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ORIGINAL ARTICLE

INHERITED THROMBOPHILIAS COULD INFLUENCE THE REPRODUCTIVE OUTCOME IN WOMEN WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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ABSTRACT

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease associated with different reproductive complications in the affected women. Inherited thrombophilias are genetic factors increasing the risk for thromboembolism and recurrent pregnancy loss, but their influence on other reproductive disturbances in SLE patients has not been completely clarified. Two hundred and twenty-three Caucasian women (112 with SLE and 111 controls) were included in the study. Complete reproductive history of all SLE patients was carefully obtained. Genotyping for the FV_{Leiden}, $FII_{G20210A}$, and $MTHFR_{C677T}$ polymorphisms was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. No significant differences in the prevalence of the FV_{Leiden}, $FII_{G20210A}$, and $MTHFR_{C677T}$ polymorphisms between patients and controls were established. Patients with FV_{Leiden} had fewer pregnancies (0.57 ± 0.98 *vs.* 2.18 ± 1.58; $p = 0.007$) than the others, while no significant differences in the reproductive history of $FII_{G20210A}$ carriers and non-carriers were observed ($p > 0.05$). In the SLE group, 41.67% of women with the $MTHFR_{C677T}$ TT genotype had at least one miscarriage in comparison to only 14.00% of the other female patients ($p = 0.030$). While the prevalence of the investigated thrombophilias was similar in patients with SLE and healthy women, a substantial influence of the inherited prothrombotic factors on the reproductive history of patients was revealed. The investigations of the FV_{Leiden} and $MTHFR_{C677T}$ polymorphisms in SLE patients could help to identify women at highest risk for reproductive failure and thus, further studies in other ethnic groups would be of strong clinical importance.

Keywords: Autoimmunity; Miscarriages; Reproduction; Systemic lupus erythematosus (SLE); Thrombophilias.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that could increase the risk for adverse pregnancy outcomes in affected women because of the associated hypercoagulability [1]. The SLE exacerbations, active SLE at conception, presence of anti-phospholipid antibodies, lupus nephritis and hypertension have been reported as important risk factors for obstetric complications [2,3]. However, the role of genetically determined thrombophilias for women with SLE has not yet been completely clarified.

A well-known prothrombotic factor is the single nucleotide substitution in the *factor V* gene (rs6025, G>A substitution at nucleotide position 1691), which encodes a resistant to activated protein C molecule (Factor V Leiden) [4]. Other common thrombophilias include a prothrombin gene G20210A variant (rs1799963) that has been associated with elevated prothrombin activity as well as C>T transition at base pair 677 of the methylenetetrahydrofolate re-ductase (*MTHFR*) gene (rs1801133) (CC, CT and TT genotypes), which has been related to increased plasma homocysteine levels [5,6].

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Several studies have investigated the influence of Factor V Leiden and prothrombin G20210A polymorphisms on thrombosis prevalence in lupus patients [7-12]. Additionally, some authors discuss the presence of recurrent miscarriages and fetal losses in SLE women with inherited thrombophilias [8,11], but no definitive conclusions occur. The present study aimed to investigate associations between the reproductive history of women with lupus and the common thrombophilic polymorphisms including Factor V Leiden (FV_{Leiden}), prothrombin G20210A (FII) and MTHFR C677T.

MATERIALS AND METHODS

Subjects. Two hundred and twenty-three Caucasian women [mean age 40.77 ± 11.94 years (20-68)] were included in the study. One hundred and twelve patients fulfilled the modified 1997 American College Rheumatology (ACR) classification criteria for SLE [13]. Secondary antiphospholipid syndrome (sAPS) according to the accepted criteria was presented in 15.18% of them [14]. All women underwent a complete general assessment and filled-out questionnaires on their reproductive history. The age of menarche, menstrual regularity (before the SLE onset and treatment), infertility, number of pregnancies, miscarriages, intentional abortions, stillbirths (fetal loss after 20 gestational weeks) and live children were self-reported. One hundred and eleven controls were collected from the medical staff and students. They were all clinically healthy women without known connective tissue diseases. The experimental protocol was explained to all participants and written informed consent was obtained. The study was approved by the institutional Ethics Committee.

