Tissue factor and human apolipoprotein H genetic variants and pro-inflammatory cytokines in systemic lupus erythematosus patients

A. Wajda[1], A. Sowińska[2], E. Haładyj[3], B. Stypińska[1], J. Nałęcz-Janik[3], P.P. Jagodziński[4], D. Majewski[5], M. Olesińska[3], A. Paradowska-Gorycka[1]

[1]Department of Molecular Biology, National Institute of Geriatrics, Rheumatology and Rehabilitation, Warsaw, Poland; [2]Department of Computer Science and Statistics, Poznan University of Medical Sciences, Poland; [3]Department of Connective Tissue, National Institute of Geriatrics, Rheumatology and Rehabilitation, Warsaw, Poland; [4]Department of Biochemistry and Molecular Biology, [5]Department of Rheumatology and Internal Diseases, Poznan University of Medical Sciences, Poland.

Abstract

Objective

Tissue factor (TF) and Human apolipoprotein H (APOH) seem to be significantly associated with a clinical manifestation in systemic lupus erythematosus (SLE) patients with or without APS, mostly because of thrombotic events and coagulation processes. Additionally, according to recent studies, these two factors appear to be an important part of immune response and inflammation.

Methods

The objective of this study was to investigate three SNPs of APOH (rs4581, rs8178835 and rs818819) and three of TF (rs958587, rs3917615, rs1361600) in SLE patients and healthy subjects using TaqMan genotyping assay and their association with inflammatory cytokines level in serum and selected clinical parameters.

Results

Present study revealed that TF rs3917615 and rs958587 and APOH rs4581 possibly predispose to joint involvement in SLE.

Conclusion

Analysed genetic variants of TF and APOH may have an impact on inflammatory processes and clinical relevance in SLE patients in the Caucasian population.

Key words

tissue factor, human apolipoprotein H, systemic lupus erythematosus

Anna Wajda, PhD
Anna Sowińska, PhD
Ewa Hałady, MD
Barbara Stypińska, MSc
Jolanta Nałęcz-Janik, PhD
Paweł Piotr Jagodziński, MD, Prof.
Dominik Majewski, PhD
Marzena Olesińska, MD, Prof.
Agnieszka Paradowska-Gorycka, PhD

Please address correspondence to:
Anna Wajda,
Department of Molecular Biology,
National Institute of Geriatrics,
Rheumatology and Rehabilitation,
Spartanska 1,
02-637 Warsaw, Poland.
E-mail: annawajda2046@gmail.com

Received on February 19, 2020; accepted in revised form on June 1, 2020.

Competing interests: none declared.

Introduction

Systemic lupus erythematosus (SLE) is a prototypic, autoimmune disease with a broad clinical spectrum of almost all organ and tissue manifestations, such as joints, skin and kidneys to infrequent manifestations, *e.g.* shrinking lung (1), transverse myelitis with spastic paraparesis (2) or necrotising scleritis (3). The aetiology of SLE includes both genetic and environmental components with female sex strongly influencing pathogenesis (4). Although the concept of an "exposome" where environmental factors affect healthy but genetically predispose individuals (5) is fully rational, there is still a need to search for specific genetic factors which trigger the different course of the disease or may have an impact on its severity. Constant development of personalised medicine is required to predict which individuals are predisposed to the disease. Thanks to large genome-wide association studies (GWAS), the importance of many genes linked with immune response and inflammation, DNA repairs, adherence of inflammatory cells to the endothelium and tissue response to injury, has been confirmed (4). Nevertheless, it has to be noted that in many cases each ethnic group can be distinguished by its unique alleles. In the case of SLE it can be reflected by the epidemiological data which revealed, *e.g.* that Hispanics and African-Americans are diagnosed with SLE at a younger age and with more severe forms of the disease than Caucasians (4, 6).

Antiphospholipid antibodies (aPL) are found in 12 to 44% of SLE patients and 1–10% of healthy subjects (7). Antiphospholipid antibodies are incorporated into SLE classification criteria and are an important risk factor for thrombosis. Other risk factors of thrombosis in specific for SLE are: high disease activity, lupus nephritis and glucocorticosteroids use. As in healthy population, cigarette smoking, hypertension, older age, immobility, previous history of arterial thrombosis, the coexistence of other acquired or inherited thrombophilia also make patients prone to thrombotic events (6, 8). aPL frequency can be underestimated in SLE due to transient aPL presence. Antiphospholipid syndrome (APS) can be diagnosed 30–70% aPL-positive patients, who develop any thrombosis or pregnancy complication during 20 years of observation (9). On the other hand, Alarcon-Segovia *et al.* (1992) have proved that 30% of SLE patients with anticardiolipin antibodies do not experience thrombosis during 7 years of observation (10). Thrombosis risk in Lupus Hopkins Cohort of SLE patients with positive lupus anticoagulant (LAC) was 50% higher compared to those without LAC in 20 years of observation (11). According to EuroLupus Cohort, thrombosis together with active disease and infections is the main cause of death of SLE patients within 10 years of observation period and its frequency arises with time (12).

Factor III or tissue factor (TF) is a membrane-bound procoagulant glycoprotein (MW47-kDa) present in the subendothelial tissue and fibroblasts. TF is also circulating on monocytes in smaller quantity. Besides direct or functional injury, sepsis or hypoxia also malignancy and inflammation may activate TF (13). The crystal structure of TF extracellular domain is bounding VIIa factor (FVIIa) (14, 15). TF is a basic initiator of coagulation *in vivo*. TF /VIIa complex activates factors IX and X, what causes fibrin and thrombin generation. The adventitia cells constitutionally express TF, which allows to initiate coagulation process straight after vessel injury (14). TF is needed to ensure homeostasis, but a pathological expression of this cytokine can lead to arterial and venous thrombosis as well as disseminated intravascular coagulation (16). Under physiological conditions TF is constitutively expressed in subendothelial cells, but not in endothelial cells and monocytes (17). One of the potent inducers of TF expression is TNFα through the activation of stress-related JNK kinase (c-Jun N-terminal kinase) (18). There is a large volume of published studies describing the role of TNF-α in SLE and its potentially useful as a disease activity and susceptibility biomarker (19, 20). Interestingly, TNFα is both a proinflammatory cytokine and an immunoregulatory cytokine. On the one hand, TNF restrains autoreactive T cell (21) and on the other decreases the stimulation of

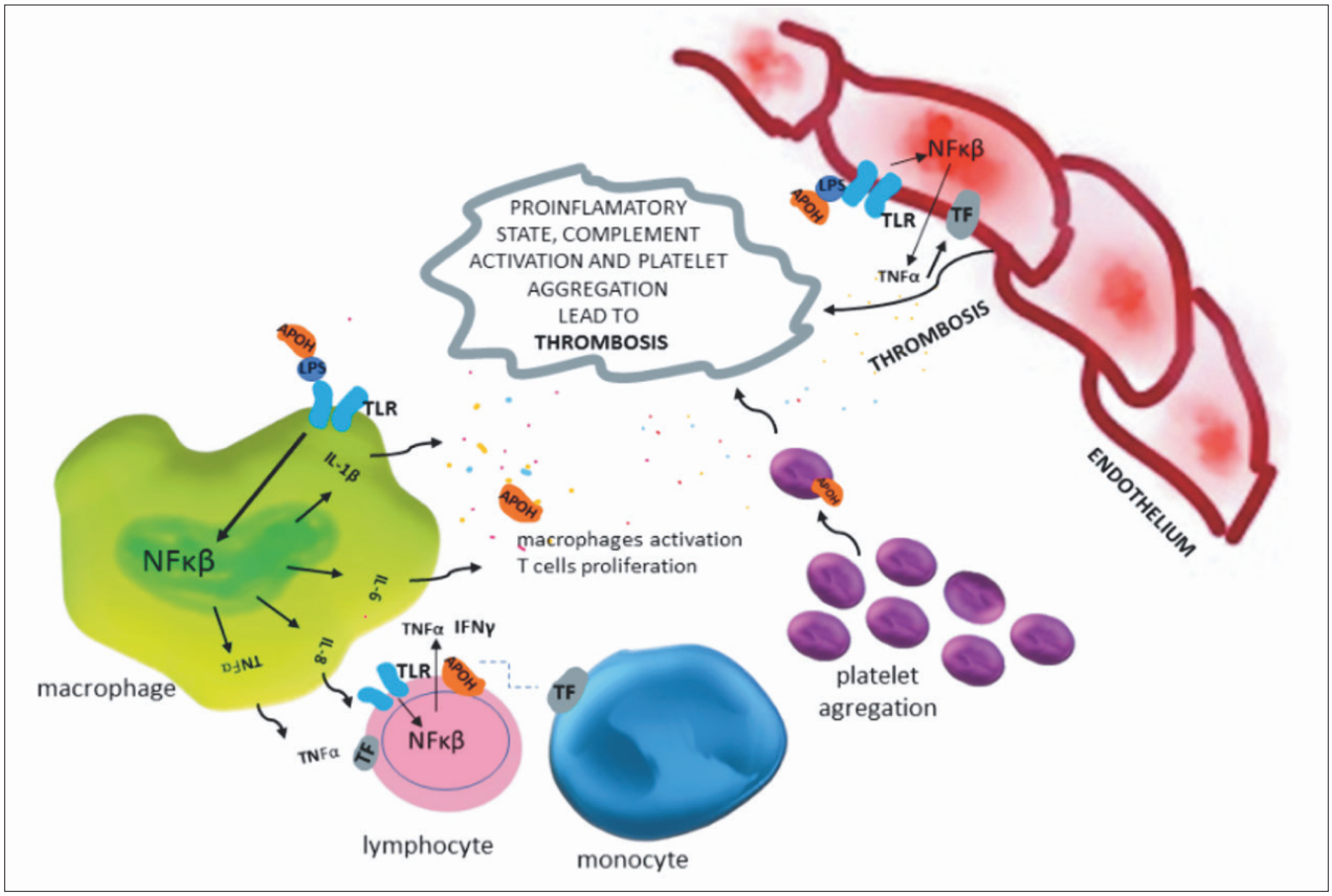


Fig. 1. Interaction of APOH and TF based on Laplante (2011) and Benagiano (2019) studies (25, 26).
APOH protein is abundant in plasma that binds to the surface of cells, activated platelets and particles expressing negatively charged lipids.
APOH may interact with TLR and with TLR4 ligands (particularly LPS) and activate TLR4. TLR4 activation triggers NF-κB pathway and TNF-α and IFN-γ are expressed. TNF-α and IFN-γ induce TF expression. Monocytes express TF after interaction with co-cultured antigen-stimulated APOH-specific T-cells.

immune system by apoptosis-derived nucleosome and inhibition of autoantibody production (22). Anti-TNF-α therapy in SLE although suppressing the local tissue destruction can lead to transient formation of anti-dsDNA and anticardiolipin antibodies (23).

