Soft, Inc., Tulsa, Oklahoma, United States). Linkage disequilibrium (LD), coefficient (D′ and r2) for haplotypes and their frequencies were performed using the genetic statistical software SHEsis (http://shesisplus.bio-x.cn/SHEsis.html) (18, 19).

Results

Patients' characteristics

The patients' clinical and laboratory characteristics were collected at the time blood sampling and they are summarised in Table I. The SLE patients were in the active stage of disease with mean±SD SLE disease activity index (SLEDAI) >6±5.82 and with the mean disease duration 10 years. Our SLE patients present several different blood autoantibody. The most frequent autoantibody was anti-dsDNA, present in 74% of our patients, while the less frequent autoantibody was anti-CEN, present in 3% of our patients with SLE. The average serum C3 concentration was 76.29±29.07 (33–162)g/L, and the average C4 was 14.05±13.49 (3.09–102). Among SLE patients the most frequent clinical manifestation was arthritis, which was observed in 63% of patients. Furthermore, the less frequent clinical manifestation in our patients was lung fibrosis, which was present only in 3 SLE patients.

TGF-β and IL-6 polymorphisms association with SLE susceptibility

Genotype frequencies for the four examined genetic variants were in Hardy-Weinberg equilibrium (HWE) with exception of *IL-6* -174G/C for the control group (p=0.001). The MAF of the *TFG β* 869 T/C as well as -509 C/T variants in our cohorts, SLE patients as well as controls, were similar to those in the Utah residents of northern and western European ancestry (HapMap database; Suppl. Table S2). Furthermore, the *IL-6* minor -174 C and -136 T alleles frequency was higher in Polish subjects (32–33% and 12–14%, respectively) than in other European populations (14% and 4%, respectively).

Table III. The disease activity and laboratory parameters in relations to TGF β -509 C/T (rs1800469). dominant model.

Parameter	CC	CT+TT	p^*
	Mean±SD (median)		
Age [years]	36 (28 - 76)	38 (21 - 72)	0.6
Disease duration [years]	3.5 (0 - 43)	8 (1 - 34)	0.2
SELENA_SLEDAI	4.5 (0 - 16)	4 (0 - 18)	0.8
SLICC	1 (0 - 5)	1 (0 - 4)	0.8
APTT	29.65 (24.1 - 78.4)	33 (21.3 - 127)	0.1
ESR [mm/h]	43 (7 - 85)	38 (5 - 114)	0.9
CRP [mg/L]	7.5 (1 - 202)	13 (3 - 395)	0.05
Haemoglobin [g/dL]	12.9 (8.3 - 16)	12.2 (4.9 - 15.4)	0.6
Pt	10.3 (8.6 - 23.3)	11.35 (9.6 - 80)	0.03
INR	0.9855 (0.7 - 2.2)	1.05 (0.89 - 5.4)	0.05
Urea	34.5 (21 - 296)	35.5 (16.7 - 78)	0.6
PLT [x10^3/mm^3]	223 (50 - 598)	209 (38 - 557)	0.7
Creatinin	0.8 (0.5 - 1.1)	0.8 (0.5 - 1.5)	0.2
C3	76.09 ± 23.36	78.79 ± 32.60	0.7
C4	13.6 (3.16 - 31)	12.1 (3.09 - 102)	0.2
ALT	29.5 (1 - 92)	44 (15 - 317)	0.1
AST	31 (20 - 62)	28 (14 - 850)	0.7
	N n (%)	N n (%)	p^{**}
anti-dsDNA	26 14 (53.84)	33 22 (66.66)	0.4
anti-SSA	24 15 (62.5)	32 10 (31.25)	0.04
anti-SSB	24 2 (8.33)	31 3 (9.67)	0.7
anti-SM	25 8 (32)	31 2 (6.45)	0.03
anti-Rib	24 2 (8.33)	30 2 (6.66)	0.7
anti-Jo	24 0 (0)	30 0 (0)	-
anti-Scl7	25 1 (4)	30 0 (0)	0.4
anti-CEN	25 2 (0)	30 2 (0)	0.2
anti-U1RNP	23 7 (30.43)	30 5 (16.66)	0.3
anti-IgM	24 2 (8.33)	32 4 (12.5)	0.9
anti-IgG	25 6 (24)	32 10 (31.25)	0.7
LAC	25 5 (20)	31 11 (35.48)	0.3
Sjögren's syndrome	26 3 (11.53)	34 7 (20.58)	0.5
Facial erythema	26 17 (65.38)	34 15 (44.11)	0.1
Arthritis	26 16 (61.53)	34 18 (52.94)	0.6
Lung fibrosis	26 1 (3.84)	34 2 (5.88)	0.8
Leucopenia	26 11 (42.3)	34 11 (32.35)	0.6
APS	21 4 (19.04)	31 7 (22.58)	0.9

N: number of patients with clinical information; n: number of patients with positive clinical manifestation. p^* Mann-Whitney U-test; p^{**} χ^2 test; p<0.05 was considered significant.