Genetic Assay. All participating women provided peripheral blood samples for DNA collected in EDTA vacutainers. Genomic DNA was extracted with a standard salt extraction procedure. Genotyping for the FV_{Leiden}, $FII_{G20210A}$ and $MTHFR_{C677T}$ polymorphisms was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis [15]. The control group of women was used only for a comparison of the genetic polymorphism prevalence. The distribution of all investigated genotypes in healthy females was in agreement with the Hardy-Weinberg equilibrium.

Statistical Analyses. The results were presented as mean ± standard deviation (SD) (median) for continuous variables or as a frequency (%). Categorical data were analyzed by the χ^2 test or Fisher's exact test. Differences between two groups were established with a Mann-Whitney test, while a Kruskal-Wallis test was used for comparisons of the three groups. Logistic regression analysis was used where appropriate. A p value of 0.05 was considered significant. Statistical analysis was conducted by the Statistical Package for the Social Sciences (SPSS), for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

No significant differences in the prevalence of the FV_{Leiden}, $FII_{G20210A}$ and $MTHFR_{C677T}$ polymorphisms between patients and controls were established (Table 1), although the frequency of heterozygous $MTHFR_{C677T}$ carriers in SLE patients was increased. The differences in the reproductive history of patients with FV_{Leiden} or $FII_{G20210A}$ polymorphisms are presented on Table 2. Patients with FV_{Leiden} had fewer pregnancies and live births than the others, while no significant differences in the reproductive history of $FII_{G20210A}$ carriers and non-carriers were observed (Table 2). The logistic regression analysis showed that the presence of FV_{Leiden} but not the $FII_{G20210A}$ decreased significantly the chance for having at least one live birth even after adjustment for the current age of patients and

Table 1. Prevalence of the investigated genetic polymorphisms in patients with SLE and healthy women. N: normal allele; M: polymorphic allele. Genotypes: NN: two normal alleles; NM: heterozygous state. No homozygous carriers of FV_{Leiden} or FII G20210A were found and none of the patients had both mutations. $MTHFR_{C677T}$ genotypes: CC: two normal alleles; TT: two polymorphic alleles; CT: heterozygous state.

	Controls			**SLE Patients**			***p* Value**
FV_{Leiden}	NN		NM	NN		NM	
n = 220	100 (91.7%)		9 (8.3%)	104 (93.7%)		7 (6.3%)	0.613
$FII_{G20310A}$	NN		NM	NN		NM	
n =223	105 (94.6%)		6 (5.4%)	106 (94.6%)		6 (5.4%)	1.000
$MTHFR_{C677T}$	CC	CT	TT	CC	CT	TT	
n =223	47 (42.3%)	46 (41.4%)	18 (16.2%)	36 (32.2%)	64 (57.1%)	12 (10.7%)	0.061

Table 2. Main reproductive characteristics of SLE patients in association with the FV_{Leiden} (2A), $FII_{G20210A}$ (2B) and $MTHFR_{C677T}$ (2C) genetic polymorphisms. SLE: systemic lupus erythematosus; ACR: American College of Rheumatology classification criteria; sAPS: secondary antiphospholipid syndrome. [The values are expressed as mean ± SD (median).]

Table 2A. Main reproductive characteristics in SLE patients with the FV_{Leiden} polymorphism

	FV_{Leiden} [+]	**FV_{Leiden} [–]**	***p* Value**
Age (years)	40.43 ± 11.18 (37.00)	42.75 ± 11.82 (42.50)	0.649
Age at diagnosis (years)	33.71 ± 15.50 (34.00)	35.03 ± 12.12 (34.50)	0.743
ACR criteria number	5.43 ± 1.27 (5.00)	5.37 ± 1.32 (5.00)	0.821
Age at menarche (years)	14.43 ± 0.79 (15.00)	13.83 ± 1.63 (14.00)	0.199
Menstrual regularity	71.43%	85.58%	0.291
Number of pregnancies	0.57 ± 0.98 (0.00)	2.18 ± 1.58 (2.00)	**0.007**
Number of miscarriages	0.00 (0.00)	0.23 ± 0.53 (0.00)	0.218
Miscarriage or stillbirth	0.00 (0.00)	22.11%	0.341
Number of abortions	0.00 (0.00)	0.55 ± 1.00 (0.00)	0.088
Number of live births	0.57 ± 0.98 (0.00)	1.33 ± 0.93 (1.00)	**0.049**
Infertility	0.00%	9.61%	1.000
sAPS	14.28%	15.38%	1.000