APOH also is known as β2-glycoprotein I (β2GPI) is a soluble blood protein involved in multiple processes involving coagulation and complement (24). Additionally, it has been proved that β2GPI induce T cell proliferation and expression of IFN-γ in T cell clones. Benagiano *et al*. (2019) proposed that via IFN-γ, β2GPI /APOH may elicit a local inflammation in lupus-antiphospholipid syndrome (25). Interestingly, Laplante *et al*. (2011) hypothesised that β2GPI may be responsible for TLR4 activation. In their study, it has been proved that both LPS and TLR4 are required for β2GPI to bind to and activate macrophages (26). TLR4 contributes via NF-κB signalling pathway in IL-6 and TNF-α production (Fig. 1).

As a strong genetic component and ethnicity factor in the autoinflammatory loop in SLE are essential, the present study is a part of our broad research on SLE (27-33). Based on the literature and population allele frequency data in population, we selected single nucleotide polymorphisms (SNPs) in the TF and APOH genes and investigated whether they may be associated with clinical parameters and outcome of patients with SLE in the Caucasian population. We decided to combine the analysis of polymorphisms in APOH and TF genes to investigate their interplay in the inflammatory changes. rs958587, rs3917615, rs1361600 in TF and their strict association with severe sepsis outcome (33) are particularly interesting hence the high risk of sepsis in SLE patients (47). In the case of APOH we present analysis of its promoter (rs818819), selected coding variant rs4581 and considering the importance of splicing effect – intron variant (rs8178835).

Moreover, serum level of proinflammatory cytokines such as INF-γ, IL-6, TNF-α and selected polymorphisms have been analysed to understand the interplay between thrombosis and inflammation. Additionally, hence it is known that TGF-β blocks NF-κβ activation, is a potent anti-inflammatory cytokine and critical in self-tolerance mechanisms, the concentration of TGF-β was measured both in healthy and in SLE patients.

Material and methods

Subjects description

147 patients diagnosed with SLE in the National Institute of Geriatrics, Rheumatology and Rehabilitation in War-

saw, Poland and in the University of Medical Sciences, Poznan, Poland. All patients were diagnosed according to 2012 SLICC criteria. 569 healthy blood donors as a control group were included in the genotyping study. Unfortunately, this paper cannot provide a comprehensive medical history of all SLE patients and comparison between genotypes and clinical date was conducted on group consisted of about 100 patients. The control group was recruited from the Regional Center for Blood Donation and Blood Treatment in Warsaw and do not show any clinical nor laboratory characteristic for autoimmune disease. The research was approved by the Research Ethics Committee of the National Institute of Geriatrics, Rheumatology and Rehabilitation. All the participants included in our study signed informed consent statements. The study was performed in accordance with the 1964 Helsinki declaration and with the ethical standards of our Institute.

DNA extraction and genotyping

DNA was extracted from the peripheral blood of SLE patients and healthy subjects using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's procedure.

Three SNPs of APOH gene: rs4581, rs8178835 and rs818819, and three SNPs of TF gene: rs958587, rs3917615, rs1361600 were analysed using the TaqMan Genotyping Assays on a Quant Studio 5 detection system (Life Technologies, Carlsbad, CA, USA). The reaction conditions were as follows: 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.

Cytokine levels in serum

Whole blood collected to the test tubes without anticoagulants was centrifuged at 1500×g for 10 minutes. Obtained serum was distributed to the test tubes and maintained at -70°C. Randomly 101 samples of patients with SLE and 124 healthy subjects were chosen to test the serum levels of IFN-γ, IL-6, TGF-β and TNF-α. The serum levels of these cytokines were assessed using Human ELISA Kits from DRG Instruments GmbH (Germany) according to the manufacturer's instructions. The absorbance (OD) was determined on LT-4000MS reader (Labtech International Ltd, Great Britain). The detection limit of human IL-6 was 2 pg/ml; TGF-β 1.9 pg/ml; IFN-γ was 0.03 IU/ml, and TNF-α was 0.7 pg/ml.

Statistical analysis

Quantitative data are presented as mean ± standard deviation (SD) or median (range) and categorical data as percentages. Data normality was checked with the Shapiro-Wilk test. Allele and genotype frequencies between cases and controls were compared using Fisher's exact test. The odds ratio (OR) and 95% confidence interval (95% CI) were calculated. All statistical analyses were performed using the software Statistica, v. 10.0. $p<0.05$ was considered as statistically significant. Haplotypes for SNPs were tested for Hardy-Weinberg equilibrium. The presence of linkage disequilibrium (LD) and a coefficient (D´ and r2) for TF and APOH haplotypes was analysed using the SHEsis software http://analysis.bio-x.cn (34). To assess the association between the analysed SNP haplotypes Fisher test was performed. Normally distributed demographic and clinical parameters were compared using t-student or Cochran

Table I. Clinical characteristics of patients with SLE.

Characteristics	SLE patients	
	N*	mean values ± SD
Age [years]	100	44.59 ± 14.17
Disease duration [years]	63	10.08 ± 10.08
SELENA_SLEDAI	63	6 ± 5.82
SLICC	63	1.17 ± 1.26
APTT	49	35.34 ± 16.52
ESR [mm/h]	59	42.80 ± 28.86
CRP [mg/L]	58	33.79 ± 68.92
Haemoglobin [g/dL]	59	11.97 ± 2.17
PLT [$x10^3/mm^3$]	59	242.14 ± 123.86
Pt	48	16.72 ± 17.86
INR	48	1.23 ± 0.79
Urea	51	41.65 ± 38.72
creatinine	56	0.83 ± 0.22
ALT	60	50 ± 54.36
AST	59	51.25 ± 108.82
Alkaline phosphatase	46	74.89 ± 44.92
C3	55	76.29 ± 29.07
C4	54	14.05 ± 13.49
	N	**n (%)**
anti-dsDNA	93	69 (74.19)
anti-SSA	59	25 (42.37)
anti-SSB	58	5 (8.62)
anti-Sm	68	20 (29.41)
anti-Rib	57	4 (7.02)
anti-Jo	57	1 (1.75)
anti-His	57	16 (28.07)
anti-Scl70	78	21 (26.92)
anti-CEN	58	2 (3.45)
anti-U1RNP	73	30 (41.09)
anti-IgM aCL	59	6 (10.17)
anti-IgG aCL	60	17 (28.33)
β_2GPI IgG	55	10 (18.2)
β_2GPI IgM	21	0 (0)
LAC	59	16 (27.12)
Facial erythema	63	35 (55.56)
Arthritis	75	47 (62.67)
Lung fibrosis	63	3 (4.76)
Leucopenia	63	23 (36.51)
APS	52	11 (21.15)
Sjögren's syndrome	63	10 (15.87)

N: number of patients with clinical information; n: number of patients with positive clinical manifestation.

Cox test for independent samples, whereas those with non-normal distribution were analysed by non-parametric Mann-Whitney test. The prevalence of variables was assessed by the Chi-square test, the Chi-square test with Yates correction, or Fisher's exact test. Differences in SLE or HC in cytokines levels among different genotypes were tested by Kruskal-Wallis and Dunn's *post hoc* or ANOVA test and Tukey *post hoc*. Computational functional prediction analysis was conducted by Mutation Taster 2.0 to check the influence of the rs4851 on the function of APOH protein (35).

Results

Demographic and clinical characteristics of SLE patients

The clinical and demographic characteristics of SLE are collected at the time blood sampling and the results were presented in Table I. The mean disease duration of our SLE patients was 10 years. Disease activity assessed by SELENA-SLEDAI index was 6 points (lowest – 0, highest 26), while damage index assessed by the SLICC index was 1.17 points (lowest – 0, highest 5). The major clinical features observed in our SLE patients were arthritis (62.7%) and facial erythema (55.6%). Anti-dsDNAs were present in 74% of patients. In our analysed group, only 11 patients suffered from APS. Unfortunately, not in every patients full antibodies profile was conducted. 28% patients were anti-IgG aCL positive and 18% β2GPI IgG positive. In our available data, there were no β2GPI IgM positive patients.

95% of analysed patients were treated with steroids, 70% received chloroquine and 32 hydroxchloroquine. 23% receive cyclophamide, only 1 patient received belimumab, azathioprine – 41% and mycophenolate 5%.

Distribution of TF and APOH gene polymorphisms in SLE patients and healthy subjects

Three SNPs of APOH rs4581, rs8178835 and rs818819 and three of TF - rs958587, rs3917615, rs1361600 were analysed in SLE patients and healthy subjects.

To detect whether there was an association between TF and APOH gene polymorphisms and risk of SLE, four genetic models, codominant, dominant, recessive and overdominant, were used. Genotype frequencies for the all analysed SNPs were in Hardy-Weinberg equilibrium (HWE). The distributions of the APOH rs4581, rs8178835 and rs818819, and TF rs958587, rs3917615, rs1361600 genotypes among patients with SLE and controls, are summarised in Table II.