The distributions of genotype and allele frequencies were shown in Table II. The analysis of the *TGF-β* 869 T/C (rs1800470) genetic variant revealed significant differences in the case–control distribution in two examined models. Under the codominant model, the frequency of the TC genotype was significantly higher in SLE patients as compared to the healthy subjects (55% *vs*. 42%; OR**:** 1.624; 95% CI: 1.066–2.473; p=0.03). Similarly, under the dominant model (TC+CC *vs*. TT) the association was also significant (OR: 1.563; 95% CI: 1.045–2.336; p=0.03). Under the overdominant (TT+CC *vs*. TC) models, we observed the tendency to more frequent present the TC genotype than TT or CC genotypes in patients with SLE (p=0.06). Under the recessive (TT+TC *vs*. CC) model genotype distributions were not statistically significant (OR: 0.806; 95% CI: 0.473–1.403). Moreover, we observed that *TGF-β* 869 C allele was more frequent in SLE patients compared to controls, however, this association was not significant (45% *vs*. 40%, OR: 1.230; 95% CI: 0.950–1.594).

As regards the TGF-β -509 C/T (rs1800469) gene polymorphism SLE patients showed genotype and allele

distributions significantly different as compared to control subjects (Table II). Under the codominant model, the frequency of the TT genotype was significantly higher in SLE patients as compared to the controls (p=0.019). Under the recessive model (CT+TT *vs*. CC) the association was also significant (OR: 1.778; 95% CI: 1.058–2.986; p=0.039). Similarly, under the dominant model we observed a tendency to the more frequent presence of TT and CT genotypes in patients with SLE than in healthy subjects (OR: 1.380; 95% CI: 0.951–2.003; p=0.093). Under the overdominant model (TT+CC *vs*. CT), genotype distributions were not statistically significant (OR: 1.059; 95% CI: 0.733–1.529).

Our analysis revealed that *TGF-β* gene -509 T allele were associated with significantly increased risk of SLE in the Polish population (p=0.025) compared with the wild-type -509 C and allele.

No significant differences were observed in genotype and allele frequencies of the *IL-6* -174 G/C (rs1800795) and -136 G/T (rs2069827) SNPs between patients with SLE and healthy subjects.

TGF-β and IL-6 genetic variants and disease activity in SLE patients

We analysed the genotype frequencies of associated genetic variants, in different genetic models (Tables III-IV and Suppl. Tables S3-S5). Our genotype-phenotype analysis showed a significant association only between TGF-β -509 C/T (rs1800469) and SLE phenotype under both dominant and recessive models (Tables III-IV). We observed that under dominant model the disease activity parameters such as the mean value of CRP, Pt, INR was higher in SLE patients with polymorphic TGF-β -509 T allele in comparison to patients with TGF-β -509 C allele (Table III, p=0.05, p=0.03, and p=0.05, respectively). In contrast, in SLE patients with TGF-β -509 C allele the autoantibody, anti-SSA and anti-Sm, presence was more frequent than in SLE patients with TGF-β -509 T allele (Table III, p=0.04 and p=0.03, respectively). Furthermore, under a recessive model, the parameters such as APTT, mean value of ESR, mean value of haemoglobin as well as the presence of anti-SSB autoantibody were higher in patients with TGF-β -509 TT genotype (Table IV, p=0.02, p=0.03, p<0.001 and p=0.009, respectively). Moreover, under a recessive model, we also observed a tendency to the higher mean value of Pt, INR and AST in SLE patients with TGF-β -509 TT genotype (Table IV, p=0.09, p=0.08, and p=0.06). The SLE patients with polymorphic TGF β -509 T allele may have a more active disease than SLE patients with wild-type TGF-β -509 C allele.

The influence of *TGF-β* 869 T/C (rs1800470) variant on clinical symptoms of SLE showed significant differences in the mean value of coagulation parameters such as APTT and INR under a recessive model (Suppl. Table S3). Both mean value of APTT and INR were significantly higher in SLE patients with *TGF-β* 869 CC genotype in comparison to SLE patients with combined genotype *TGF-β* 869 CT+TT (p=0.01 and p=0.05, respectively). Moreover, in this model, we also observed a tendency to the higher mean value of CRP, creatinine, and AST in SLE patients with *TGF-β* 869 CC genotype (p=0.07, p=0.09 and p=0.09, respectively).

The analysis of *IL-6* -174 G/C (rs1800795) and -136 G/T (rs2069827) gene polymorphisms showed some significant differences under a recessive

Table IV. The disease activity and laboratory parameters in relations to TGF β -509 C/T (rs1800469). recessive model.