Table 2B. Main reproductive characteristics of SLE patients in association with the $FII_{G20210A}$ polymorphism

	$FII_{G20210A}$ [+]	**$FII_{G20210A}$ [–]**	***p* Value**
Age (years)	42.83 ± 12.78 (44.00)	42.50 ± 11.73 (42.00)	0.959
Age at diagnosis (years)	29.83 ± 13.15 (29.50)	35.06 ± 12.31 (34.50)	0.386
ACR criteria number	5.67 ± 1.50 (5.00)	5.38 ± 1.31 (5.00)	0.607
Age at menarche (years)	15.00 ± 2.28 (14.50)	13.80 ± 1.52 (14.00)	0.290
Menstrual regularity	83.33%	84.91%	1.000
Number of pregnancies	2.50 ± 1.05 (2.50)	2.04 ± 1.63 (2.00)	0.342
Number of miscarriages	0.33 ± 0.52 (0.00)	0.21 ± 0.51 (0.00)	0.322
Miscarriage or stillbirth	33.33%	19.81%	0.601
Number of abortions	0.67 ± 0.82 (0.50)	0.50 ± 0.99 (0.00)	0.313
Number of live births	1.33 ± 0.52 (1.00)	1.26 ± 0.97 (1.00)	0.870
Infertility	0.00%	9.43%	1.000
sAPS	16.67%	15.09%	1.000

Table 2C. Main reproductive characteristics of SLE patients in association with the $MTHFR_{C677T}$ polymorphism

$MTHFR_{C677T}$	**CC**	**CT**	**TT**	***p* Value**
Age (years)	42.53 ± 11.46 (40.50)	41.73 ± 12.17 (42.00)	46.67 ± 9.99 (47.50)	0.400
Age at diagnosis (years)	34.67 ± 11.86 (35.00)	34.20 ± 12.91 (32.50)	38.17 ± 11.08 (37.50)	0.599
ACR criteria number	5.44 ± 1.38 (5.00)	5.30 ± 1.28 (5.00)	5.75 ± 1.36 (6.00)	0.504
Age at menarche (years)	13.64 ± 1.46 (14.00)	13.91 ± 1.60 (14.00)	14.33 ± 1.87 (14.50)	0.307
Menstrual regularity	91.67%	81.25%	83.33%	0.398
Number of pregnancies	2.25 ± 1.42 (2.00)	1.88 ± 1.73 (1.00)	2.50 ± 1.31 (2.00)	0.146
Number of miscarriages	0.19 ± 0.47 (0.00)	0.16 ± 0.44 (0.00)	0.58 ± 0.79 (0.00)	**0.040**
Miscarriage or stillbirth	19.44%	17.18%	41.66%	0.181
Number of abortions	0.64 ± 0.96 (0.00)	0.50 ± 1.05 (0.00)	0.17 ± 0.39 (0.00)	0.223
Number of live births	1.36 ± 0.99 (1.00)	1.16 ± 0.95 (1.00)	1.58 ± 0.79 (2.00)	0.287
Infertility	16.67%	4.69%	8.33%	0.085
sAPS	16.66%	10.94%	33.33%	0.135

the presence of sAPS [0.041 (0.004-0.400), $p = 0.006$]. The FV_{Leiden} or $FII_{G20210A}$ polymorphisms did not influence significantly the risk for at least one unsuccessful pregnancy (miscarriage or stillbirth) ($p > 0.05$).

The reproductive history of patients according to the presence of $MTHFR_{C677T}$ polymorphism is presented in Table 2. Among women with the TT genotype, 41.67% had at least one miscarriage in comparison to 14.00% of the other female patients ($p = 0.030$), while the prevalence of self-reported infertility and number of pregnancies was similar in $MTHFR_{C677T}$ TT homozygotes in comparison to $MTHFR_{C677T}$ C allele carriers. The presence of $MTHFR_{C677T}$ TT increased more than three times the risk for at least one miscarriage in SLE patients after adjustment for age [odds ratio (OR) 3.827 (1.013-14.459), $p = 0.048$] but the association was attenuated after adjustment for age and sAPS [OR 2.765 (0.607-12.604), $p = 0.189$].