The analysis of the APOH rs8178819 showed significant differences in the case-control distribution in three genetic models. Under the codominant model, the frequency of the CT genotype was significantly lower in patients with SLE than in controls (OR: 0.338; 95% CI=0.178–0.603; $p<0.0001$). Similarly, under the dominant model (CT+TT *vs*. CC) as well as under the overdominant model (CC+TT *vs*. CT) the association was also significant (OR: 0.309; 95% CI=0.163–0.550; $p<0.0001$ and OR: 0.348; 95% CI=0.184–0.621; $p=0.0001$, respectively). Our analysis also revealed that APOH rs8178819 T allele was more frequent in healthy subjects compared to SLE patients ($p<0.0001$). Moreover, in our SLE patients, the APOH rs8178819 TT genotype was not observed. For the other APOH genetic variants in position rs4581 and rs8178835, we found no differences in genotype/allele distribution between SLE patients and controls.

Concerning TF rs958587, rs3917615 and rs1361600 genetic variants there were no significant differences in the genotype/allele frequencies between SLE patients and healthy subjects ($p>0.05$, Table II).

Association of APOH gene polymorphisms with the clinical phenotype of SLE patients

We conducted a stratified analysis of combined genotypes under different genetic models, dominant and recessive, for APOH gene polymorphisms at position rs4581, rs8178835 and rs818819. Our analysis showed that APOH rs818819 CC genotype was associated with higher ESR levels and lower haemoglobin levels in SLE patients under the dominant model ($p=0.038$ and $p=0.007$, respectively, Table III). Joint inflammation/arthritis was observed only in SLE patients with APOH rs4581 C allele ($p=0.003$). In SLE patients with APOH rs4581 AA genotype, we did not observe arthritis presence. Additionally, an association between APOH rs8178835 A allele and arthritis presence in our SLE patients ($p=0.0028$) has been revealed. In contrast, in SLE patients with APOH rs8178835 GG genotype association with arthritis was not observed.

Association of TF gene polymorphisms with the clinical phenotype of SLE patients

Next, we investigated whether TF genetic variants may have an impact on SLE activity/phenotype. We analysed the potential association between all examined polymorphisms, in two genetic models: dominant and recessive, and SLE phenotype according to clinical and laboratory parameters of SLE.

The genotype-phenotype analysis revealed a significantly longer time of disease duration in SLE patients with TF rs958587 C allele than in SLE patients with rs958587 TT genotype (Table IV and V). Our data showed that joint inflammation/arthritis was more frequently observed in SLE patients with TF rs958587 T allele than in SLE patients with rs958587 C allele ($p=0.002$, Table IV and Table V). We also observed the association of the TF rs958587 CC genotype with Sjögren's syndrome presence ($p=0.02$) and erythema presence ($p=0.03$) under recessive model (CC *vs*. TC+TT, Table V).

Our analysis also demonstrated that SLE patients with TF rs1361600 TT genotype were characterised by a longer time of disease duration compare to SLE patients with combined TF rs1361600 TC+CC genotype under recessive model (13.5 (1–43) *vs*. 3 (0–30), $p=0.003$, Table V). Results also showed that SLE patients with TF rs1361600 CC genotype revealed significantly higher haemoglobin levels as well as lower ESR levels (average level 16.5mm/h) than SLE patients with TF rs1361600 CT+TT genotype (haemoglobin level: 13±1.32 *vs*. 11.62±2.36, $p=0.009$ and ESR level: 16.5 *vs*. 51,

Table II. Genotype and allele frequencies of APOH and TF SNPs in SLE and healthy control group.

APOH rs8178819		SLE n (%)	Controls n (%)	OR (95% CI)	*p*-value
	genotype				
Codominant	CC	132 (89.8)	416 (73.11)	References	
	CT	15 (10.2)	140 (24.6)	0.338 (0.178 – 0.603)	**<0.0001**
	TT	0 (0)	13 (2.28)	0.000 (0.000 – 1.052)	0.059
Dominant	CC	132 (89.8)	416 (73.11)	References	
	CT+TT	15 (10.2)	153 (26.89)	0.309 (0.163 – 0.550)	**<0.0001**
Recessive	CC+CT	147 (100)	556 (97.72)	References	
	TT	0 (0)	13 (2.28)	0.000 (0.000 – 1.259)	0.098
Overdominant	CC+TT	132 (89.8)	429 (75.4)	References	
	CT	15 (10.2)	140 (24.6)	0.348 (0.184 – 0.621)	**0.0001**
	Alleles				
	C	279 (94.9)	972 (85.41)	References	
	T	15 (5.1)	166 (14.59)	0.315 (0.169 – 0.546)	**<0.0001**
APOH rs4581		SLE n (%)	Controls n (%)**	OR (95% CI)	*p*-value
	genotype				
Codominant	AA	8 (6.25)	23 (4.05)	References	
	AC	49 (38.28)	178 (31.34)	0.791 (0.317 – 2.179)	0.742
	CC	71 (55.47)	367 (64.61)	0.556 (0.229 – 1.500)	0.261
Dominant	AA	8 (6.25)	23 (4.05)	References	
	AC+CC	120 (93.75)	545 (95.95)	0.633 (0.266 – 1.679)	0.386
Recessive	AA+AC	57 (44.53)	201 (35.39)	References	
	CC	71 (55.47)	367 (64.61)	0.682 (0.455 – 1.028)	0.068
Overdominant	AA+CC	79 (61.72)	390 (68.66)	References	
	AC	49 (38.28)	178 (31.34)	1.359 (0.891 – 2.057)	0.161
	Alleles				
	A	65 (25.39)	224 (19.72)	References	
	C	191 (74.61)	912 (80.28)	0,722 (0.521 – 1.008)	0.056
APOH rs8178835		SLE n (%)	Controls n (%)**	OR (95% CI)	*p*-value
	genotype				
Codominant	AA	88 (59.06)	364 (64.08)	References	
	AG	54 (36.24)	180 (31.69)	1.241 (0.828 – 1.848)	0.314
	GG	7 (4.7)	24 (4.23)	1.206 (0.425 – 3.004)	0.820
Dominant	AA	88 (59.06)	364 (64.08)	References	
	AG+GG	61 (40.94)	204 (35.92)	1.237 (0.839 – 1.816)	0.301
Recessive	AA+AG	142 (95.3)	544 (95.77)	References	
	GG	7 (4.7)	24 (4.23)	1.117 (0.398 – 2.742)	0.946
Overdominant	AA+GG	95 (63.76)	388 (68.31)	References	
	AG	54 (36.24)	180 (31.69)	1.225 (0.822 – 1.815)	0.339
	Alleles				
	A	230 (77.18)	908 (79.93)	References	
	G	68 (22.82)	228 (20.07)	1.177 (0.852 – 1.613)	0.335
TF rs958587		SLE n (%)	Controls n (%)*	OR (95% CI)	*p*-value
	genotype				
Codominant	TT	28 (18.79)	112 (20.11)	References	
	TC	72 (48.32)	288 (51.70)	1.000 (0.601 – 1.663)	1.000
	CC	49 (32.89)	157 (28.19)	1.248 (0.719 – 2.196)	0.486
Dominant	TT	28 (18.79)	112 (20.11)	References	
	TC+CC	121 (81.21)	445 (79.89)	1.088 (0.676 – 1.793)	0.819
Recessive	TT+TC	100 (67.11)	400 (71.81)	References	
	CC	49 (32.89)	157 (28.19)	1.248 (0.827 – 1.868)	0.308
Overdominant	CC+TT	77 (51.68)	269 (48.30)	References	
	TC	72 (48.32)	288 (51.70)	0.873 (0.598 – 1.275)	0.521
	Alleles				
	T	128 (42.95)	512 (45.96)	References	
	C	170 (57.05)	602 (54.04)	1.130 (0.866 – 1.475)	0.389
TF rs3917615		SLE n (%)	Controls n (%)**	OR (95% CI)	*p*-value
	genotype				
Codominant	TT	25 (16.78)	96 (16.93)	References	
	TC	69 (46.31)	300 (52.91)	0.883 (0.518 – 1.542)	0.723
	CC	55 (36.91)	171 (30.16)	1.235 (0.704 – 2.207)	0.525
Dominant	TT	25 (16.78)	96 (16.93)	References	
	TC+CC	124 (83.22)	471 (83.07)	1.011 (0.614 – 1.712)	1.000
Recessive	TT+TC	94 (63.09)	396 (69.84)	References	
	CC	55 (36.91)	171 (30.16)	1.355 (0.909 – 2.007)	0.141
Overdominant	CC+TT	80 (53.69)	267 (47.09)	References	
	TC	69 (46.31)	300 (52.91)	0.768 (0.526 – 1.120)	0.179
	Alleles				
	T	119 (39.93)	492 (43.39)	References	
	C	179 (60.07)	642 (56.61)	1.153 (0.882 – 1.509)	0.314
TF rs1361600		SLE n (%)	Controls n (%)**	OR (95% CI)	*p*-value
	genotype				
Codominant	CC	28 (18.79)	117 (20.53)	References	
	CT	71 (47.65)	294 (51.58)	1.009 (0.607 – 1.710)	1.000
	TT	50 (33.56)	159 (27.89)	1.314 (0.759 – 2.304)	0.369
Dominant	CC	28 (18.79)	117 (20.53)	References	
	CT+TT	121 (81.21)	453 (79.47)	1.116 (0.695 – 1.836)	0.732
Recessive	CC+CT	99 (66.44)	411 (72.11)	References	
	TT	50 (33.56)	159 (27.89)	1.306 (0.867 – 1.949)	0.211
Overdominant	CC+TT	78 (52.35)	276 (48.42)	References	
	CT	71 (47.65)	294 (51.58)	0.855 (0.586 – 1.246)	0.446
	Alleles				
	C	127 (42.62)	528 (46.32)	References	
	T	171 (57.38)	612 (53.68)	1.162 (0.891 – 1.517)	0.282

Table III. The disease activity and laboratory parameters in relations to APOH polymorphisms.