Parameter	TT	TC+CC	p^*
	Mean±SD (median)		
Age [years]	33 (25-49)	39 (21-76)	0.1
Disease duration [years]	7 (1-25)	7 (0-43)	0.9
SELENA_SLEDAI	4 (0-14)	4 (0-18)	0.8
SLICC	1 (0-4)	1 (0-5)	0.4
APTT	42.55 (29.9-48)	31.7 (21.3-127)	0.02
ESR [mm/h]	78 (18-114)	41 (5-104)	0.03
CRP [mg/L]	32 (4-262)	10 (1-395)	0.1
Haemoglobin [g/dL]	9.26 ± 2.84	12.31 ± 1.90	<0.0001
Pt	12.1 (10.3-62)	10.7 (8.6-80)	0.09
INR	1.155 (0.9-5.4)	1 (0.7-3.3)	0.08
Urea	39.3 (16.7-78)	34 (18-296)	0.3
PLT [x10^3/mm^3]	209 (111-405)	216 (38-598)	0.6
Creatinin	0.8 (0.7-1.3)	0.8 (0.5-1.5)	0.2
C3	78.3 ± 43.17	77.53 ± 27.03	0.9
C4	12 (3.9-15.6)	13.25 (3.09-102)	0.5
ALT	55 (17-317)	36 (1-298)	0.1
AST	57 (20-850)	28 (14-167)	0.06
	N n (%)	N n (%)	p^{**}
anti-dsDNA	7 5 (71.43)	52 31 (59.62)	0.8
anti-SSA	7 2 (28.57)	49 23 (46.94)	0.6
anti-SSB	7 3 (42.86)	48 2 (4.17)	0.009
anti-SM	7 2 (28.57)	49 8 (16.33)	0.7
anti-Rib	7 0 (0)	47 4 (8.51)	1.0
anti-Jo	7 0 (0)	47 0 (0)	-
anti-Scl7	7 0 (0)	48 1 (2.08)	1.0
anti-CEN	7 0 (0)	48 2 (4.17)	1.0
anti-U1RNP	7 2 (28.57)	46 10 (21.74)	0.9
anti-IgM	7 1 (14.29)	49 5 (10.2)	0.7
anti-IgG	7 3 (42.86)	50 13 (26)	0.6
LAC	7 2 (28.57)	49 14 (28.57)	0.6
Sjögren's syndrome	7 1 (14.29)	53 9 (1698)	0.7
Facial erythema	7 3 (42.86)	53 29 (54.72)	0.8
Arthritis	7 6 (85.71)	53 28 (52.83)	0.2
Lung fibrosis	7 1 (14.29)	53 2 (3.77)	0.7
Leucopenia	7 3 (42.86)	53 19 (35.85)	0.9
APS	7 2 (28.57)	45 9 (20)	0.9

N: number of patients with clinical information; n: number of patients with positive clinical manifestation. p^* Mann-Whitney U-test; p^{**} χ^2 test; p<0.05 was considered significant.

Table V. HLA-DRB1 genotype distributions in SLE patients and healthy controls (HC).

Genotype	HC n(f%) n=398	SLE n(f%) n=192	*p*-value
01:01	24 (6.03)	5 (2.6)	0.1
04:01	4 (1.01)	0 (0)	0.3
04:04	14 (3.52)	4 (2.08)	0.4
04:07	10 (2.51)	6 (3.13)	0.8
04:09	1 (0.25)	1 (0.52)	0.5
04:15	22 (5.53)	5 (2.6)	0.1
07:01	13 (3.27)	0 (0)	0.007
07:07	40 (10.05)	4 (2.08)	**0.0003**
07:09	2 (0.5)	1 (0.52)	1
07:15	26 (6.53)	10 (5.21)	0.6
09:01	2 (0.5)	0 (0)	1
09:09	1 (0.25)	2 (1.04)	0.2
09:10	0 (0)	1 (0.52)	0.3
09:15	0 (0)	3 (1.56)	0.03
15:10	0 (0)	1 (0.52)	0.3
15:01	17 (4.27)	1 (0.52)	0.01
15:15	49 (12.31)	20 (10.42)	0.5
52.1:01	19 (4.77)	9 (4.69)	1
52.1:04	14 (3.52)	19 (9.9)	**0.003**
52.1:07	37 (9.3)	9 (4.69)	0.05
52.1:09	1 (0.25)	4 (2.08)	0.04
52.1:10	0 (0)	3 (1.56)	0.03
52.1:15	53 (13.32)	31 (16.15)	0.4
52.1:52.1	49 (12.31)	53 (27.6)	**<0.0001**

Bonferroni-corrected $p<0.0028$ was considered statistically significant.

Table VI. Cytokines serum levels in SLE patients and controls.

Parameter	SLE group		Control group		*p*-value
	N	median (IQR)	N	median (IQR)	
TGF-β [pg/ml]	101	724.649 (210.508)	93	562.720 (300.803)	<0.0001
IL-6 [pg/ml]	92	46.058 (47.959)	124	22.962 (21.816)	<0.0001

model and under a dominant model, respectively (Suppl. Tables S4-S5). Our analysis demonstrated that *IL-6* -174 C allele was specifically associated with SLICC (p=0.05), *IL-6* -174 GG genotype with the anti-SSA (p=0.05) and anti-SSB (p=0.05) autoantibody presence under a recessive model. We found an association between the *IL-6* -136 GG genotype and higher age of SLE patients (p=0.05) as well as the lower mean value of ALT in SLE patients (p=0.04) under a dominant model (Suppl. Table S5).

Distribution of HLA-DRB1 alleles in SLE patients and healthy subjects

HLA-DRB1 genotyping was performed in 216 SLE patients and 552 healthy subjects. First, we explored the distribution of HLA-DRB1 genotypes in all study group and results are presented in Suppl. Table S6. The most frequent HLD-DRB1 genotype in the Polish population is HLA-DRB1*52.1:52.1 genotype, which was observed in 17% of subjects. HLA-DRB1*52.1:15 and HLA-DRB1*15:15 genotypes were observed in 14% and 11% of study subjects, respectively. In contrast, HLA-DRB1*52.1:09, *04:01, *52.1:10, *09:01, *09:09, *09:15, *07:09, *04:09, *10:15, *09:10 geno-