Recurrent pregnancy loss (two or more miscarriages) was reported by six (5.4%) of the patients. It was not related to any of the investigated polymorphisms ($p > 0.1$ for all).

DISCUSSION

Factor V Leiden and $FII_{G20210A}$ are the most frequently investigated genetic polymorphisms related to hypercoagulability, venous thromboembolism and recurrent pregnancy loss [16,17]. Both polymorphisms were thoroughly investigated in SLE patients, but the studies were focused primarily on disease-related thrombotic events [9,11]. The largest study that investigated risk factors for thrombosis in SLE patients suggested that the genetic predisposition for obstetric and non-obstetric thrombosis in lupus patients might be similar [11]. Accordingly, the present study aimed to investigate the influence of inherited thrombophilias on some reproductive aspects of SLE women. The results showed that the FV_{Leiden} allele carriers reported a lower number of pregnancies and live births irrespective of the presence of the sAPS. As the self-reported prevalence of menstrual irregularities was similar in both groups, an increased frequency of unrecognized chemical pregnancy loss is one of the possible hypotheses. Factor V Leiden was already associated with a higher rate of preclinical pregnancy losses and very early recurrent miscarriages in otherwise healthy women [18,19]. The mutant allele was not associated with fetal losses in the investigated SLE patients, although in healthy women FV_{Leiden} increased the risk for second trimester miscarriages and stillbirths due to uteroplacental insufficiency [19-21]. As FV_{Leiden} and lupus are both independently related to a resistance against activated protein C and hypercoagulability, their synergistic effect could lead to an earlier miscarriage than in women without the autoimmune disease [22]. Ineffective blood flow caused by a vascular insufficiency in the trophoblast might impair the developing pregnancy in mothers with several prothrombotic risk factors [18]. Contrary to our results, Regéczy *et al.* [8] found significantly higher prevalence of fetal losses in FV_{Leiden} carriers than in other SLE women. The discrepancies could be explained by the low number of mutation carriers, different characteristics of SLE patient groups and ethnic peculiarity, but nevertheless, both studies showed that FV_{Leiden} could be an important genetic factor in the deteriorating reproductive outcomes in lupus patients.

The prevalence of the G20210A polymorphism in the prothrombin gene did not increase the risk for SLE development and did not influence the thrombotic risk in patients with lupus [9,11,23]. $FII_{G20210A}$ was related to increased prevalence of recurrent miscarriages in the general population [17], but data concerning SLE patients were scarce. The present study did not find any significant associations between the mutant allele and the reproductive outcomes in SLE patients. However, no definitive conclusions could be drawn, because of the small number of $FII_{G20210A}$ carriers.

The $MTHFR_{C677T}$ polymorphism was widely investigated in different thrombotic and cancer diseases, and ethnic-dependent associations were found [24,25]. The prevalence of the $MTHFR_{C677T}$ polymorphism was also investigated in SLE patients [26,27]. Afeltra *et al.* [26] found an increased frequency of TT but not CT genotypes in lupus patients compared to controls, while Summers *et al.* [27] did not find any differences. Our data did not show significant differences between patients and controls, although the prevalence of heterozygous CT carriers in SLE patients was increased. Further larger studies are needed to establish the possible influence of the $MTHFR_{C677T}$ polymorphism on SLE development.

According to our results the $MTHFR_{C677T}$ TT genotype increased the risk of at least one miscarriage in lupus women. $MTHFR_{C677T}$ was significantly associated with recurrent miscarriages in Asian women, while the results in Caucasians were conflicting [28-30]. After adjustment for sAPS, the association between $MTHFR_{C677T}$ polymorphism and spontaneous abortion was attenuated, thus, synergistic effects of the immunological and genetic factors in SLE could not be excluded.

The main limitations of the study were the small number of pregnancies in the group of SLE patients as well as the self-reported reproductive history without data from medical records where other confounding factors could

be included. Nevertheless, the present data showed that inherited thrombophilias could influence the reproductive outcome in women with SLE. Interestingly, the same polymorphisms modulate the thrombosis risk in European-American SLE patients [11]. Thus, further studies in other ethnic groups would be of strong clinical importance.

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