APOH rs4581	Median (range)	*p*	APOH rs8178835	Median (range)	*p*	APOH rs8178819	Median (range)	*p*
ESR [mm/h]								
AA	53 (49 - 86)	0.268	AA	35.5 (7 - 114)	0.215	CC	49 (5 - 114)	**0.038**
AC+CC	42 5 - 114)		AG+GG	52 (5 - 114)		CT+TT	16 (11 - 38)	
Haemoglobin [g/dL]								
AA	11.4 (8.9 - 13.9)	0.472	AA	12.5 (4.9 - 16)	0.330	CC	12.2 (4.9 - 16)	**0.007**
AC+CC	12.7 (4.9 - 15.3)		AG+GG	11.5 (8.9 - 14.8)		CT+TT	14.2 (12.8 - 15.4)	
Arthritis								
	positive clinical manifestation n(%)	*p*		positive clinical manifestation n(%)	*p*		positive clinical manifestation n(%)	*p*
AA	0 (0)	**0.003**[b]	**AA**	22 (57.89)	0.827[a]	**CC**	31 (59.61)	0.116[a]
AC+CC	28 (73.68)		**AG+GG**	12 (57.14)		**CT+TT**	1 (16.66)	
CC	18 (72)	0.719[a]	**GG**	0 (0)	**0.028**[b]	**TT**	-	-
AC+AA	3 (16.66)		**AG+AA**	34 (61.81)		**CT+CC**	32 (55.1)	

Mann Whitney; a: chi-squared with Yates correction; b: Fisher's exact test; c: student t-test, d: Cochran-Cox test.

Table IV. The disease activity and laboratory parameters in relations to TF polymorphisms in a dominant model.

TF rs958587	Median (range)	*p*	TF rs3917615	Median (range)	*p*	TF rs1361600	Median (range)	*p*
Disease duration [years]								
TT	1 (0-23)	**0.001**	TT	9.5 (1 - 22)	**0.004**	CC	1 (0 - 30)	**0.020**
TC+CC	10 (1-43)		TC+CC	10 (1 - 43)		CT+TT	10 (1 - 43)	
ESR [mm/h]								
TT	33 (5 – 104)	0.165	TT	21 (18 - 26)	0.306	CC	16.5 (5 - 104)	**0.014**
TC+CC	50 (7 – 114)		TC+CC	49 (7 - 114)		CT+TT	51 (9 - 114)	
Haemoglobin [g/dL]								
TT	13.3 (9.2 –14.8)	0.2819	TT	12.25 (10 - 13.3)	0.292	CC	13 ± 1.32	**0.009c**
TC+CC	12.2 (4.9 – 16)		TC+CC	12.2 (4.9 - 16)		CT+TT	11.62 ± 2.36	
Arthritis								
TF rs958587	positive clinical manifestation n (%)	*p*	TF rs3917615	positive clinical manifestation n (%)	*p*	TF rs1361600	positive clinical manifestation n (%)	*p*
TT	14 (50)	**0.002**	TT	13 (92.86)	**0.004**[a]	CC	12 (85.71)	**0.023**[a]
TC+CC	19 (15.7)		TC+CC	20 (44.44)		CT+TT	21 (46.67)	

Mann Whitney; a: chi-squared with Yates correction; b: Fisher's exact test; c: Cochran-Cox test.

p=0,01; Table IV). Moreover, we also observed that TF rs1361600 C allele has shown an association with arthritis presence (*p*=0.002), Sjögren's syndrome presence (*p*=0.02) and erythema presence (*p*=0.02) under the recessive model (Table V).

The analysis of disease activity and laboratory parameters in relation to TF rs3917615 gene SNP have shown association this genetic variant with a time of disease duration, arthritis presence, Sjögren syndrome presence and erythema presence. We observed that SLE patients with TF rs3917615 CC genotype had a longer time of disease duration comparing to SLE patients with combined TF rs3917615 TC+TT genotype (15 years *vs*. 3 years, *p*=0.002, Table V). Arthritis and erythema were observed more frequently in SLE patients with combined TF rs3917615 TC+TT than in SLE patients with TF rs3917615 CC genotype (*p*=0.001 and *p*=0.05, respectively). In contrast, the Sjögren syndrome was more frequently present in SLE patients with TF rs3917615 CC than in SLE patients with combined TF rs3917615 TC+TT genotype (*p*=0.03).

APOH and TF haplotypes and risk of SLE

APOH and TF haplotypes were assessed for patients with SLE and healthy subjects. Haplotypes with frequency <0.03 are ignored. Very strong linkage disequilibrium (LD) and r2 has been observed between APOH rs4581 and rs8178835 (D'=1.0 r2=0.99) but not between rs8178835 and rs8178819 or rs4581 and rs8178819 (Fig. 2A). The most frequent haplotype observed in both groups was CAC (*p*=0.11). Haplotype AGC revealed the significantly higher risk for SLE (OR 3.46, 95% CI 2.386-5.03) whereas AGT significantly reduced risk for SLE (Fig. 2C).

High LD has been observed between three of analysed SNPs in TF. The highest r2 (0.91) was noted between rs958587 and rs1361600 (Fig. 2B). However, CCT and TTC in TF did not show significant difference (*p*=0.55 and *p*=0.053, respectively) (Fig. 2C).

Table V. The disease activity and laboratory parameters in relations to TF polymorphisms in a recessive model.

TF rs958587	Median (range)	*p*	TF rs3917615	Median (range)	*p*	TF rs1361600	Median (range)	*p*
Age [years]								
CC	46 (25 - 76)	**0.021**	CC	43 (25 - 76)	**0.043**	TT	41.5 (25 - 76)	0.081
TC+TT	35 (21 - 59)		TC+TT	35 (21 - 59)		CT+CC	35 (21 - 72)	
Disease duration [years]								
CC	15.5 (1 - 43)	**0.001**	CC	15 (1 - 43)	**0.002**	TT	13.5 (1 - 43)	**0.003**
TC+TT	3 (0 - 30)		TC+TT	3 (0 - 30)		CT+CC	3 (0 - 30)	
TF rs958587	positive clinical manifestation n(%)	*p*	TF rs3917615	positive clinical manifestation n(%)	*p*	TF rs1361600	positive clinical manifestation n(%)	*p*
Facial erythema								
CC	6 (30)	0.027[a]	CC	7 (33.33)	0.054[a]	TT	6 (30)	0.027[a]
TC+TT	25 (64.1)		TC+TT	24 (63.15)		CT+CC	25 (64.1)	
Arthritis								
CC	5 (25)	**0.002[a]**	CC	5 (23.8)	**0.001[a]**	TT	5 (25)	**0.002[a]**
TC+TT	28 (71.79)		TC+TT	28 (73.68)		CT+CC	28 (71.79)	
Sjögren's syndrome								
CC	7 (35)	**0.023[a]**	CC	7 (33.33)	**0.033[a]**	TT	7 (35)	**0.023[a]**
TC+TT	3 (7.69)		TC+TT	3 (7.89)		CT+CC	3 (7.69)	

Mann Whitney; a: chi-squared with Yates correction; b: student t-test; c: Cochran-Cox test.

Impact of TF gene polymorphisms on serum TGF-β, IL-6, IFN-γ and TNF-α concentrations

Concentration of selected cytokines in serum in SLE patients and their association with clinical parameters are shown in Table VI.

Next, we investigated whether TF genetic variants may have an impact on TGF-β, IL-6, IFN-γ and TNF-α levels in serum (Table VII).

A significantly higher concentration of TGF-β was detected in serum of SLE patients with all analysed TF gene polymorphisms in comparison to healthy subjects (Table VII). In the comparison between three different genotypes of SLE or control, no significant differences were observed. However, in the case of TF rs3917615 TT and rs1361600 CC genotypes average concentration in serum of the healthy subject was around 490pg/ml whereas in the other genotypes were around 560-590 pg/ml.

Patients with TF SNPs such as rs958587 and rs1361600 were characterised by significantly higher level of IFN-γ in serum in comparison to healthy subjects (Table VII). In the case of TF rs3917615 TC difference was not statistically significant. Moreover, SLE patients with longer than or equal to 10 years of disease duration revealed significantly lower average IFN-γ concentration in serum in comparison with patients with less than 10 years of disease duration (Table VI).

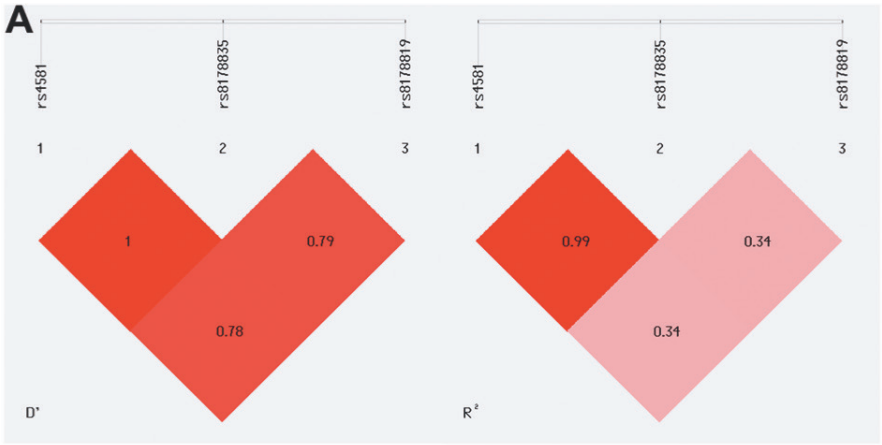
It has been found that SLE carriers of anti-Sm positive revealed significantly higher level of IL-6 in serum as compared with anti-Sm negative SLE patients (average level 61.85 and 39.54, respectively; p=0,048) (Table VI).