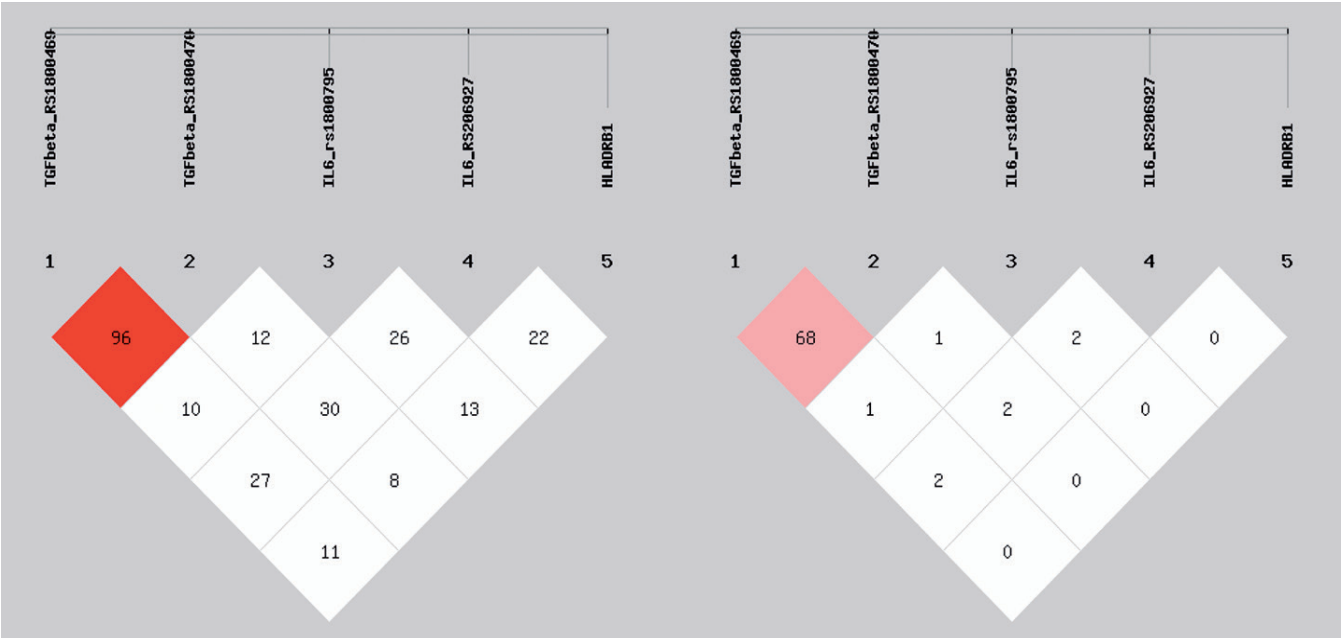

Fig. 1. Linkage disequilibrium (LD) between TGF-β rs1800469, TFG-β rs1800470, IL-6 rs2069827, IL-6 rs1800795 and HLA-DRB1. The plot illustrates LD based on D′ (left) and R-squared (r2; right) scores.

types was found in less than 1% of the study subjects.

In the next step, we investigated the association between HLA-DRB1 genotypes and the risk of developing SLE in Polish population (Table V). The distribution of two HLA-DRB1 genotypes showed significant differences between patients with SLE and healthy controls. The most significant allele associated with SLE in our population was HLD-DRB1*52.1, which was presented in 27% of SLE patients and 12% of controls (p<0.0001). While HLA-DRB1*07 allele was identified as protecting against SLE in the Polish population. This allele was observed in 10% in controls and in 2% in SLE patients (p=0.0003). We have also observed that HLA-DRB1 52.1:04 genotype showed a tendency to frequent occurrence in patients with SLE than in controls (9.9% *vs*. 3.5%, p=0.003). Additionally, the carrier frequency of the HLA-DRB1*01 was higher in healthy controls as compared to SLE patients (6% *vs*. 2.5%), however, this difference was not statistically significant.

HLA-DRB1 genotypes and clinical phenotype of SLE

We also tested whether examined HLA-DRB1 genotypes were associ-

Table VII. Correlation of TGF-β serum level in serum with the various clinical characteristics in SLE patients.

Parameter	Serum level			Serum level			*p*-value
	parameter group I	N	median (IQR)	parameter group II	N	median (IQR)	
Age	age ≥ 56	*9*	705.806 (171.028)	age <56	*44*	790.834 (211.908)	0.3
sex	women	*50*	755.595 (223.591)	men	*3*	806.575 (219.111)	0.7
SELENA_SLEDAI	≥6	*23*	766.835 (211.730)	<6	*30*	767.249 (210.163)	0.6
disease duration	≥10	24	799.05 (180.953)	<10	29	737.01 (196.985)	0.4
ESR	≥30	28	768.132 (175.646)	<30	21	806.575 (219.111)	0.8
SLICC	≥3	9	811.479 (158.742)	<3	44	755.595 (213.088)	0.3
CRP	≥13	19	769.429 (164.658)	<13	29	791.525 (244.617)	0.6
anti-dsDNA	+	*31*	742.423 ± 153.952	-	*21*	742.601 ± 137.429	0.8
anti-SSA	+	22	731.147 (296.573)	-	27	744.355 (153.358)	0.5
anti-SSB	+	5	790.143 (189.43)	-	43	744.355 (224.884)	0.9
anti-SM	+	10	813.004 (172.118)	-	39	717.355 (224.884)	0.2
anti-Rib	+	3	811.479 (396.777)	-	44	755.595 (213.088)	0.5
anti-Jo	+	1	619.21 (0)	-	46	768.132 (210.163)	-
anti-Scl7	+	*0*	0	-	48	755.595 (213.088)	-
anti-CEN	+	2	674.185 (56.786)	-	46	768.132 (224.884)	-
anti-U1RNP	+	12	798.359 (295.673)	-	34	755.595 (189.43)	0.9
anti-IgM	+	6	758.643 (285.95)	-	43	766.835 (212.085)	0.8
anti-IgG	+	13	806.575 (188.467)	-	37	744.355 (201.291)	0.7

p: Mann Whitney; p<0.05 was considered significant.