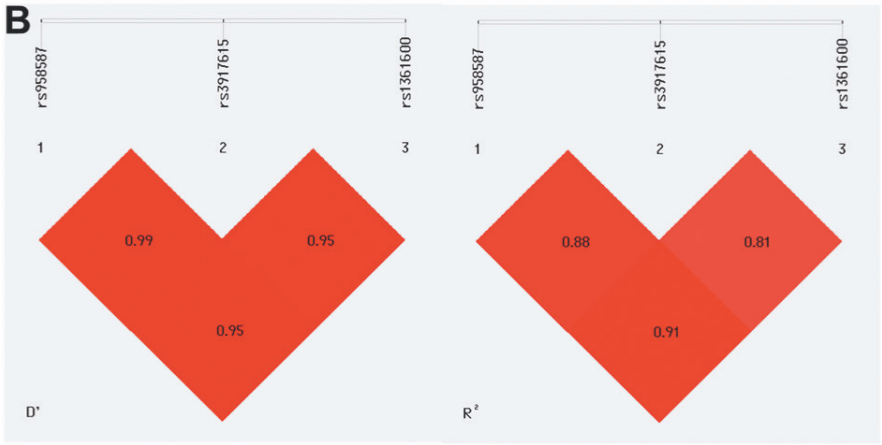
Excluding TF rs3917615TT, rs1361600CC, in all analysed TF polymorphisms, higher concentration of IL-6 in serum was noted in SLE patient as compared to healthy subjects (Table VII). SLE patients, TF rs958587CC carriers were characterised by almost two-times higher IL-6 average level in serum than TT carriers (63 and 32 pg/ml, respectively). Interestingly in both in SLE patients and healthy subject with TF rs3917615 TT or TF rs1361600 CC genotype concentration of IL-6 in serum was at a similar level. In other cases, IL-6 concentration in serum was higher in SLE patients. Regardless of the genotype in the TF gene, in healthy subjects, IL-6 concentration was at a similar level.

Almost two-times higher TNF-α concentration in serum was observed in SLE patients with Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI) score of greater than or equal to 6 (p=0,008) than in patients with SELENA-SLEDAI score lower than 6 (Table VI). SLE patients with TF rs958587 and rs3917615 polymorphism, in all examined genotypes, were characterised by higher TNF-α concentration than HC, although in the case of TF rs958587 TT the difference was not statistically significant. In SLE patients and healthy subjects with TF rs1361600 CC genotype, the serum level of TNF-α was similar.

Impact of APOH gene polymorphisms on serum TGF-β, IL-6, IFN-γ and TNF-α concentrations

The last step of our analysis was to establish the impact of APOH genetic variants on TGF-β, IL-6, IFN-γ and TNF-α serum level in SLE patients and healthy subjects. We observed that in the serum of SLE patients with all analysed APOH gene polymorphisms TGF-β concentration was higher in comparison to healthy control. However, in the case of APOH rs4581 AA and rs8178835 GG genotypes difference was not statistically significant. In the case of APOH rs8178819 TT genotype, TGF-β concentration could not be compared, because of no subjects with this genotype. Generally, in all analysed SNPs of APOH IFN-γ level was higher in SLE patients than in healthy control. In the case of rs4581AA and rs8178835GG





C

GENE	Haplotype	Case(freq)	Control(freq)	Fisher's p	OR [95% CI]
APOH	CAC	183(0.732)	871(0.78)	0.113	0.768 [0.561~1.051]
	AGC	55(0.22)	84(0.075)	4.22e-10	3.465 [2.386~5.03]
	AGT	6(0.024)	138(0.123)	2.87e-07	0.174 [0.076~0.399]
TF	CCT	153(0.52)	592(0.54)	0.554	0.923 [0.713~1.195]
	TTC	107(0.363)	468(0.427)	0.053	0.767 [0.588~1.001]

Fig. 2. Linkage disequilibrium (LD) between genetic variants based on D' (left) and R^2 (right) scores of APOH (**A**) and TF (**B**) between SLE and healthy subjects.
C) Haplotype analysis results among chosen loci rs4581, rs8178835, rs8178819 in APOH and rs95858, rs391761, rs1361600 in TF.

IFN-γ concentration was below quantification or was absent in the serum.

In the case of rs8178819 IFN-γ concentration could be compared only in CC genotype.

In the case of SNPs APOH, a higher concentration of TNF-α in serum was observed in SLE patients at the level 30–38 pg/ml whereas concentration was in the average range from 6 to 16 in healthy subjects (Table VIII). We could not analyse TNF-α concentration in rs8178819TT carriers.

Discussion

The risk of developing a thrombotic event is higher in SLE patients than in the general population. Moreover, the susceptibility to thrombosis may further increase when associated with other risk factors, or in the presence of inherited or acquired thrombophilia. Although thrombotic events are not included in the SLE classification criteria, they have been studied in patients with risk factors both from a pathophysiological and a clinical point of view to define the therapeutic strategies of prophylaxis and treatment. It seems to be a reasonable approach due to relatively frequent thrombotic incidents in SLE patients and serious complication of the natural history of the disease (6). Therefore, the objective of this study was to investigate whether selected SNPs in two genes taking part in thrombosis and coagulation processes, the tissue factor (TF) and Human apolipoprotein H (APOH), were associated with other clinical parameters and outcome for patients with SLE in the Polish population.

Up to date, about 151 SNPs of APOH and several of TFs were discovered. The literature shows that APOH and TF are involved in inflammatory pathways (13, 15, 36-40) . The connection between inflammation, coagulation and thrombosis processes is still under delineating, and novel insight has also an impact on other, more specific and safer therapeutic strategies (14, 41). A strong association between TF polymorphisms (rs1361600, rs3917615 and rs958587) and severe sepsis has been reported (16, 42). However, reports on the impact of APOH on SLE course are contradictory.

The aetiology of inflammation in SLE is undoubtedly associated with genetic abnormalities (1). APOH and TF seem to be good candidates for genetic markers implicated in the clinical state not only because of the thromboembolic incidents but also because of the potential of other clinical disease activities. Much of the current literature on inflammation and autoimmunological disorders pays attention to the role of Toll-Like Receptors (TLRs) in them. APOH and as well as TF revealed cross-talks with TLRs, which makes them even more attractive target for further research.

Despite many substantive premises on the importance of APOH in the pathogenesis of SLE, it seems that polymorphism rs4581 (Val266Leu) in this gene is not associated with increased risk of developing SLE. However, it may have an impact on the clinical features of the disease. The result of the current study conducted on Caucasian population is in agreement with the findings of Kamboh *et al*. (1999) (43, 44). Nevertheless, studies on Chinese population revealed that the Val allele may be one of the genetic risk factors for the development of thrombosis in patients with SLE (25, 36). Recently, Shishkova *et al*. (2019)

Table VI. Concentration of TGF-β, IFN-γ, TNF-α and IL-6 in serum and the selected clinical parameters (SLE).

Parameter	**TGF-β (pg/ml)**			**TGF-β (pg/ml)**			*p*
	parameter group I	N	median (IQR)[a] mean ± SD[b]	parameter group II	N	median (IQR)[a] mean ± SD[b]	
Age	age ≥56	*9*	697.865 ± 152.748 705.806 (171.028)	age <56	*44*	749.418 ± 143.99 790.834 (211.908)	0.337[b]
sex	women	*50*	738.772 ± 147.709 755.595 (223.591)	Men	*3*	772.187 ± 113.531 806.575 (219.111)	0.703[b]
SELENA-SLEDAI	≥6	*23*	749.943 ± 147.708 766.835 (211.730)	<6	*30*	733.549 ± 145.570 767.249 (210.163)	0.688[b]
disease duration	≥10	24	756.125 ± 147.306 799.05 (180.953)	< 10	29	727.867 ± 144.97 737.01 (196.985)	0.486[b]
CRP	≥13	19	769.533 ± 117.392 769.429 (164.658)	<13	29	748.478 ± 146.066 791.525 (244.617)	0.601[b]
Parameter	**IFN-γ (IU/ml)**			**IFN-γ (IU/ml)**			*p*
	parameter group I	N	median (IQR)[a] mean ± SD[b]	parameter group II	N	median (IQR)[a] mean ± SD[b]	
Age	age ≥56	*3*	0.366 (2.001)	age <56	*11*	0.697 (1.044)	0.678[a]
sex	women	14	0.748 ± 0.629 0.668 (1.045)	Men	*0*	-	
SELENA_SLEDAI	≥6	8	0.643 ± 0.596 0.619 (1.069)	<6	6	0.887 ± 0.701 0.907 (0.778)	0.494[b]
disease duration	≥10	7	0.399 ± 0.43 0.366 (0.697)	<10	7	1.096 ± 0.624 1.132 (0.993)	**0.031[b]**
CRP	≥13	5	0.886 ± 0.743 0.697 (0.533)	<13	7	0.772 ± 0.614 1.094 (1.056)	0.776[b]
Parameter	**TNF-α (pg/ml)**			**TNF-α (pg/ml)**			*p*
	parameter group *I*	N	median (IQR)	parameter group II	N	median (IQR)	
Age	age ≥56	10	38.357 (26.58)	age <56	53	33.399 (52.754)	0.903[a]
sex	women	59	36.872 (52,938)	Men	4	34.694 (9.764)	0.855[a]
SELENA-SLEDAI	≥6	28	42.133 (74.537)	<6	35	28.832 (24.478)	**0.008[a]**
disease duration	≥10	28	31.237 (33.904)	<10	35	37.045 (55.14)	0.557[a]
CRP	≥13	24	36.781 (42.872)	<13	34	32.12 (46.795)	0.881[a]
Parameter	**IL-6 (pg/ml)**			**IL-6 (pg/ml)**			*p*
	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)	0.215[a]
sex	women	51	45.219 (45.321)	Men	4	46.698 (25.201)	0.502[a]
SELENA-SLEDAI	≥6	25	42.184 (38.117)	<6	30	46.058 (43.22)	0.872[a]
disease duration	≥10	27	57.272 (47.884)	<10	28	38.288 (35.78)	0.210[a]
CRP	≥13	20	40.145 (74.184)	<13	30	45.396 (36.417)	0.469[a]
anti-dsDNA	positive	32	38.374 (43.823)	Negative	22	53.078 (37.468)	0.705[a]
anti-SSA	positive	24	47.843 (36.809)	Negative	28	44.556 (54.647)	0.776[a]
anti-SSB	positive	5	32.047 (12.464)	Negative	46	47.459 (47.079)	0.289[a]
anti-Sm	positive	10	61.854 (33.104)	Negative	41	39.536 (38.086)	0.048[a]

a: Mann-Whitney test; b: student t-test.