Table VIII. Correlation of IL-6 serum level with the clinical parameters of SLE patients.

Parameter	Serum level			Serum level			*p*-value
	parameter group I	N	median (IQR)	parameter group II	N	median (IQR)	
Age	age ≥56	8	40.33 (43.195)	age <56	47	48.02 (38.889)	0.2
sex	women	51	45.219 (45.321)	men	4	46.698 (25.201)	0.5
SELENA_SLEDAI	≥6	25	42.184 (38.117)	<6	30	46.058 (43.22)	0.8
disease duration	≥10	27	57.272 (47.884)	<10	28	38.288 (35.78)	0.2
ESR	≥30	28	45.396 (60.931)	<30	23	45.219 (34.86)	0.9
SLICC	≥3	9	36.766 (44.137)	<3	46	47.459 (38.251)	0.4
CRP	≥13	20	40.145 (74.184)	<13	30	45.396 (36.417)	0.4
anti-dsDNA	+	32	38.374 (43.823)	-	22	53.078 (37.468)	0.7
anti-SSA	+	24	47.843 (36.809)	-	28	44.556 (54.647)	0.7
anti-SSB	+	5	32.047 (12.464)	-	46	47.459 (47.079)	0.2
anti-SM	+	10	61.854 (33.104)	-	41	39.536 (38.086)	0.04
anti-Rib	+	4	33.347 (44.689)	-	46	46.058 (38.889)	0.7
anti-Jo	+	*0*		-	50	44.556 (38.889)	-
anti-Scl7	+	*0*		-	51	45.219 (38.889)	-
anti-CEN	+	2	31.788 (51.187)	-	49	45.219 (38.251)	0.3
anti-U1RNP	+	11	48.884 (40.217)	-	38	44.556 (39.137)	0.2
anti-IgM	+	5	35.071 (64.305)	-	48	46.058 (38.184)	0.7
anti-IgG	+	15	67.099 (89.084)	-	39	42.184 (32.995)	0.3

p: Mann Whitney; p<0.05 was considered significant.

ated with the clinical phenotype of SLE in our patients. A significant positive association was found for HLA-DRB1*52.1:52.1 genotype and disease duration; the mean disease duration was significantly higher in SLE patients with HLA-DRB1*52.1:52.1 genotype (15.1 years) than in SLE patients without this genotype (8.5 years) (p=0.03, date not shown). We also observed that HLA-DRB1*52.1 allele have shown positive association with production of anti-Scl70 antibodies in our SLE patients; the *52.1 allele was more frequent observed in anti-Scl70 positive SLE patients than anti-scl70 negative SLE patients (75% *vs*. 19%, OR=11.84, CI=673.37–0.85, p=0.03). No significant correlation was found between other HLA-DRB1 genotypes and other SLE clinical parameters.

Linkage disequilibrium (LD) between TGF-β and IL-6 polymorphisms and HLA-DRB1 alleles

To estimate the extent of LD, both D' and r2 score between TGF-β and IL-6 alleles and HLA-DRB1 alleles were calculated. D' can be very important for population genetics, but for substituting or tagging one SNP for another r2 is the important measure. The analysis results revealed very high LD between TGF-β rs1800469 and TGF-β rs1800470 (D'=0.96 and r^2=0.68), but indistinct LD between TGF-β SNPs and IL-6 SNPs as well as between TGF-β SNPs and HLA-DRB1alleles, and IL-6 SNPs and HLA-DRB1 alleles (Fig. 1).

45 haplotypes with frequency >0.03%, were observed in our study groups, whereas only eight haplotypes have shown statistical differences between SLE patients and controls (Suppl. Table S7). The most frequent haplotype identified in SLE patients was G A G G 52.1:52.1, which was estimated with frequency 24% in SLE patients and 6% in controls, and it have shown association with risk of SLE in the Polish population (p=0.0007; OR= 4.376, CI=1.780–10.754). In contrast, the most frequent haplotype identified in healthy subjects was G A G G 15:15, which was estimated with frequency 3% in SLE patients and 13% in controls (p=0.02; OR= 0.179, CI=0.036–0.878).

Table IX. Variation in TGF-β and IL-6 expression levels in SLE patients and control group in relation to gene polymorphisms.

SNPs	SLE median (IQR)	Controls median (IQR)	*p*-value
	TGF-β rs1800469		
AA	790.143 (103.943)	402.725 (334.68)	0.002
AG	740.683 (223.591)	524.553 (263.548)	<0.0001
GG	733.828 (221.525)	596.213 (226.295)	0.002
	TGF-β rs1800470		
AA	705.806 (259.215)	586.13 (226.295)	0.01
AG	766.835 (218.39)	548.323 (265.106)	<0.0001
GG	796.043 (93.171)	488.115 (334.68)	0.001
	IL-6 -174 G/C		
GG	42.975 (38.696)	21.481 (21.619)	<0.0001
GC	45.219 (51.666)	23.919 (23.021)	0.001
CC	30.91 (120.038)	17.656 (13.36)	0.5
	IL-6 -136 G/T		
GG	52.621 (33.923)	23.096 (19.794)	<0.0001
GT	48.02 (27.154)	18.708 (35.983)	0.3
TT	41.962 (61.957)	4.836 (0)	-

Table X. TGF-β serum levels in study subjects with different HLA-DRB1 genotypes.