did not reveal an association of APOH (rs4581) with first ischaemic stroke in the Russian population, which may be interesting in the aspect of our studies because of common ethnicity studied groups (47). Rs4581 is localised in the exon 7 of APOH gene, which encodes domain V of mature protein (48). Domain V contains the major phospholipid binding site (49) and it may have a role in the elimination of apoptotic cells from circulation (50). Mutation Taster analysis revealed that change valine by leucine in APOH rs4851 may affect protein features by losing region in the

Table VII. Variation in selected serum cytokines levels in SLE patients and control group in relation to TF gene polymorphisms.

	Genotype	SLE	Controls	*p*-value
	TGF-β (pg/ml)			
TF rs958587		median (IQR)[a] Mean ± SD[b]	median (IQR)[a] Mean ± SD[b]	
Codominant	TT	725.513 (211.706)	560.402 (252.566)	0.0008[a]
	TC	723.543 (271.57) 736.613 ± 164,107	596.213 (277.591) 564.657 ± 214,76	0.0002[b]
	CC	796.734 (135.669)	560.402 (252.566)	0.00001[a]
TF rs3917615		median (IQR)[a] Mean ± SD[b]	median (IQR)[a] Mean ± SD[b]	
Codominant	TT	745.22 (211.706) 717,083 ± 119,271	496.526 (235.452) 485,609 ± 191,064	0.0008[b]
	TC	720.372 (271.57) 733.649 ± 163,715	592.701 (308.953) 559.925 ± 223,93	0.0002[b]
	CC	801.943 (117.867)	562.72 (255)	0.00001[a]
TF rs1361600		median (IQR)[a] Mean ± SD[b]	median (IQR)[a] Mean ± SD[b]	
Codominant	CC	725.513 (211.706)	496.526 (235.452)	0.0008[a]
	CT	723.543 (271.57) 736.613 ± 164,107	596.213 (277.591) 564.657 ± 214,76	0.0002[b]
	TT	796.734 (135.669)	560.402 (252.566)	0.00002[a]
	IFN-γ (IU/ml)			
TF rs958587		median (IQR)[a]	median (IQR)[a]	
Codominant	TT	1.113 (0.291)	0.043 (0.237)	0.002[a]
	TC	0.455 (1,58)	0 (0.226)	0.035[a]
	CC	0.638 (1.541)	0 (0.222)	0.047[a]
TF rs3917615				
Codominant	TT	1.113 (0.291)	0.021 (0.195)	0.002[a]
	TC	0.455 (1.58)	0.004 (0.356)	0.057[a]
	CC	0.638 (1.541)	0 (0.158)	0.035[a]
TF RS1361600				
Codominant	CC	1.113 (0.291)	0.043 (0.225)	0.003[a]
	CT	0.455 (0.455)	0 (0.282)	0.040[a]
	TT	0.638 (1.541)	0 (0.222)	0.047[a]
	IL-6 (pg/ml)			
TF rs958587		median (IQR)[a]	median (IQR)[a]	
Codominant	TT	32.499 (41.939)	18.879 (29.51)	0.042[a]
	TC	44.703 (34.101)	21.192 (18.446)	0.0002[a]
	CC	62.866 (49.648)	26.99 (21.173)	0.0003[a]
TF rs3917615		median (IQR)[a]	median (IQR)[a]	
Codominant	TT	29.927 (48.734)	22.248 (24.939)	0.083[a]
	TC	44.703 (34.101)	21.192 (19.257)	0.0003[a]
	CC	59.612 (46.445)	26.559 (19.881)	0.0002[a]
TF rs1361600		median (IQR)[a]	median (IQR)[a]	
Codominant	CC	32.499 (45.158)	22.248 (30.415)	0.175[a]
	CT	46.898 (37.923)	20.953 (18.446)	0.00004[a]
	TT	62.866 (50.155)	26.99 (21.173)	0.0006[a]
	TNF-α (pg/ml)			
TF rs958587		median (IQR)[a]	median (IQR)[a]	
Codominant	TT	39.725 (63.634)	23.983 (4.786)	0.068[a]
	TC	31.598 (19.48)	16.084 (11.176)	0.00001[a]
	CC	32.459 (32.016)	13.301 (11.084)	0.00001[a]
TF rs3917615		median (IQR)[a]	median (IQR)[a]	
Codominant	TT	40.975 (63.634)	23.102 (20.876)	0.027[a]
	TC	31.109 (17.589)	16.084 (11.176)	0.00001[a]
	CC	32.697 (31.835)	13.301 (11.084)	0.000009[a]
TF rs1361600		median (IQR)[a]	median (IQR)[a]	
Codominant	CC	29.361 (63.715)	23.102 (20.876)	0.057[a]
	CT	32.222 (18.09)	16.084 (11.176)	0.000004[a]
	TT	35.807 (51.111)	13.301 (11.084)	0.00001[a]

a: Mann Whitney test; b: student t-test.

sushi-like domain. The sushi domain is also known as the complement control protein (CCP) module and is involved in many recognition processes like the binding of several complement factors to complement fragments C3b and C4b (51). According to Manderson *et al*. (2004) abnormal activation of complement pathway is associated with a high risk (>80%) of developing SLE (52). Additionally, in APS patients rs4581 allele C (variant Val) is associated with increased risk of disease development and in the present study, we notice that this allele may affect the clinical course of SLE. In the present study, we observed that carriers of C allele but not A allele carrier, are characterised with significantly higher serum concentration of analysed cytokines.

Significance of the genetic variants in APOH promoters has been described by Suresh *et al*. (2009). The authors revealed that APOH rs8178819 genetic variant is significantly associated with the risk of SLE (53). In the present study, we observed that SLE patients with rs8178819 genotype CC did not reveal higher concentration of inflammatory cytokines in serum as compared to SLE patients with the CT or TT genotypes. Nevertheless, in comparison to the healthy individuals, significantly higher IL-6 and TGFβ serum levels were observed in carriers of CC genotype but not CT. Moreover, we noticed that APOH rs8178819 genotype CC seems to be associated with high value of ESR in SLE patients. In several studies, an association of ESR with SLE activity has been reported and in fact, patients in remission revealed lower values of ESR (38, 53). In most cases, inflammatory processes are associated with ESR marker, which strongly depends on high fibrinogen concentration allowing the sticking between erythrocytes (48). ESR is an easy, fast and cheap diagnostic method for observation of changes in plasma proteins. Higher concentration of globulins, including immunoglobulins and interaction between erythrocytes and fibrinogen raised viscosity plasma, elevates RBC aggregation and ESR value (40, 54).

Very little is known about the significance of APOH rs8178835, which is

Table VIII. Variation in selected cytokines concentration in SLE patients and control group in relation to APOH gene polymorphisms.

	genotype	SLE	Controls	*p*-value
	TGF-β (pg/ml)			
APOH rs4581		median (IQR)[a] Mean ± SD[b]	median (IQR)[a] Mean ± SD[b]	
Codominant	AA	751.767 ± 62.046	497.371 ± 318.598	0.062[b]
	AC	763.577 (189.28)	612.892 (186.38)	**0.002**[a]
	CC	727.454 ± 164.622	532.297 ± 223.902	**0.00008**[b]
APOH rs8178835		median (IQR)[a] Mean ± SD[b]	median (IQR)[a] Mean ± SD[b]	
Codominant	AA	744,355 (210,16)	542.943 (302.79)	**<0.00001**[a]
	AG	790,143 (177,13)	612.892 (186.38)	**0.001**[a]
	GG	759,427 ± 64,24	497.371 ± 318.598	0.079[b]
APOH rs8178819		median (IQR)[a] Mean ± SD[b]	median (IQR)[a] Mean ± SD[b]	
Codominant	CC	766.835 (186.75)	554.529 (306.04)	**<0.00001**[a]
	CT	692.806 ± 190.466	591.687 ± 189.113	0.295[b]
	TT	-	-	
	IFN-γ (IU/ml)			
APOH rs4581		median (IQR)[a]	median (IQR)[a]	
Codominant	AA	0 (0)	0 (0.113)	0.724[a]
	AC	0.858 (0.517)	0 (0.089)	**0.018**[a]
	CC	1.138 (1.838)	0.043 (0.347)	**0.013**[a]
APOH rs8178835		median (IQR)[a]	median (IQR)[a]	
Codominant	AA	1.113 (1.327)	0.043 (0.356)	**0.001**[a]
	AG	0.697 (0.479)	0 (0.089)	**0.0004**[a]
	GG	0 (0)	0 (0.113)	-
APOH rs8178819		median (IQR)[a]	median (IQR)[a]	
Codominant	CC	0.697 (0,931)	0 (0.226)	**0.00006**[a]
	CT	-	0 (0.166)	
	TT	-	-	
	IL-6 (pg/ml)			
APOH rs4581		median (IQR)[a]	median (IQR)[a]	
Codominant	AA	72.94 (96.31)	27.061 (34.534)	0.171[a]
	AC	58.802 (53.709)	22.63 (28.358)	**0.0006**[a]
	CC	31.091 (35.819)	20.643 (18.919)	**0.002**[a]
APOH rs8178835		median (IQR)[a]	median (IQR)[a]	
Codominant	AA	42.184 (37.337)	22.946 (19.445)	**0.00002**[a]
	AG	48.884 (45.64)	22.63 (28.358)	**0.002**[a]
	GG	88.286 (77.894)	27.061 (34.534)	0.156[a]
APOH rs8178819		median (IQR)[a]	median (IQR)[a]	
Codominant	CC	43.894 (40.652)	23.15 (21.471)	**<0.00001**[a]
	CT	56.358 (49.067)	21.137 (16.791)	0.314[a]
	TT	-	-	-
	TNF-α (pg/ml)			
APOH rs4581		median (IQR)[a]	median (IQR)[a]	
Codominant	AA	29.776 (60,618)	6.133 (0,941)	0.057[a]
	AC	36.605 (17,935)	16.626 (15,41)	**0.0008**[a]
	CC	31.311 (42,887)	16.026 (13,885)	**<0.00001**[a]
APOH ra8178835		median (IQR)[a]	median (IQR)[a]	
Codominant	AA	30.663 (33,839)	16.084 (16,09)	**<0.00001**[a]
	AG	36.872 (16,597)	16.626 (15,41)	**0.0002**[a]
	GG	31.775 (60,618)	6.133 (0,941)	0.067[a]
APOH rs8178819		median (IQR)[a] mean ± SD[b]	median (IQR)[a] mean ± SD[b]	
Codominant	CC	31.642 (33,914)	14.766 (13,659)	**<0.00001**[a]
	CT	52.426 ± 35,536	16.334 ± 10,334	**0.036**[b]
	TT	-	-	-