HLA-DRB1 genotype	carries of HLA-DRB1 genotype		non-carries of HLA-DRB1 genotype		*p*-value
	N	median (min-max)	N	median (min-max)	
*07:07	44	468.33 (241.45 – 596.21)	546	649.49 (56.2 – 1026.1)	0.01
*52.1:52.1	102	746.34 (439.46 – 949.04)	488	624.22 (56.2 – 1026.1)	0.01
*04:04	18	357.1 (163.69 – 550.66)	572	644.61 (56.2 – 1026.1)	0.08

Correlation between TGF-β and IL-6 polymorphisms and HLA-DRB1 genotypes

The possible association between study TGF-β and IL-6 polymorphisms and HLA-DRB1 genotypes was evaluated, but this analysis did not reveal an independent effect of TGF-β and IL-6 and HLA–DRB1 on genetic predisposition either to the disease (date not shown).

Correlation of the TGF-β and IL-6 protein level with SLE risk and disease activity

In the next step, we determined both cytokines protein levels in serum, which was assessed in 101 SLE patients and 93 healthy subjects for TGF-β and 92 SLE patients and 124 controls for IL-6 recruited from the genetic study cohort. As shown in Table VI a significantly higher TGF-β as well as IL-6 protein levels were found in SLE patients (725 pg/ml and 46 pg/ml, respectively) compared with that in healthy subjects (563 pg/ml and 23 pg/ml, respectively; both p<0.0001). We also carried out a comparative analysis between TGF-β and IL-6 protein levels in serum and clinical phenotype of SLE (Tables VII-VIII). We divided our SLE patients into two groups: I group included the patients with higher disease activity and with autoantibody presence; while II group included the patients with the lowest disease activity and without autoantibody. Association analysis did not show any significant correlation between TGF-β levels and clinical phenotype of SLE in our patients (Table VII).

In the case of IL-6, we found significant association only between IL-6 protein levels and anti-Sm autoantibody presence. The IL-6 serum level was higher in SLE patients with anti-Sm autoantibody (Table VIII, p=0.04). No significant correlation was found between the IL-6 serum levels and SLE patients

demographics including age, sex, and disease duration. Also, no significant association was observed with disease activity parameters such as SELENA_SLEDAI or inflammatory markers as well as autoantibody presence.

Association analysis of TGF-β and IL-6 polymorphisms with their serum levels

Finally, we examined whether the presence of the genetic variants in the *TGF-β*, as well as IL-6 gene, may have an impact on their serum levels in SLE patients and healthy subjects. First, we evaluated a comparative analysis between SLE patients controls in relation to *TGF-β* and *IL-6* polymorphisms (Table IX). We observed that there was a significant interaction between both TGF-β and IL-6 serum levels and examined *TGF-β* and *IL-6* genetic variants. Increased serum levels of TGF-β was observed in SLE patients with all analysis *TGF-β* genotypes compared to healthy individuals. Furthermore, in case of IL-6 we observed that the IL-6 serum levels in SLE patients with -174 GG and -174 GC genotypes as well as in SLE patients with -136 GG genotype were significantly higher than controls with the same genotypes (Table IX; $p<0.0001$, $p=0.001$ and $p<0.0001$, respectively). In carriers of the other *IL-6* genotypes, serum IL-6 levels were not significantly different from those observed in the serum of controls with the same genotypes.

Next, we determined the relationship between TGF-β and IL-6 expression in serum in SLE patients according to *TGF-β and IL-6* gene polymorphisms, respectively. In this case, although, we find some differences among SLE patients in relation to *TGF-β* -509 C/T, *IL-6* -174 G/C and -136 G/T variants, this association was not significant. We observed that in SLE patients with the TGF-β -509 TT genotype the TGF-β serum levels were higher than in SLE patients with TGF-β -509 CC and TGF-β -509 CT genotypes (data not shown). SLE patients with the *IL-6* -174 CC genotypes had the lowest IL-6 serum levels comparing with SLE patients with -174 GG and -174 GC genotypes (data not shown). Furthermore, SLE patients with -136 GT genotype had the highest and SLE patients with -136 TT genotype had the lowest IL-6 serum levels (data not shown). In the case of the TGF-β 869 T/C polymorphisms, we do not observe differences in TGF-β serum levels between genotypes.

Association of HLA-DRB1 genotypes with the TGF-β and IL-6 serum levels in SLE patients and healthy subjects

Finally, a possible association between TGF-β and IL-6 serum levels and HLA-DRB1 genotypes was investigated. In the first stage, we analysed the correlation of the TGF-β and IL-6 serum levels with the HLA-DRB1 genotypes for the all study groups (SLE patients together with healthy subjects). We observed that HLA-DRB1*07:07 and *52.1:52.1 genotypes may be associated with TGF-β serum levels (Table X). The HLA-DRB1*07:07 genotype was associated with reducing TGF-β levels in serum. Furthermore, HLA-DRB1*52.1:52.1 display a suggestive association with increased TGF-β levels in serum. But when we did the above comparisons separately for the SLE patients and separately for the controls, we did not find the abovementioned relationships (Fig. 2).

We do not observed any significant differences in IL-6 serum levels between HLA-DRB1 genotypes (Fig. 3).

Discussion

The present study was based on the hypothesis that cytokines through the dysregulation of the immune system and tissue damage play an essential role in the pathogenesis of SLE. We chose two pleiotropic cytokine genes with a wide range of biological activities that may be a key component to maintaining the auto-inflammatory loop in lupus as candidates for replication. Although the SLE pathogenesis is still incompletely deciphered, now we know that genetic predisposition and environmental factors interact with each other and they are ultimately responsible for regulation of the pro- and anti-inflammatory cytokines balance as well as disease development. However, the extent of genetic factors in the immunity imbalance and its possible relation to disease activity remain unknown. We believe that better understanding of SLE genetics components can be used in predicting the risk of developing and/or course of the SLE as well as gives the opportunity to explore potential new therapeutic strategies and diagnostic tests for the disease.

Considering the very important role of the TGF-β and IL-6 in the regulation of inflammatory processes as well as differences in genetic predispositions between different populations we decided to carry out an analysis of selected *TGF-β and IL-6* genetic variants in relation to SLE. In the current study, we demonstrated that the *TGF-β* genetic variants may be associated with susceptibility to and severity of SLE as well as TGF-β serum levels in the Polish population. Our analysis revealed that *TGF-β* -509 T as well as *TGF- β* 869 C allele alleles may be associated with increased risk of SLE in the Polish population. Further, we have shown that SLE patients with polymorphic *TGF-β* -509 T allele may have a more active disease than SLE patients with wild-type *TGF-β* -509 C allele. Our detailed genotype-phenotype analysis indicated that *TGF-β* -509 T allele was associated with APTT/INR, mean value of CRP, ESR, haemoglobin, Pt, AST as well as with anti-SSB autoantibodies presence. Moreover, we also demonstrated that *IL-6* gene polymorphisms did not play a significant role in the susceptibility to as well as disease activity in our patients with SLE. The *TGF-β and IL-6* polymorphisms, including all examined here, have been already studied in the different ethnic group (20-33), however, the results are rather conflicting. Discrepancies in the incidence of allele/genotype frequencies between studies can be explained in part by the heterogeneity of the studied diseases, genetic trait differences, various ethnic groups, and the limited sample size.

Several studies have shown that certain HLA-DRB1 alleles are associated with SLE, in different ethnic groups: DRB1*03:01, *08:01 and *15:01 in European (34-36), *15:01, *15:02 and *09:01 in Asian populations (37-40),

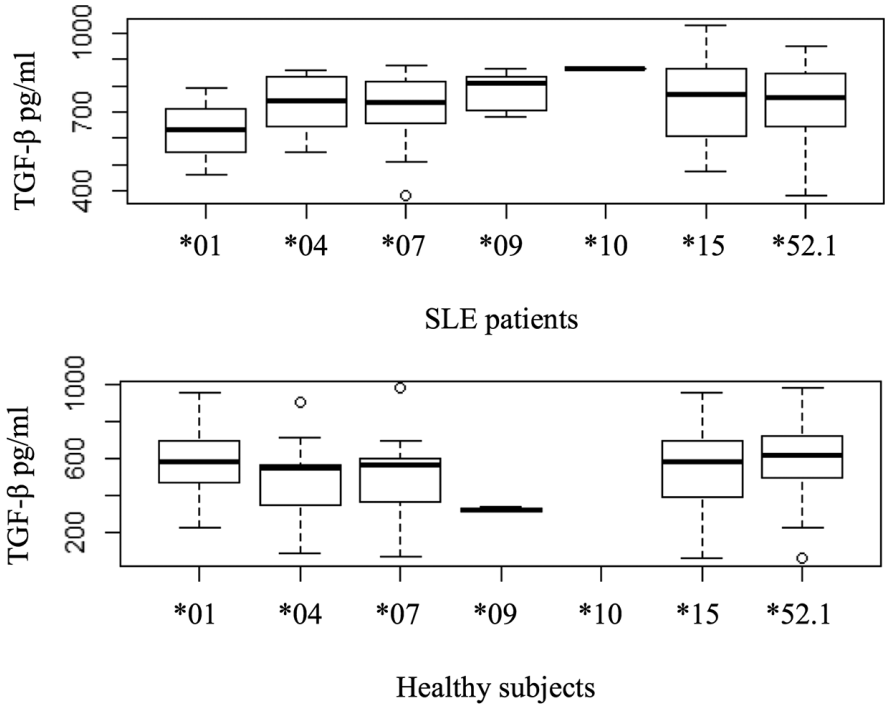

Fig. 2. TGF-β serum levels in relation to HLA-DRB1 alleles in SLE patients and healthy subjects.