a: Mann Whitney test; b: student t-test.

localised in intron 3. Cooper (2010) pointed out that some SNPs may influence the expression of remote genes at distance, rather than the expression of those genes which actually host them (55). Certainly, the development of new technologies, deep sequencing and bioinformatic tools, will contribute to understanding the structure and function of introns. Nevertheless, rs8178835 has been classified as a marker capable of being associated with cardiovascular diseases, particularly coronary heart disease (especially myocardial infarction) or hypertension, a greater than 51% probability (US20130030051A1) (56). Present study, revealed that homozygous rs8178835 GG patients did not suffer from arthritis. Moreover, IL-6 and TGF-β serum concentrations in these patients were not statistically different from the outcomes noted in healthy subjects. Additionally, IFN-γ serum level in GG patients was not detected or it was below quantification. Further studies will be necessary to delineate the possibly protective function of the APOH rs8178835 genotype GG in pathology and clinical course of SLE.

The most important clinically relevant finding of the present study is that in our population, almost three-quarters of SLE patients with TF rs3917615 TC and TT genotypes and halve of SLE patients with TF rs958587 TT genotype suffered from joint inflammation/arthritis. These results provide further support for the statement that some polymorphic variants may to predispose to more severe inflammatory processes and consequently modify susceptibility for disease (17, 57). TF expression is found to be regulated by several inflammatory factors including TNF-α, IFN-γ, IL-1 and IL-6 (15). Moreover, in the present study we observed that serum TNF-α levels was not significantly different between healthy subjects and patients with TF rs958587 TT genotype and rs1361600 CC variants, what might be also the result of the treatment or other cytokines concentration/ratio impact (18, 58). On the other hand, differences in production of TNF-α can be in part explained by its gene polymorphisms which are beyond the scope

of present study. Further studies with more focus on this aspect are therefore recommended.

Another important finding in the present study was similar level of IL-6 in serum of healthy subjects and SLE patients with TF rs3917615 TT and rs1361600 CC variants. Ott *et al*. (2004) reported that TF -603G (rs1361600) is associated with an enhanced TF expression, higher plasma TF concentration and consequently with an increased risk for myocardial infarction (59). Moreover, TF rs3917615 TT and rs1361600 CC variants were characterised by the lowest concentration of TGF-β among SLE patients. However, SLE patients revealed a higher level of TGF-β in serum compared with healthy individuals. Generally, SLE patients have an increased level of IL-6 (19, 60). Yoshito *et al*. (1997) reported elevated concentration of IL-6 during cardiopulmonary complication of SLE (61) and Eilertsen *et al*. (2011) found involvement of IL-6 in joint damage in SLE patients (62). On the other hand, numerous studies have revealed the raised value of inflammatory cytokines in blood donors (22, 23, 27, 62-64) which appears to be another limitation of this study. It is also worth noting that these three SNPs have been implicated into novel treatment strategies and treatment prediction for patients having or at risk of developing an inflammatory condition or hypertension US20090148458A1 (65), and for predicting patients response to treatment with activated protein C or protein C like compound US20110027184A1 (66).

Conclusions

In summary, our data indicate that genetic variants of TF and APOH may have an impact on inflammatory processes and clinical relevance in SLE patients in the Caucasian population.

References

1. ROSE T, DÖRNER T: Drivers of the immunopathogenesis in systemic lupus erythematosus. *Best Pract Res Clin Rheumatol* 2017; 31: 321-33.
2. CERVERA R, KHAMASHTA MA, FONT J *et al*.: Morbidity and mortality in systemic lupus erythematosus during a 10-year period: A comparison of early and late manifestations in a cohort of 1,000 patients. *Medicine* (Baltimore) 2003; 82: 299-308.
3. PEPONIS V, KYTTARIS VC, TYRADELLIS C, VERGADOS I, SITARAS NM: Ocular manifestations of systemic lupus erythematosus: A clinical review. *Lupus* 2006; 15: 3-12.
4. BERTSIAS G *et al*.: Systemic lupus erythematosus: pathogenesis and clinical features. *Eular On-line Course Rheum Dis* 2012.
5. WILD CP: Complementing the genome with an "exposome": The outstanding challenge of environmental exposure measurement in molecular epidemiology. *Cancer Epidemiol Biomarkers Prev* 2005; 14: 1847-50.
6. BAZZAN M, VACCARINO A, MARLETTO F: Systemic lupus erythematosus and thrombosis. *Thromb J* 2015; 13: 16.
7. BIGGIOGGERO M, MERONI PL: The geoepidemiology of the antiphospholipid antibody syndrome. *Autoimmun Rev* 2010; 9: A299-304.
8. AL-HOMOOD IA: Thrombosis in systemic lupus erythematosus: a review article. *ISRN Rheumatol* 2012; 2012: 428269,
9. MEHRANI T, PETRI M: Chapter 2 Epidemiology of the antiphospholipid syndrome. *Handb Syst Autoimmune Dis* 2009; 10: 13-34.
10. ALARCÓN-SEGOVIA D, PÉREZ-VÁZQUEZ ME, VILLA AR, DRENKARD C, CABIEDES J: Preliminary classification criteria for the antiphospholipid syndrome within systemic lupus erythematosus. *Semin Arthritis Rheum* 1992; 21: 275-86.
11. PETRI M: Epidemiology of the antiphospholipid antibody syndrome. *J Autoimmun* 2000; 15: 145-51.
12. CERVERA R, KHAMASHTA MA, FONT J *et al*.: Morbidity and mortality in systemic lupus erythematosus during a 10-year period: A comparison of early and late manifestations in a cohort of 1,000 patients. *Medicine* (Baltimore) 2003; 82: 299-308,
13. PALTA S, SAROA R, PALTA A: Overview of the coagulation system. *Indian J Anaesth* 2014; 58: 515-23.
14. MACKMAN N: Regulation of the tissue factor gene. *FASEB J*. 1995; 9: 883-9.
15. BANNER DW, D'ARCY A, CHÈNE C *et al*.: The crystal structure of the complex of blood coagulation factor VIIa with soluble tissue factor. *Nature* 1996; 380: 41-6.
16. EGORINA EM, SOVERSHAEV MA, OLSEN JO, ØSTERUD B: Granulocytes do not express but acquire monocyte-derived tissue factor in whole blood: Evidence for a direct transfer. *Blood* 2008; 111: 1208-16.
17. STEFFEL J, LÜSCHER TF, TANNER FC: Tissue factor in cardiovascular diseases: molecular mechanisms and clinical implications. *Circulation* 2006; 113: 720-31.
18. STEFFEL J, HERMANN M, GREUTERT H *et al*.: Celecoxib decreases endothelial tissue factor expression through inhibition of c-Jun terminal NH 2 kinase phosphorylation. *Circulation* 2005; 111: 1685-9.
19. MCCARTHY EM, SMITH S, LEE RZ *et al*.: The association of cytokines with disease activity and damage scores in systemic lupus erythematosus patients. *Rheumatology* 2014; 53: 1586-94.
20. IDBORG H, EKETJÄLL S, PETTERSSON S *et al*.: TNF-α and plasma albumin as biomarkers of disease activity in systemic lupus erythematosus. *Lupus Sci Med* 2018; 5: e000260
21. MCDEVITT H, MUNSON S, ETTINGER R, WU A: Multiple roles for tumor necrosis factor-alpha and lymphotoxin alpha/beta in immunity and autoimmunity. *Arthritis Res* 2002; 4 (Suppl. 3): S141-52.
22. ARINGER M, SMOLEN JS: Complex cytokine effects in a complex autoimmune disease: Tumor necrosis factor in systemic lupus erythematosus. *Arthritis Res Ther* 2003; 5: 172-7.
23. ARINGER M, GRANINGER WB, STEINER G, SMOLEN JS: Safety and efficacy of tumor necrosis factor α blockade in systemic lupus erythematosus: An open-label study. *Arthritis Rheum*. 2004; 50: 3161-9.
24. MCDONNELL T, WINCUP C, BUCHHOLZ I *et al*.: The role of beta-2-glycoprotein I in health and disease associating structure with function: More than just APS. *Blood Rev* 2020; 39: 100610.
25. BENAGIANO M, BORGHI MO, ROMAGNOLI J *et al*.: Interleukin-17/interleukin-21 and interferon-γ producing T cells specific for β2 glycoprotein I in atherosclerosis inflammation of systemic lupus erythematosus patients with antiphospholipid syndrome. *Haematologica* 2019; 104: 2519-27.
26. LAPLANTE P, AMIREAULT P, SUBANG R, DIEUDÉ M, LEVINE JS, RAUCH J: Interaction of β2-glycoprotein I with lipopolysaccharide leads to Toll-like receptor 4 (TLR4)-dependent activation of macrophages. *J Biol Chem* 2011; 286: 42494-503.
27. PARADOWSKA-GORYCKA A, ROSZAK M, STYPINSKA B *et al*.: IL-6 and TGF-β gene polymorphisms, their serum levels, as well as HLA profile, in patients with systemic lupus erythematosus. *Clin Exp Rheumatol* 2019; 37: 963-75.
28. HAŁADYJ E, PARADOWSKA-GORYCKA A, FELIS-GIEMZA A, OLESIŃSKA M: Immunity and early atherosclerosis in the course of systemic lupus erythematosus, mixed connective tissue disease and antiphospholipid syndrome. *Reumatologia* 2016; 54: 187-95.
29. PARADOWSKA-GORYCKA A, WAJDA A, STOLAREK I *et al*.: Is the T-G-CT-G SNRNP70 haplotype another proof that mixed connective tissue disease is distinct from systemic lupus erythematosus and systemic sclerosis? A novel gene variant in SNRNP70 gene. *Clin Exp Rheumatol* 2019; 37: 32-40.
30. PARADOWSKA-GORYCKA A, SOWINSKA A, STYPINSKA B *et al*.: Impact of the IL-17F, IL-23 and IL-23R on susceptibility and phenotype of systemic lupus erythematosus. *Autoimmunity* 2016; 49: 373-82.
31. PARADOWSKA-GORYCKA A, SOWINSKA A, STYPINSKA B *et al*.: Genetic variants in IL-12B and IL-27 in the Polish patients with systemic lupus erythematosus. *Scand J Immunol* 2016; 84: 49-60.
32. RZESZOTARSKA E, SOWINSKA A, STYPINSKA B *et al*.: The role of MECP2 and CCR5 polymorphisms on the development and course of systemic lupus erythematosus. *Biomolecules* 2020; 10: 494.
33. WOJDASIEWICZ P, WAJDA A, HAŁADYJ E *et al*.: IL-35, TNF-α, BAFF, and VEGF serum levels in patients with different rheumatic diseases. *Reumatologia* 2019; 57: 145-50.
34. SHI YY, HE L: SHEsis, a powerful software

platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. *Cell Res* 2005; 15: 97-8.
35. LUBECK E, COSKUN AF, ZHIYENTAYEV T, AHMAD M, CAI L: MutationTaster2: mutation prediction for the deep-sequencing age. *Nat Methods* 2012; 9: 743-8.
36. LIANG W, ZHOU J, ZHOU S *et al*.: Val/Leu247 polymorphism of β2-glycoprotein I and thrombosis in Chinese patients with SLE. *Int J Lab Hematol* 2015; 37: 22-8.
37. FABBRI M, CROCE CM, CALIN GA: MicroRNAs in the ontogeny of leukemias and lymphomas. *Leuk. Lymphoma* 2009; 50: 160-70.
38. GEORGE J, HARATS D, GILBURD *B et a*l.: Immunolocalization of β2-glycoprotein I (apolipoprotein H) to human atherosclerotic plaques: Potential implications for lesion progression. *Circulation* 1999; 99: 2227-30.
39. NIESSEN HW, LAGRAND WK, RENSINK HJ, MEIJER CJ, AARDEN L, HACK CE: Apolipoprotein H, a new mediator in the inflammatory changes ensuing in jeopardised human myocardium. *J Clin Pathol* 2000; 53: 863-7.
40. CHU AJ: Tissue factor mediates inflammation. *Arch Biochem Biophys* 2005; 440: 123-32.
41. FOLEY JH, CONWAY EM: Cross talk pathways between coagulation and inflammation. *Circ Res* 2016; 118: 1392-408.
42. SHI D, SONG Z, YIN J *et al*.: Genetic variation in the tissue factor gene is associated with clinical outcome in severe sepsis patients. *Crit Care* 2014; 18: 631.
43. SCHOUSBOE I: β2-glycoprotein I: A plasma inhibitor of the contact activation of the intrinsic blood coagulation pathway. *Blood* 1985; 66: 1086-91.
44. KAMBOH MI, MANZI S, MEHDI H *et al*.: Genetic variation in apolipoprotein H (β2-glycoprotein I) affects the occurrence of antiphospholipid antibodies and apolipoprotein H concentrations in systemic lupus erythematosus. *Lupus* 1999; 8: 742-50.
45. JONES JV, JAMES H, TAN MH, MANSOUR M: Antiphospholipid antibodies require beta 2-glycoprotein I (apolipoprotein H) as cofactor. *J Rheumatol* 1992; 19: 1397-402.
46. ZHOU L: AHR function in lymphocytes: emerging concepts. *Trends Immunol* 2016; 37: 17-31.
47. SHISHKOVA VN, REMENNIK AY, VALYAEVA VV *et al*.: Study of associations of polymorphic variants of lipid metabolism genes and angiogenesis with first ischemic stroke. *Cons Medicum* 2019; 21: 48-52.
48. VEAS F: Acute phase proteins as early nonspecific biomarkers of human and veterinary diseases; VEAS F (Ed.) 2011; InTech; ISBN 9789533078731.
49. PERNAMBUCO-CLIMACO JM, BROCHADO MJF, FREITAS MVC, ROSELINO AMF, LOUZADA-JUNIOR P: Val/Leu247 polymorphism of β2-glycoprotein I in Brazilian patients with antiphospholipid syndrome - A genetic risk factor. *Proc Ann NY Acad Sci* 2009; 1173: 509-14.
50. PENGO V, RUFFATI A: Lupus anticoagulant testing. *Autoantibodies* 2007; 733-9.
51. SCHALLER J, GERBER S, KÄMPFER U, LEJON S, TRACHSEL C: *Human Blood Plasma Proteins: Structure and Function*; 2008; ISBN 9780470016749.
52. MANDERSON AP, BOTTO M, WALPORT MJ: The role of complement in the development of systemic lupus erythematosus. *Annu Rev Immunol* 2004; 22: 431-56.
53. SURESH S, DEMIRCI FY, JACOBS E *et al*.: Apolipoprotein H promoter polymorphisms in relation to lupus and lupus-related phenotypes. *J Rheumatol* 2009; 36: 315-22.
54. SCHÄFER VS, WEIß K, KRAUSE A, SCHMIDT WA: Does erythrocyte sedimentation rate reflect and discriminate flare from infection in systemic lupus erythematosus? Correlation with clinical and laboratory parameters of disease activity. *Clin Rheumatol* 2018; 37: 1835-44.
55. COOPER DN: Functional intronic polymorphisms: Buried treasure awaiting discovery within our genes. *Hum Genomics* 2010; 4: 284-8.
56. US20130030051A1 - Genetic polymorphisms associated with cardiovascular diseases, methods of detection and uses thereof - Google Patents Available online: https://patents.google.com/patent/US20130030051A1/en?oq=US20130030051A1 (accessed May 1, 2020).
57. LOZA MJ, MCCALL CE, LI L, ISAACS WB, XU J, CHANG BL: Assembly of inflammation-related genes for pathway-focused genetic analysis. *PLoS One* 2007; 2: e1035.
58. MÓZES T, BARÁTH I, GORNICSAR K *et al*.: Deviations in circulating TNF-α levels and TNFα production by mononuclear cells in healthy human populations. *Mediators Inflamm* 2011; 2011: 972609.
59. OTT I, KOCH W, VON BECKERATH N *et al*.: Tissue factor promotor polymorphism -603 A/G is associated with myocardial infarction. *Atherosclerosis* 2004; 177: 189-91.
60. OHL K, TENBROCK K: Inflammatory cytokines in systemic lupus erythematosus. *J Bio-med Biotechnol* 2011, 2011: 432595.
61. YOSHIO T, MASUYAMA JI, KOHDA N *et al*.: Association of interleukin 6 release from endothelial cells and pulmonary hypertension in SLE. *J Rheumatol* 1997; 24: 489-95.
62. EILERTSEN G, NIKOLAISEN C, BECKER-MEROK A, NOSSENT JC: Interleukin-6 promotes arthritis and joint deformation in patients with systemic lupus erythematosus. *Lupus* 2011; 20: 607-13.
63. SAHDO B, FRANSÉN K, ASFAW IDOSA B *et al*.: Cytokine profile in a cohort of healthy blood donors carrying polymorphisms in genes encoding the NLRP3 inflammasome. *PLoS One* 2013; 8: e75457.
64. VON STEMANN JH, RIGAS AS, THØRNER LW *et al*.: Prevalence and correlation of cytokine-specific autoantibodies with epidemiological factors and C-reactive protein in 8,972 healthy individuals: Results from the danish blood donor study. *PLoS One* 2017; 12: e0179981.
65. US20090148458A1 - Coagulation factor iii polymorphisms associated with prediction of subject outcome and response to therapy - Google Patents Available online: https://patents.google.com/patent/US20090148458?oq=rs958587 (accessed May 1, 2020).
66. US20110027184A1 - Protein c pathway associated polymorphisms as response predictors to activated protein c or protein c-like compound administration - Google Patents Available online: https://patents.google.com/patent/US20110027184?oq=rs958587 (accessed May 1, 2020).