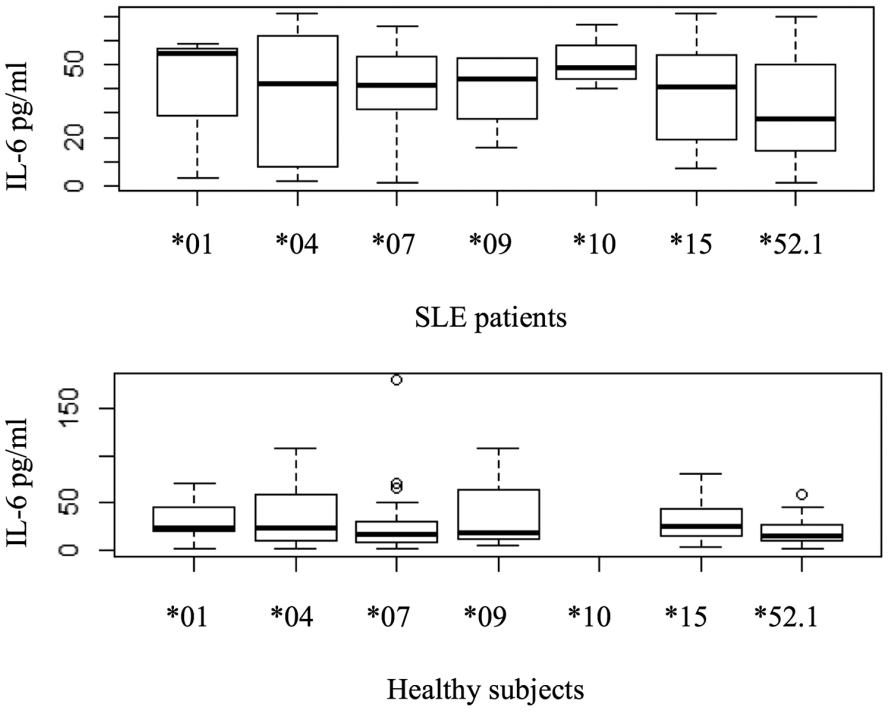

Fig. 3. IL-6 serum levels in relation to HLA-DRB1 alleles in SLE patients and healthy subjects.

and *15:03 in African-American (41). In the current study, we have shown that HLA-DRB1*15 and *09 are not a risk gene for SLE in the Polish population. Furthermore, to the best our knowledge, this is the first report showing a positive association of HLA-DRB1*52.1:52.1 with SLE risk. In contrast to other studies (42), we also do not confirmed that HLA-DRB1*04 play a protective role in humans against SLE. Because HLA-DRB1 is the strong genetic factor for SLE, these discrepancies may stem from differences in ethnic background, environmental background and/or genetic heterogeneity. In addition, this study is the first to identify a HLA-DRB1*07:01 allele as a protective against SLE. Our results are in contrast to study by Hrycek *et al*. (43), who do not find any association of this HLA allele with SLE susceptibility. However, they have a very small study population, 24 SLE patients and 36 controls, and this may cause above differences. Moreover, we also do not observed that examined HLA-DRB1 alleles may have an impact on the clinical phenotype of SLE. We have only found that HLA-DRB1*52.1 correlated with longer mean disease duration in SLE patients and with production of anti-Scl70 antibodies in our SLE patients.

In this study, we also determined both cytokines protein levels in serum of SLE patients. We demonstrated that TGF-β, as well as IL-6 protein levels in SLE patients, were significantly higher than in healthy subjects reflecting the chronic inflammatory processes in patients. Our findings showing the increased IL-6 serum levels in patients are consistent with the findings of other studies (44-50). Furthermore, in the case of TGF-β, our results are not concordance with other studies (51-55), where the authors found the decreased production of TGF-β in SLE patients. In contrast to other studies described the TGF-β serum levels, our study is one with the very well described group of patients, and we believe that this is a very strong side of our study. We think that the increased production of TGF-β in our SLE patients could be explained by the very high disease active (SLEDAI >6), the ongoing inflammatory process in patients and the attempts to keep it under control. Moreover, we also observed that subjects with HLA-DRB1*52.1 allele had a higher TGF-β serum levels than subjects without this allele. The occurrence of the HLA-DRB1* 52.1 allele significantly influences the TGF beta concentration. The importance of the HLA-DRB1* 52.1 allele in the whole population may be significant because, the HLA-DRB1* 52.1 allele is the most frequent in SLE patients than in controls (28% *vs*. 12%) as well as in the SLE patients the concentration of this cytokine is gener-

ally higher. In our opinion high levels of IL-6 in SLE patients may favour TGF-β pro-inflammatory properties and both these cytokines together may enhance an immune response through the induction of Th17 cells, promote inflammation as well as augment autoimmune conditions. Moreover, the higher production of the TGF-β in our SLE patients may be also associated with the TGF-β polymorphisms, which may affect its transcription and cause inter-individual variations in cytokine production (56-58). TGF-β -509 C/T gene polymorphisms are located within a Yin-Yang1 consensus binding site and TGF-β -509 T allele has been associated with higher TGF-β1 serum level (57, 59, 60), what is concordance with our study.

The present study is not strong enough to draw the final conclusion. Sample size may not be large enough to detect a relationship between examined genetic variants and some effect on SLE. Since the stratification analysis reduces statistical power, there may be a risk that the presented observation is due to chance. However, in contrast to the other studies carried out on SLE patients, our population has a homogenous origin and it is a clinically well-defined group including biochemical parameters. More studies with larger numbers of SLE patients are needed to confirm the relationship.

In conclusion, this study suggested that the *TGF-β* -509 C/T (rs1800469) variant may be considered as a genetic marker for SLE in the Polish population as the polymorphic allele of the *TGF-β* -509 T may be associated with both susceptibilities to SLE as well as the more active stage of the disease. Current study also clearly shows that SLE has strong positive association with HLA-DRB1*52.1, and negative with HLA-DRB1*07:01 allele.

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